Methods of Ion Generation

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I. Introduction

Mass spectrometry separates ions according to their mass-to-charge ratio. Except for the quadrupole ion trap, all conventional mass analyzers require ions in the gas phase in a sufficiently good vacuum that collisions with background gas are insignificant. With few exceptions, such as flames and plasmas, samples of interest are not charged and are present in condensed phases or as gases at atmospheric pressure or above. The challenge in selecting an ionization method for mass spectrometry is to choose a technique that preserves the properties of the sample that are of interest while at the same time converting it to ions which can be mass analyzed. This review will concentrate on ionization methods of most practical utility at present but will also include a brief summary of some that are of historical or fundamental importance.

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Mass spectrometry traces its roots to the work of J. J. Thomson[1] beginning nearly a century ago. During the first half of the century, the technique was developed and practiced by physicists and the applications were mainly limited to precise determination of the masses of the stable nuclides and natural abundance of isotopes.[2] During and immediately following World War II, analytical applications and fundamental physical measurements on molecules began to emerge. The early work required intense beams of atomic ions. The fact that most of the elements of interest were naturally available as components of molecules or crystalline solids was a nuisance to be overcome by the choice of suitably energetic means of producing ions such as gaseous discharges, arcs, and sparks. During the last half of the century, there has been a relatively continuous shift toward applications involving molecules of greater and greater complexity, and within the past decade biological applications have become, by far, the most important and fastest growing segment.

II. Atomic Ions

A. Thermal Ionization

This is one of the earliest techniques in mass spectrometry and was originally used for producing beams of positive and negative atomic ions for precise measurements of masses of the nuclides and relative intensities of the isotopes. This ionization source produces intense, stable beams that can be maintained for long periods to allow precise measurements but is limited to elements of relatively low ionization potential. An excellent review of the theory of thermal ionization and early experimental results has been given by Ivanov.[3]

In thermal ionization, one or more metal filaments are heated, directly or indirectly, by passage of an electrical current. The sample may be either in the gas phase or deposited directly on the surface. A widely used practical source is the triple-filament design of Inghram and Chupka.[4] In this arrangement the sample is deposited on the outer two filaments that are heated to produce a neutral vapor of the sample and the inner, hotter filament causes the ionization. The ratio of ions to neutrals at the surface of the hotter filament is given by the well-known Langmuir–Saha equation. As discussed in detail by Ivanov,[3] the effective work function is strongly dependent on the chemical and physical properties of the surface and may not be simply related to those for the clean, crystalline metal surface. This mode accounts satisfactorily for ionization of alkali metal and other easily ionized atoms on heated metal surfaces. This source is still widely used for accurate determinations of isotope ratios in geochemistry applications[5] and in nuclear technology.[6]

B. Spark Source

The thermal ionization source is simple and well-behaved but is highly selective. Elements with low ionization potentials are efficiently ionized, but others such as most transition metals are difficult or impossible to ionize thermally, and the ionization efficiency may vary by many orders of magnitude. The vacuum spark was originally developed by Dempster[7] to extend mass spectrometry to analysis of metals. In this source an electrical spark is produced between a rod electrode of the material to be analyzed and the wall of an aperture in a tantalum disk. Both Tesla-coil and pulsed-RF sources have been used for excitation of the spark. Ions passing through the aperture in the tantalum electrode are accelerated to ca. 15 kV and analyzed in a double-focusing mass spectrometer.[8] The ions from the source have a very wide energy spread (ca. 1000 eV), so double focusing is required to obtain useful resolution. Another problem with this source is that the spark is subject to random fluctuations in ion intensity and produces considerable RF noise which may interfere with electrical detection. Despite these limitations, the spark source provides a very sensitive method for the analysis of trace impurities in solids that give approximately equal sensitivity for all elements.[9] It has been replaced for some applications by more recently developed plasma ionization sources but is still used in many laboratories, particularly for determination of trace impurities in metals.

C. Plasma Sources

The dominant techniques presently in use for elemental analysis by mass spectrometry fall into the general category of plasma sources. An excellent review with an extensive bibliography has been published by Hieftje.[10] These may be separated into those operating at reduced pressure, ca. 1 Torr, producing a glow discharge (GD), and those operating at atmospheric pressure using inductively coupled plasma (ICP) generators. Glow discharge ionization has a long history in mass spectrometry, beginning with its early use in isotopic studies and as a means of ionizing volatile organic compounds. These applications generally employ other techniques at present, but glow discharges are still used extensively in the analysis of trace elements in semiconductors, metals, and especially high-purity metals.[11]

D. Glow Discharge

A schematic diagram of a glow discharge ion source for a magnetic mass spectrometer is shown in Figure 1.[12] The cathode is formed as a metal pin, ca. 2 mm in diameter, mounted on a removable probe. The stainless steel anode contains the ion exit aperture, typically 0.5 mm in diameter. The anode is separated
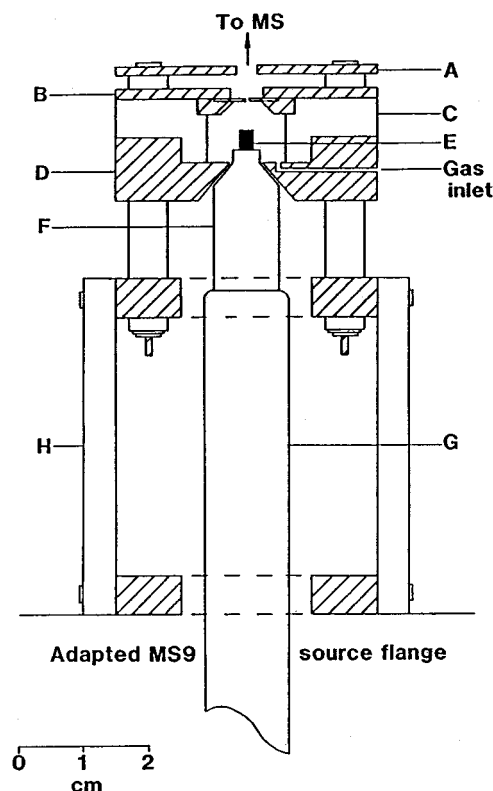
Figure 1. Schematic diagram of the glow discharge source for an MS9 magnetic mass spectrometer: A, beam focusing plates; B, ion exit (anode) plate; C, machined ceramic insulator; D, source block; E, sample pin (cathode); F, insulated cathode "cap"; G, stainless steel probe shaft; H, quartz support plates. (Reprinted with permission from ref 12. Copyright 1989.)

from the source block by a machined ceramic insulator. The anode to cathode gap is about 5 mm. The cathode is insulated from the source block by a shaped cap of either ceramic or vespel through which the sample pin protrudes. The discharge gas, usually high-purity argon, is supplied through a needle valve and a length of fused silica capillary to maintain a pressure of about 1 Torr in the discharge. A floating power supply provides a voltage of about -1000 V to the cathode, and a series resistor limits the discharge current to $1-2$ mA. The sample of interest is either the cathode material itself, in the case of metals, or a layer of material depositied on the surface of the cathode. In this configuration the glow discharge is limited to analysis of conductive solids, but a RF-excited version has also been developed and applied to nonconductive solids.[13]

The processes occurring within a glow discharge have been studied extensively[14,15] For a given discharge voltage and gas pressure, the voltage drop adjacent to the cathode (the cathode fall) occurs mostly within the thin dark space near the cathode. The plasma in the remaining "negative glow" is almost electrically neutral overall and is close in potential to the anode. Sputtering is caused by both ionic and neutral argon bombardment of the probe with an energy which is estimated to be about 1/4 of the discharge voltage at a pressure of 1 Torr. Neutral atoms or molecules are sputtered into the gas phase from the surface of the cathode. Any cations formed would not be able to escape the electric field of the cathode fall. The neutrals are then ionized by electrons, excited atoms (Penning ionization), or by ion–molecule reactions (chemical ionization). The ions sampled by the mass spectrometer are those formed close to the ion exit that are swept out by the flowing gas. The major disadvantage of the glow discharge for elemental analysis is that it takes relatively long times (ca. several minutes) for the extracted ion beam to reach equilibrium with the elemental concentration of the original sample. Thus, sample throughput may be low.

E. Inductively Coupled Plasma (ICP)

This plasma source was originally developed as a superior excitation source for elemental analysis of solutions by atomic emission spectroscopy (AES).[16] Techniques for sampling flames and plasmas into mass spectrometers were developed earlier for fundamental studies.[17] Gray[18] demonstrated that useful mass spectra of elemental constituents in solution could be obtained from a DC plasma, and Houk and co-workers[19] produced the first analytically useful spectra from an ICP.

A typical example of an ICP source coupled to a quadrupole mass spectrometer is shown in Figure 2.[20] The excitation portion of the source is identical to that used in AES, but instead of viewing the emission with a spectrograph, the ions produced at atmospheric pressure are sampled into the vacuum of the mass spectrometer. The plasma is sustained by RF energy applied to the load coil. The electromagnetic field transfers energy from the coil to the plasma. Most of the energy is added to the outer, annular
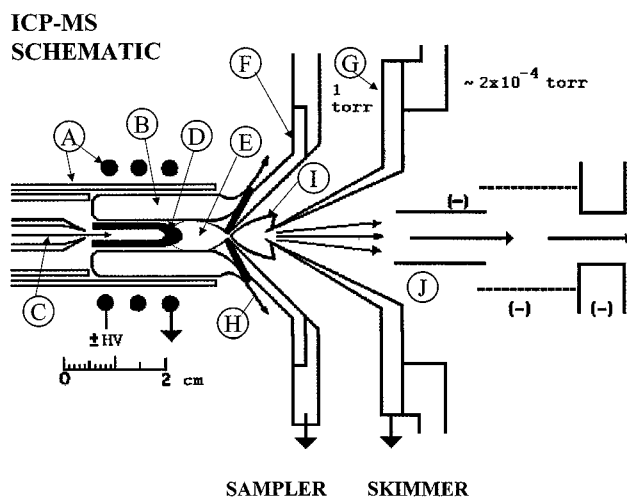
Figure 2. ICP ion source and sampling interface: A, torch and load coil; B, induction region of ICP; C, solution aerolsol being injected in axial channel; D, initial radiation zone; E, normal analytical zone; F, nickel cone with sampling orifice in tip; G, skimmer cone; H, boundary layer of ICP gas deflected outside sampling orifice; I, expanding jet of gas sampled from ICP; J, first element of ion lens. (Reprinted with permission from ref 20. Copyright 1986 American Chemical Society).

portion of the plasma inside the load coil. The sample traverses only the axial channel, which is heated by energy transferred from the outer, induction region. The physical separation of the sample from the region where the energy is added has important analytical implications. The sample composition can change substantially and have little effect on the electrical processes that sustain the plasma. Also, the absence of electrodes in physical contact with the ICP prevents contamination by sputtered electrode material.

The challenge with ICP-MS is to efficiently transfer ions from a plasma at atmospheric pressure and ca. 5000 K into the vacuum of the mass spectrometer. As shown in Figure 2, the plasma flows around the tip of a water-cooled metal cone. This cone has a circular orifice of ca. $0.5-1$ mm in diameter at its tip. Gas from the ICP enters this orifice from a cross section of the axial channel which is approximately three times the orifice diameter; thus, a large fraction of the gas in the axial channel is sampled. The central orifice of a conical skimmer is located beyond the sampler at an appropriate distance to transmit as much of the sampled beam as possible into a second vacuum chamber without overloading the second vacuum system. The pressure here is low enough (ca. 10^{-5} Torr or less) so that ion lenses can be used to collect, focus, and transmit the ions to the mass analyzer with minimal losses by scattering.

The ICP is a very efficient source for producing singly charged elemental ions. Positive ions have been detected for all of the naturally occurring elements except F, Ne, and He, all with ionization potentials greater than Ar used to form the plasma. On theoretical grounds, some 54 elements are expected to be ionized with efficiencies of 90% or more. Even metalloid or nonmetallic elements such as P and As are ionized with useful efficiency. Doubly charged ions are produced from some elements having low second ionization potentials, such as Ba and

several rare earths, but these are generally less than 10% of the singly charged ions of the same element. Oxide ion peaks (MO^+) are observed for some elements.[21] In some cases, doubly charged ions or oxide ions from abundant elements may interfere with detection of ions from elements present at lower abundance.

For some applications, helium may be used in place of argon in the ICP.[22] This eliminates the spectral interferences due to polyatomic argon-containing species and provides more efficient ionization of elements with high ionization potential, such as halogens.[23] Helium microwave-induced plasma sources operating at subatmospheric pressure have also been developed, particularly for the detection of halogenated hydrocarbons.[24]

III. Molecular Ions from Volatile Samples.

A. Electron Ionization (EI)

The electron ionization source was first used by Dempster,[25] and most commercially available sources are based on the design of Nier.[26] A beam of electrons is directed into molecular vapor at reduced pressure, one or more secondary electrons are ejected, and relatively unstable odd-electron or multiply charged positive ions are produced. Negative ions may also be produced when samples have high electron affinities.

A schematic diagram of a "Nier-type" ion source is shown in Figure 3. Electrons are produced by heating a metal filament, usually formed from a fine wire or ribbon of tungsten or rhenium. These electrons are accelerated by a potential difference between the filament and the ions source box, pass through entrance and exit apertures in the box, and are collected by a trap electrode. Voltage is applied to a repeller electrode within the box that accelerates ions toward the ion exit aperture. A collimating magnetic field is applied parallel to the electron beam axis, and the field strength is chosen to provide high transmission of the electrons with minimal perturbation of the ion beam. A field on the order of 100 G is typically used. The electron current is stabilized by monitoring either the total emission current or the current to the trap with feedback to the filament heater power supply to maintain a constant current. Samples are introduced directly into the ion source box, and for most analytical applications, the vapor input rate and the orifices in the source box must be chosen so that the probability of ion–molecule reactions is minimal. In the "chemical ionization" (CI) source, discussed
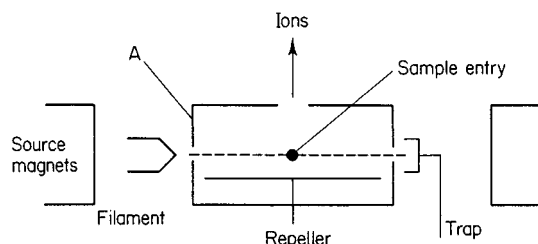
below, the pressure in the ion source box is elevated and EI is used to generate reagent ions that react with the sample molecules to produce CI spectra. To obtain pure EI spectra it is usually necessary to maintain the ion source pressure below about 0.1 milliTorr. This is particularly important if the results are to be used for compound identification by comparing spectra with those in a database.

The major applications of EI are the determination of molecular weight and structure of molecules that are sufficiently stable to survive vaporization without decomposition. Most of the advances since Nier have been in improved techniques for handling difficult samples, either by chemical derivatization to increase volatility and/or thermal stability or by novel sample introduction methods which minimize unwanted pyrolysis. The cross section for ionization of molecules is a strong function of the electron energy. Ions are first detected at the "appearance potential", and the ion current increases with electron energy to a maximum that typically occurs for singly charged ions in the range of 50−100 eV. Measurements of appearance potentials by EI for both parent and fragment ions was a major source of data on ion energetics in the past,[27] but this has been supplanted by photoionization techniques in more recent work.

For analytical applications, an electron energy of 70 eV has been adopted as the standard. This is a reasonable, though arbitrary, choice in that it corresponds approximately to the maximum cross section for most molecules and provides nearly the maximum absolute intensity for molecular ions while providing relatively intense fragment ions which carry structural information. Fragmentation of the odd-electron molecular ions produced by EI is well understood in principle using the "quasi-equilibrium theory" (QET) of Wahrhaftig and co-workers,[28] which is equivalent to the more general RRKM theory of unimolecular kinetics.[29] While this theory has been used to explain many of the features of ionic fragmentation processes,[30] it is difficult to apply in practice without detailed information on the energetics and structures of the ions involved. There exists a vast literature on the interpretation and prediction of EI mass spectra,[31] and not surprisingly, considering the complexity of the processes involved, the former is much more successful than the latter.

Despite difficulties in interpreting and predicting EI mass spectra, the spectra observed under standard conditions are reproducible and largely independent of the instrument used. Furthermore, the spectra are highly correlated with molecular structure even though rapid isomerization may, in some cases, occur before fragmentation. Thus, fragment ions are often observed which clearly must be formed following rearrangement of the neutral molecule ionized. Nevertheless, the relative intensities of the observed fragment ions may still reflect the isomeric structure even though the connection between the observed masses and the structure may not be obvious. For these reasons, the spectra of compounds to be identified are often treated as molecular fingerprints that are used in a library search to generate a "hit list" of compounds arranged in order of similarity to the

Figure 3. Schematic diagram of electron impact ion source adapted from the original design of Nier. (Adapted with permission from ref 26. Copyright 1947.)

unknown. Extensive libraries of EI mass spectra have been generated over the past 50 years and now contain some 180 000 entries. Recent work has been aimed at improving the reliability of this approach by critical evaluation and correction of the existing libraries[32] and by developing better methods for generating higher quality data by extracting pure component mass spectra from complex GC/MS data files.[33]

The major limitation of EI mass spectrometry is the requirement for stable neutral molecules in the gas phase prior to ionization. Many molecules are simply not stable at the elevated temperatures required for vaporization. Chemical derivatization techniques have been highly developed for at least partially overcoming this limitation, particularly for molecules of biological interest,[34] but these techniques are of limited use with trace amounts of material present in complex mixtures. Another approach is based on the observation that intact molecular ions could be observed from relatively nonvolatile samples if they were vaporized rapidly from weakly bonding surfaces such as fluorocarbons.[35] This approach, which became known as "direct" or "desorption" EI, is illustrated in Figure 4, where it is compared with sample introduction by conventional solids probe. In this approach samples are vaporized from a heated wire, which is generally coated with an inert substrate such as a polyimide, and the filament is inserted directly into the path of the electron beam.[36] This approach extends the EI technique to somewhat less volatile molecules but is

Figure 4. Illustration of (a) conventional solids probe method for introducing a solid sample into and ion source and (b) the direct exposure method. The conventional method requires vaporization of the sample prior to it entry into the active region of the ion source, while the direct method places the solid sample into the ionizing region. (Adapted with permission from ref 36. Copyright 1980 American Chemical Society.)
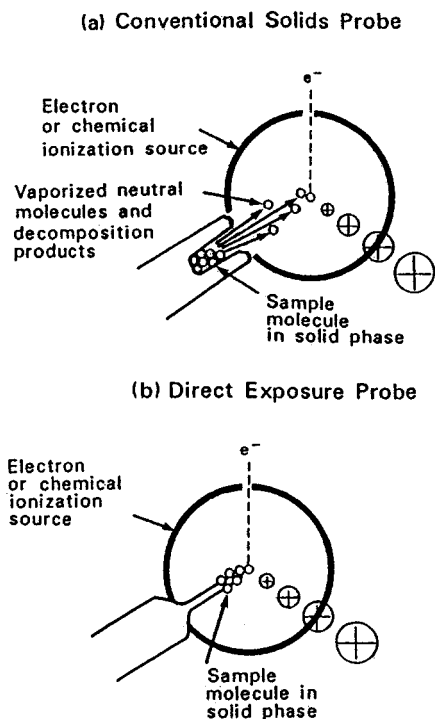
limited in utility for molecules of biological interest since many of these appear not to yield useful intensities of molecular ions even when vaporized as intact molecules.
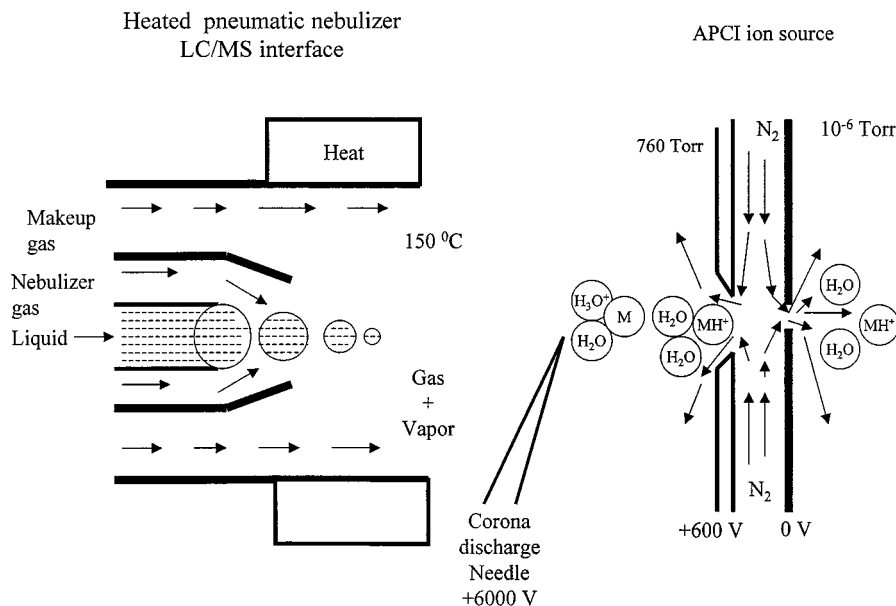
B. Chemical Ionization (CI)

The chemical ionization technique was developed by Munson and Field[37] and initially applied to hydrocarbons and petrochemicals. The technique uses an ion source similar to that for EI, as illustrated in Figure 3, except that it is designed to work at higher ion source pressures of ca. 1 Torr. This is accomplished by using small ion source apertures, introducing a reaction gas directly into the ion source box in addition to the sample, and by using a larger vacuum pump on the ion source housing to maintain adequate vacuum in the mass analyzer. The electron energy is generally increased to 500−1000 eV so that the electron beam penetrates into the center of the ion source box. The essential requirements for the reaction gas are that it produces a set of ions which are essentially nonreactive with the reaction gas itself but can undergo exoergic ion−molecule reactions with the samples of interest. The reaction gas used initially was methane, which produces the stable reagent ions CH_5^+ and $C_2H_5^+$. These react by either proton transfer or hydride abstraction to form either MH^+ or $(M − H)^+$ ions, and the former predominate for samples of higher proton affinity. This new technique aroused some academic interest but was not widely accepted as an analytical technique until the utility for biologically important samples not yielding molecular ions by EI was demonstrated by Fales and co-workers at NIH using a commercial prototype CI source.[38]

While the ion source for CI is superficially quite similar to the EI source, the nature and energetics of the ions produced are quite different. In EI, a relatively high-energy incoming particle is used (ca. 70 eV) and the energy lost in the ionizing collision is shared between the ion and the secondary electron. This leads to a rather broad internal energy distribution (typically 10−20 eV in nominal width), and virtually all energetically allowed fragments are observed. In CI, the collisions between reactant ion and analyte molecule occur at thermal energies and the energy available to drive fragmentation is limited to the exoergicity of the reaction. For proton transfer reactions, this is simply the difference in proton affinity between neutral sample molecule and that of the neutral reactant gas. Furthermore, ionization occurs in a gas at ca. 1 Torr, where collisional relaxation may compete with fragmentation. Also, except for rare gas reactants where charge exchange may be the dominant reaction, the ions formed are even-electron species which are inherently much more stable than the odd-electron molecular ions produced by EI. Thus, it is not surprising that CI yields molecular ions from many molecules for which molecular ions are very weak or absent by EI.

The degree of fragmentation can be controlled by the choice of reactant gas. This can be correlated with the gas-phase acidity of the reactant. Thus, hydrogen or methane may be used when fragmentation is

Figure 5. Simplified schematic diagram of a heated pneumatic nebulizer LC/MS interface combined with an atmospheric pressure chemical ionization source. (Adapted with permission from ref 44. Copyright 1986 American Chemical Society.)

desired and a weaker acid such as ammonia when predominately protonated molecular ions are the goal. Of course, if the proton affinity of the sample molecule is less than that of the reactant, proton transfer will not occur although complex formation with the reactant may be observed. This selectivity is an important feature of CI when the goal is to determine molecular weight but may be a distinct disadvantage when the goal is determination of molecular structure. The problem is that the fragmentation pattern is very dependent on the operating conditions such as choice of reactant and the temperature and pressure in the ion source. Furthermore, it may be influenced by uncontrolled variables such as trace impurities, which may drastically change the energy transferred in the ionization process. For example, traces of water vapor in reactants such as methane or hydrogen will cause H_3O^+ to be the major reagent ion reacting with samples which will substantially reduce the energy transferred and hence reduce the observed fragmentation. Thus, libraries of CI spectra have not been generated for compound identification because this lack of reproducibility has generally limited their potential utility. Dual sources are available on most commercial GC/MS systems with CI used to determine molecular weight and EI for identification by searching the MS library. The direct vaporization probe illustrated in Figure 4 is particularly useful with CI since the combination of controlled vaporization and soft ionization often allows determination of molecular weight on difficult samples.

The CI source has been particularly useful for interfacing with liquid chromatography. Several approaches to interfacing LC with EI mass spectrometry have been described, including the original direct coupling technique of Talroze[39] and the particle beam interface described by Willoughby and Browner.[40] These systems have not been widely used mainly because of the difficulty of vaporizing liquid and

efficiently transferring samples into the low-pressure ion source required in EI. In contrast, the higher pressures used with CI improves heat transfer and allows larger amounts of solvent vapor to be vaporized in the ion source. Successful interfaces with CI mass spectrometry include direct liquid introduction (DLI)[41] and thermospray.[42]

In recent years these earlier LC interfaces have been largely replaced by atmospheric pressure ion sources. These have the distinct advantage that the liquid is vaporized and the sample is ionized at atmospheric pressure and ideally only the ions in a bath of relatively dry gas enter the mass spectrometer. This approach was pioneered by Horning and co-workers[43] and has become widely available commercially, typically on instruments that also provide electrospray ionization. A schematic diagram of an atmospheric pressure chemical ionization (APCI) source with a heated pneumatic nebulizer is shown in Figure 5.[44] In this system the LC effluent passes through the central tube while the nebulizer gas and makeup gas are introduced coaxially into the heated region. The combination of heat and gas flow desolvates the droplets to produce a dry vapor of solvent and analyte molecules. Ionization of solvent molecules is initiated by corona discharge, and the ionized solvent serves as the chemical ionization reagent to ionize the samples by ion−molecule reactions. The ions are focused and declustered in a dry nitrogen curtain gas and pass through as small (ca. 100 μm) orifice into the high-vacuum region of the mass spectrometer. This interface performs well for samples that survive vaporization as neutral molecules without decomposition but is not suitable for very nonvolatile or thermally labile samples. For these electrospray is the method of choice for LC interfacing, and since the interface with the mass spectrometer is similar, both techniques are frequently installed on the same MS.

C. Photoionization (PI)

The ion source for photoionization is similar to that shown in Figure 3,[45] with the beam of electrons replaced by a beam of monoenergetic vacuum UV photons. The photon beam may be produced by a monochromator with either a conventional discharge light source or by radiation from a synchrotron. More recently, laser multiphoton ionization of vibrationally and rotationally cooled beams of molecules has been used extensively.[46] These ionization methods have been primarily used to obtain accurate values of ionization and appearance thresholds for both positive and negative ions. The results provide much of the basic data required in gas-phase ion and neutral thermochemistry.[47]

D. Field Ionization (FI)

The earliest example of a "soft" ionization technique that produces molecular ions from gas-phase molecules with little or no fragmentation is the field ionization technique originally described by Inghram and Gomer[48] and further developed by Beckey and co-workers.[49] In this technique a sharp edge or small diameter wire is held at high positive potential relative to a grounded counter electrode. Chemical etching or growing of "whiskers" on the metal surface may be used to further increase the local field strength by introducing points with very small radii. At field strengths on the order of 10^{10} V/m, electrons may be extracted from gas-phase molecules that approach within a few angstroms of the metal surface and transferred to the metal. Very little excess energy is transferred to the molecules by the ionization process, and very little fragmentation is observed. With condensable samples such as water or methanol, protonated molecular ions were observed which were shown to be produced by condensation on the emitter tip followed by field desorption of protonated ions, including under some conditions protonated clusters. These observations lead to the development of the field desorption technique for analyzing nonvolatile molecules discussed below.

IV. Molecular Ions from Nonvolatile Samples

By the mid-1970s, mass spectrometry with electron ionization (EI) and chemical ionization (CI) was widely recognized as a powerful analytical tool, particularly in combination with on-line separation of samples by gas chromatography and with dedicated minicomputer systems for acquiring and processing the data. Applications were limited to those involving relatively volatile samples, and despite some success with chemical derivatization to increase volatility and direct introduction techniques to minimize pyrolysis, it was generally understood that mass spectrometry was of little use in biology. Even for small nonvolatile biological samples such as the amino acid arginine, it was nearly impossible to produce detectable intensities of molecular ions and, except for small peptides containing volatile residues, biological polymers were out of the question. It appeared that mass spectrometry was reaching maturity, and while the applications of GC-MS were

expanding rapidly, many felt that the exciting work in developing analytical mass spectrometry was finished. Then Macfarlane and co-workers[50] made the astounding discovery that intact molecular ions of cysteine and arginine could be desorbed from thin layers of these compounds excited by high-energy fission fragments from ^{252}Cf-radioactive decay. This was followed by the observation of molecular ions from "difficult" molecules such as tetrodotoxin,[51] peptides with molecular weights in excess of 3000,[52] and protected oligonucleotides up to m/z 12 500.[53] These observations revitalized mass spectrometry for biological applications, and many other techniques were rapidly developed.

A. Spray Techniques

Static electrification processes were extensively studied in the laboratory of Professor Leonard Loeb before and after World War II.[54] In one of those studies, Chapman[55] measured ion mobilities produced at atmospheric pressure by vaporization of charged liquid droplets that were consistent with molecular ions in the gas phase, although they could also be explained as relatively small droplets with an unusually high charge. Dole and co-workers[56] developed an electrospray apparatus aimed at producing molecular ions from nonvolatile polymers. Spraying solutions containing high molecular weight polystyrene produced mobility spectra that were consistent with molecular ions of these molecules, but attempts to observe ions in a time-of-flight mass spectrometer were unsuccessful. Iribarne and Thompson[57] repeated the measurements of Chapman with a higher resolution mobility apparatus and concluded that desorption of small molecular ions did indeed occur. Subsequently they showed that molecular ions from a wide variety of solute species could be produced by spray electrification and that these species could be detected and analyzed in a mass spectrometer.[58] Independently, in connection with developing the "thermospray" interface between liquid chromatography and mass spectrometry, it was found that ions were produced in the spray process when the hot filament normally used for initiating ionization failed and the ion beam persisted. Furthermore, intact molecular ions were produced by the spray ionization process from molecules which were not amenable to normal CI even with direct vaporization.[59] Following these earlier observations, Fenn and co-workers[60] applied their expertise in supersonic molecular beams to developing a new approach, superficially similar to that described earlier by Dole but with spectacularly different results.

B. Electrospray

The work by Fenn and co-workers in developing electrospray ionization was largely ignored by the mass spectrometry community until, before a small audience at the ASMS meeting in 1988, they presented their first results on proteins.[61] A small sample of the early results is shown in Figure 6. The fact that these highly charged molecular ions from proteins as large as 40 000 molecular weight could
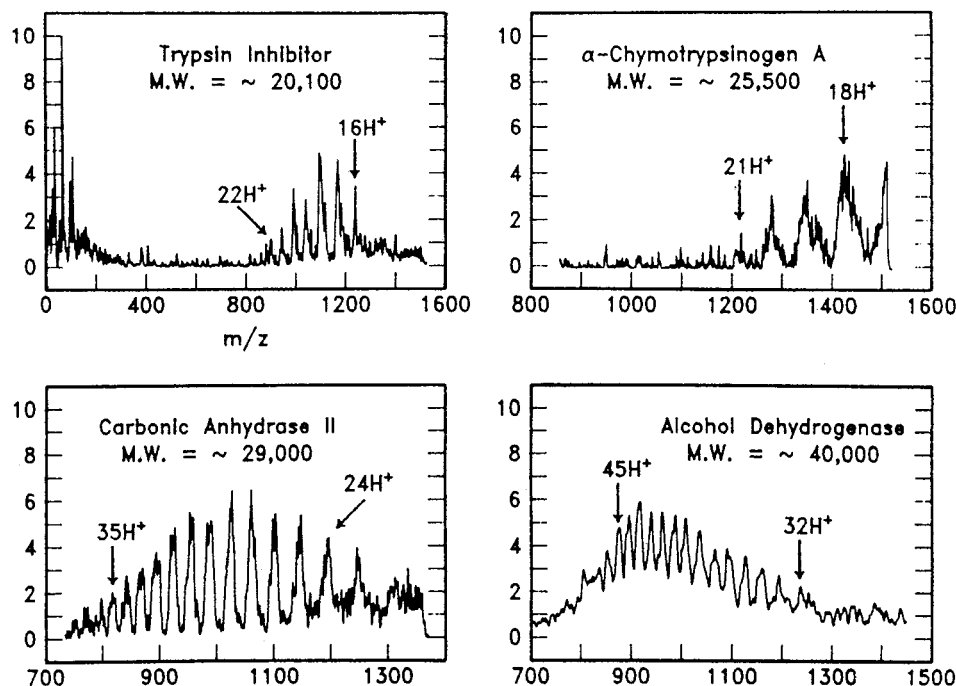
Figure 6. Examples of the first electrospray ionization spectra of proteins. Each spectrum is the result of a single 30 s scan over the indicated range of *m/z* values and comprises a family of multiply charged peaks from the same nominal mass. In this case the charge carrier is a proton, and the molecular weight of the molecule and the charge state for each peak can be calculated from measured *m/z* values for two or more peaks. (Reprinted with permission from ref 61. Copyright 1988.)

be observed with no evidence of fragmentation was truly astounding. The apparatus used in these experiments is shown schematically in Figure 7. This system employs a glass capillary in place of the simple nozzle used in an earlier configuration.[62] This allows the electrospray needle to be operated at ground potential and is convenient for interfacing to LC. For positive ions with the needle at ground, typical applied voltages are as follows: cylindrical electrode, −3500; metallized inlet end of the glass capillary, −4500; exit end, +40; skimmer, +20; and entrance lens in front of the quadrupole, ground. Negative ions are produced by reversing the polarity of the voltages; in addition, it is useful to add a small stream of oxygen or other electron scavenger near the needle to inhibit the onset of corona discharge, which occurs at lower voltages in the negative-ion mode. The mobility of ions in the capillary is sufficiently low that the gas flow through the capillary

can drag the ions up the potential gradient and deliver them to the exit. This approach has also been used successfully for interfacing with magnetic mass spectrometers where the ion source may be as much as +15 kV relative to the needle at ground potential.[63]

Sample solution enters through the stainless steel needle at flow rates usually between 1 and 40 μL/ min. The field at the needle tip charges the surface of the emerging liquid, and Coulomb forces disperse it into a fine spray of charged droplets. The droplets are driven by the electric field toward the inlet end of the capillary through a countercurrent flow of bath gas (dry nitrogen) typically at 800 Torr, entering temperature of 320−350 K, and flow rate of about 100 mL/min. The solvent vapor from the evaporating droplets along with any other uncharged material is swept away from the capillary inlet by the bath gas. As the highly charged droplets vaporize, they become unstable, subdivide, and eventually produce molecular ions. If species are present as solutes which can accommodate large numbers of elemental charges, such as proteins, these are observed in the mass spectrometer as shown in Figure 6. Some of these ions are entrained in the flow of dry bath gas that enters the glass capillary and emerge at the exit end, carried along in a supersonic free jet into the first of two vacuum chambers. A core portion of this free jet passes through a skimmer into a second vacuum chamber and delivers ions to the mass analyzer.

The details of the mechanisms by which molecular ions are desorbed from evaporating charged liquid droplets are still somewhat controversial, but the major features are now generally accepted. The droplets are initially formed with a charge near but
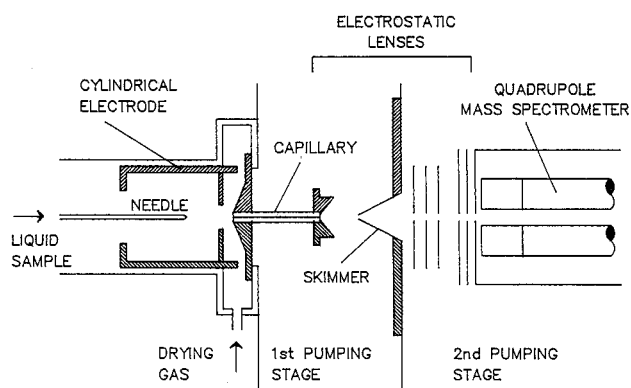
Figure 7. Schematic diagram of and electrospray ion source. (Reprinted with permission from ref 62. Copyright 1985 American Chemical Society.)

somewhat below the Rayleigh limit,[64] which corresponds to the droplet radius at which the forces of surface tension holding the droplet together are equal to the Coulomb repulsive forces between the charges. As the droplet vaporizes, this limit is exceeded and the droplet becomes unstable. This hydrodynamic instability, at least for larger droplets, results in release of a jet of small charged droplets. As the initial droplet vaporizes further, this process is repeated until the liquid is entirely dispersed. There is general agreement about the first part of this process, but the mechanism leading to molecular ions from very small charged droplets has been the source of some debate. Iribarne and Thomson[58] proposed the model of field-induced ion evaporation in which the high electric field at the surface of a small charged droplet was sufficient to make ion evaporation competitive with solvent vaporization. Roellgen[65] challenged this model on the basis that such high localized fields would cause the onset of hydrodynamic instability on the surface producing a jet releasing a number of charged droplets. Roellgen's view is that the hydrodynamic disintegration continues until the droplets are so small that they contain only a single solvated ion, and further desolvation leads to the ions observed in the mass spectrometer. It is fairly clear that the observed energetics for the ion evaporation model requires that the emitted ions must be heavily solvated. If so, the two models become almost indistinguishable and mostly semantic since in the one case we have a solvated ion and in the other a very small charged droplet containing a single species which can compete with the solvent for the charge as vaporization proceeds. In either case, to efficiently produce ions by electrospray it is desirable to produce small charged droplets with as much charge as possible and cause those droplets to vaporize in a bath of gas. If the concentration of sample or nonvolatile salts is too high, then the efficiency of ionization is low since the droplets may dry to a solid particle without releasing much of the sample into small droplets that can produce molecular ions.

A number of variations on the design of electrospray ion sources have been described in the literature, and many of these are in use on commercial instruments. These variations are mostly concerned with the sampling of the electrospray ions into the mass spectrometer to maximize sensitivity and improve reliability. In some of these, the countercurrent flowing bath gas is not used and residual droplets and some of the solvent vapor enters the nozzle interfacing with the mass spectrometer. In these systems vaporization is completed and resolution prevented by using either a heated capillary nozzle[66] or a heated chamber downstream of the skimmer.[67] In applications requiring continual sampling of the effluent from an HPLC, a major concern has been contamination of surfaces in the interface or mass spectrometer by nonvolatile components which may cause the system to fail and require it to be disassembled and cleaned frequently. Several ingenious schemes have been described recently in an attempt to efficiently sample the ions but reject any liquid droplets or macroscopic particles which might be present.[68,69]

Electrospray is a concentration-dependent ionization method that works best at low flow rates. By reducing the diameter of the electrospray needle, stable sprays of very fine droplets can be produced at flow rates of 1 nL/min or even less.[70] Furthermore, these "nanospray" needles work at very low voltages (ca. 1000 V or less) because of the very high field produced at the small tip, and they can be located very close to the nozzle sampling ions into the mass spectrometer since the flow of solvent is so low. This approach allows very high sensitivity for small amounts of sample, but because of the small orifices involved, special care must be taken to avoid plugging.

C. Desorption from Surfaces

With the benefit of 30 years of hindsight, it is now intuitively obvious that spray techniques should be very soft ionization methods. The concepts of disrupting liquids to produce very fine droplets, charging the droplets, and gently vaporizing the droplets to remove the neutral solvent leaving behind a multiply charged protein ion in the gas phase now seems perfectly reasonable. Most of the surface desorption techniques do not. One exception is the field desorption technique (FD) developed primarily by Beckey and co-workers.[71] This might be considered a forerunner of the spray techniques in that it involves application of a high electric field to a liquid surface resulting in the production of intact molecular ions from nonvolatile samples. The major difference is that FD is done in the vacuum of the mass spectrometer and the spray techniques require a bath gas at higher pressure. The mechanism of ion production for both positive and negative ions by FD has been extensively studied by Roellgen and co-workers.[72] Even though this technique is not widely practiced at present, these studies have provided much of the fundamental understanding of the processes involved in spray ionization.

## D. High-Energy Particle Impact

The idea that Mev fission fragments impacting on surfaces can produce molecular ions from nonvolatile and thermally labile molecules is certainly not intuitive. This ionization method was discovered as a byproduct of studies on beta decay by Macfarlane and co-workers.[50] These studies used time-of-flight techniques to measure the time between emission of the electron and detection of the recoil partner at a detector several meters away. Peaks were observed in these spectra that were not correlated with beta decay. The conclusion was that contaminants on the surface were also being ionized and emitted. The beta emitter was replaced by a ^{252}Cf fission fragment source to increase the energy deposition on the surface, and the surface was doped with known nonvolatile samples. In the earliest experiments, protonated molecular ions were observed from arginine, a molecule generally considered impossible by techniques then in use.
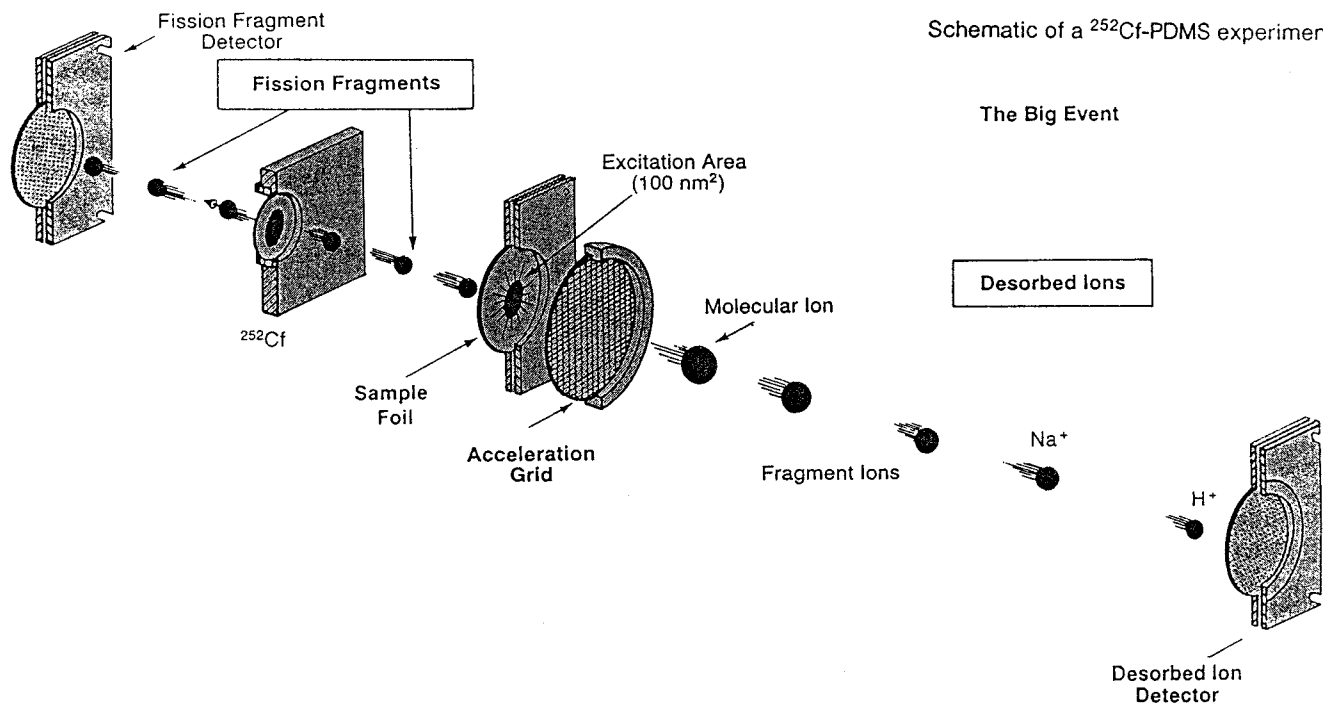
Figure 8. Schematic of a ^{252}Cf—PDMS experiment. (Reprinted with permission from ref 74. Copyright 1985.)

A schematic diagram of a plasma desorption system is shown in Figure 8.[74] Ionization is initiated by spontaneous fission of a ^{252}Cf nucleus which produces fission fragments with energies of about 100 Mev and charges of +20 or so. Results have also been obtained using similar beams of particles from an accelerator.[73] The linear energy transfer is these ions in a solid is so high (0.1—1 keV/Å) that it is important that the source be thin. A typical source strength is 10 microcuries spread over a circular area 3—5 mm in diameter. The thickness of the californium layer is about 1 ng/cm^2. The foils covering the source must be thin enough to allow one of the fission fragments to reach the "start" detector and the other fragment to pass through the foil and ionize the sample deposited on the surface of the foil. A grounded grid is placed 2—5 mm in front of the sample surface, and a high voltage (ca. 5—10 kV) is applied to the sample to accelerate ions toward the detector.

Prior to electrospray and MALDI, this technique was becoming a standard tool in mass spectrometry laboratories focusing on biological samples[74] and was commercially available. At that time it was the only technique available that could reliably produce molecular ions from proteins with molecular weight over 10 000. The spectrum of one of the larger proteins measured is shown in Figure 9; this illustrates both the power and the problems with the technique. The rather low quality of the spectrum, by contemporary standards, is now understood to be primarily due to metastable decomposition of the ions in flight. The plasma desorption technique has been largely supplanted by electrospray and MALDI but marked an extremely important milestone in the development of ionization techniques for mass spectrometry of difficult samples. It both renewed interest in the largely dormant field of time-of-flight mass spectrometry, particularly for large molecules, and showed for
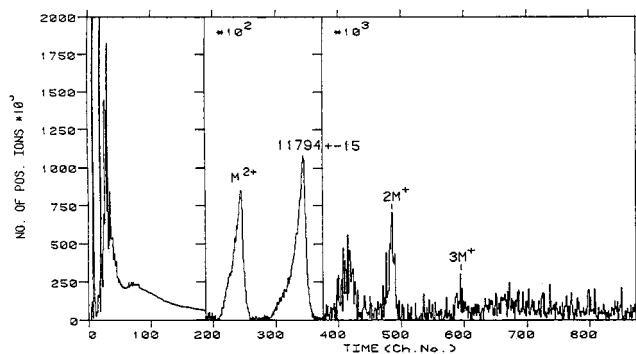
Figure 9. Positive-ion ^{252}Cf—PDMS of PSP (pancreatic spasmolytic polypoptide). Each time channel is 32 ns wide; counting time 8 h with a source producing 1450 fragments/ sec. (Reprinted with permission from ref 129. Copyright 1984.)

the first time that such incredible ions could exist in the gas-phase long enough to be measured.

E. Low-Energy Particle Impact

Shortly after the publication of Macfarlane's work, Benninghoven and co-workers presented the first results on ionization of nonvolatile molecules by low-energy ion impact.[75] Mass spectrometric analysis of ions produced by bombarding surfaces with keV ions was already well established as a technique for analysis of surfaces.[76] The early applications were to fundamental studies which were based on the fact that secondary-ion mass spectrometry (SIMS) provided high absolute sensitivity for many surface components and unlike other available techniques could detect hydrogen and determine isotopic compositions of elements on the surface.[77] Benninghoven et al.[78] showed that SIMS was a very sensitive technique for the detection and identification of organic compounds provided it is operated in the "static" mode. The main problem in detecting intact

molecules in SIMS can be the damaging effect of the primary-ion beam. This effect can be minimized by operating in the "static" mode in which the surface is bombarded at very low primary current density. Damage cross sections are typically on the order of 10^{-14} cm^2; keeping the total dose of primary ions below about 10^{12}/cm^2 ensures a small probability that a surface area damaged by the impact of an ion is struck by another primary ion during the measurement. The static SIMS technique has been applied to a wide variety of organic compounds including amino acids, peptides, nucleosides, nucleotides, vitamins, and many others.[79] Almost all of the spectra show high abundances of protonated or cationized molecular ions. Secondary yields as high as 0.1 molecular ion per incident primary ion have been measured, but the yields are strongly dependent on the substrate and the properties of the primary beam. Noble metal substrates generally produce the highest yields.

Most of the early SIMS work employed either magnetic deflection or quadrupole analyzers, and the need to work in the "static" mode together with the ion losses caused by the duty cycle of scanning analyzers made the sensitivity lower than desired. One solution to this problem was the use of the time-of-flight analyzer that allowed nearly every ion desorbed by a pulse of primary ions to be detected.[80] Also, the linear time-of-flight analyzer only requires that the ions be stable for the time required to traverse the accelerating field; thus, ions that would go undetected in the scanning instruments may be detected as the result of fragments arriving with essentially the same time-of-flight as intact molecular ions. Significant yields of molecular ions were obtained from a number of intractable compounds. Spectra obtained by TOF−SIMS are very similar to those from ^{252}Cf TOF, as shown by detailed comparisons on the same samples.[81,82] In both cases, ions of higher molecular weight are predominately metastable. Neutral particles in the keV range have also been used, but the results are similar to those with ions of comparable energy.[83] Since it is generally understood that ions approaching a surface with keV energies are probably neutralized by long-range electron transfer as they approach the surface, it is not surprising that the charge on the particle is unimportant.

F. Low-Energy Impact with Liquid Surfaces

Another successful approach to improving the utility of low-energy impact ionization for nonvolatile molecules was developed by Barber and co-workers.[84] This technique differs from SIMS in two respects. First, the primary beam is a keV-energy neutral beam produced by charge exchange neutralization of an ion beam, and second, the sample is introduced as a solution or suspension in a relatively nonvolatile liquid such as glycerol. It is now clear that the presence or absence of charge on the incident particle has little effect on the desorption process, but the use of the neutral beam is more convenient with magnetic instruments where the source is at high potential. Replacing the solid surface with a liquid matrix has pronounced effects.

The most striking effect of the liquid matrix is removal of the requirement for working at low current densities to avoid damage to molecules on the surface. Current densities several orders of magnitude larger than those suggested by Benninghoven[78] have been used routinely, and in many cases, production of protonated molecular ions from nonvolatile samples has persisted at stable intensities for 30 min or more.[85] The rationale for this effect is that solution presents a mobile, constantly renewed surface to the bombarding beam. This provides continuous replenishment of undamaged sample molecules to the surface to be ionized. Work by Field[86] showed that substantial radiation damage to the glycerol does occur; his results indicate that approximately 100 molecules of radiation-damaged products are produced per incident 5-keV argon atom.

One disadvantage of the liquid matrix is that a substantial background is produced by ionization of the glycerol and its radiation-induced fragments. Also, substantial clustering of glycerol with sample ions is observed together with mixed cluster ions when impure samples or mixtures are analyzed. This technique, known as fast atom bombardment (FAB) or liquid SIMS, depending of the charge on the incident beam, has been successfully applied to a wide variety of difficult samples.[87] Part of this success can be ascribed to its compatibility with high-performance magnetic instruments; these were the instruments most widely used at the time this technique was developed, and FAB or LSIMS sources were commercially available from all of the major manufacturers almost immediately after the initial publications.

G. Flow FAB

Continuous-flow FAB was developed to correct some of the difficulties with the standard FAB technique while retaining the essential advantages.[88] This technique uses a sample introduction probe that provides a continuous flow of liquid into the mass spectrometer ion source and onto the sample stage where atom bombardment occurs. This reduces the requirement for a viscous carrier and allows the use of more volatile solvents such as water, methanol, and acetonitrile. The liquid flow rate is generally in the range of $1-20$ μL/min, and samples may be analyzed by flow injection, or following on-line separation by capillary LC,[89] or capillary electrophoresis.[90] In addition to the obvious advantages of convenience and speed of sample introduction, the flow technique also provides lower limits of detection[91] and decreased ion suppression effects[92] compared to conventional FAB.

H. Laser Ionization–MALDI

Laser ionization of organic solids was investigated by Mumma and Vastola[93] in the late 1960s and early 1970s, but most of the early work on laser ionization was focused on inorganic samples. The early work has been reviewed by Conzemius and Capellen.,[94] During the next decade, several groups conducted research on improved methods for applying lasers to
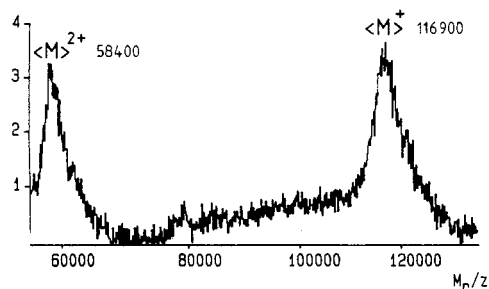
Figure 10. MALDI mass spectrum of β-D-galactosidase in the parent molecule range. Sum of 100 laser shots (266 nm) with nicotinic acid matrix. This is the first mass spectrum reported showing intact molecular ions in the gas phase with MW greater than 100 000. (Reprinted with permission from ref 98. Copyright 1989.)

ionization of organic samples,[95,96] but until the breakthrough by Karas and Hillenkamp in 1988, these techniques were not widely applied.[97,98] By embedding large bioorganic molecules in a suitable matrix which strongly absorbs the radiation from a UV laser, they showed that singly charged molecular ions with masses greater than 100 000 could be efficiently desorbed and measured in a time-of-flight mass spectrometer. An example of one of the spectra from the original publication is shown in Figure 10.[98] At the time, this example of β-D-galactosidase, molecular mass 116 900, was by far the largest intact molecular ion that had been observed. This matrix-assisted laser desorption/ionization (MALDI) technique has rapidly evolved to become one of the predominant methods for analyzing large, nonvolatile molecules. Since the ions are produced in short pulses using a pulsed laser, the technique is particularly well suited for time-of-flight analyzers, but it is also used quite successfully with FT-ICR and with quadrupole ion traps.

A schematic diagram of a MALDI source is shown in Figure 11.[103] Samples in solution are mixed with a matrix solution containing a large molar excess (ca. 10^4–10^5) of a UV-absorbing matrix material. This liquid is deposited in a small droplet, a few μL or less in volume, on a sample probe or plate and allowed to dry. The early work employed a single sample probe, similar to solids probes used in other areas, but most commercial systems now use a sample plate holding a number of samples and a mechanism for sequentially positioning the samples in front of the laser. A laser with pulse duration of a few nanoseconds is preferred. Nitrogen lasers emitting at 337 nm
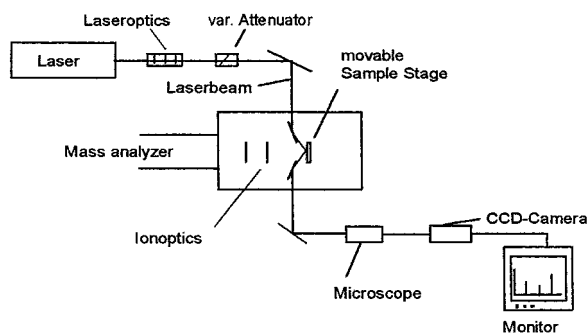
Figure 11. Schematic drawing of a MALDI ion source. (Reprinted with permission from ref 103. Copyright 1997.)

are the most widely used, but frequency tripled and quadrupled Nd:YAG lasers emitting at 355 and 266 nm are also used. The angle of irradiance to the sample surface varies between 15° and 70° and is not critical. The laser is focused to a spot size between 30 and 500 μm, and the required irradiance is typically between 10^6 and 10^7 W/cm² depending on the spot size and matrix employed.[101] Both neutral and ion desorption show a strong dependence on applied laser irradiance. For a given sample, there is a distinct threshold value for producing detectable ion intensities and above that value the ion yield increases rapidly with increasing irradiance. Typically, the ion current is proportional to the fifth or sixth power of irradiance above threshold.[101] Best results are obtained with laser irradiances not more than 20–50% above threshold; thus, a carefully controlled attenuator is required to adjust the laser intensity.

Some of the details of the mechanisms involved in MALDI are still somewhat controversial and are the subject of research by several investigators.[99–102] Nevertheless, the major features are established and have been reviewed recently by Karas and Bahr.[103] The matrix serves several functions. First, it must provide strong absorption of the incident laser light. This allows transfer of energy from the laser to the solid sample in a controllable way. Typical operating conditions are laser irradiance in the 10^6–10^7 W/cm² range, laser pulse length of a few nanoseconds, and molar absorption coefficients for the matrix of a few thousand L mol^{-1} cm^{-1}. This corresponds typically to the order of 1 photon absorbed per matrix molecule in the outermost layer. This results in exponentially decaying in-depth excitation with typical penetration depths of ca. 100 nm. This energy is sufficient to induce the ablation of a small volume, setting free intact matrix and analyte neutrals and ions. Another important function of the matrix is to isolate individual sample molecules and prevent aggregation. The analyte is typically diluted in a large excess of matrix (ca. 10 000-fold or more on a molar basis), and it appears that molecules are isolated as a "solid solution" in the matrix. Incorporation of analyte molecules into matrix crystals formed upon evaporation of the solvent is desirable and for some matrixes may be essential. The matrix also appears to play a role in the ionization process, but this is not fully understood.

The understanding of the MALDI process is not yet sufficient to allow selection of a matrix on theoretical considerations alone. The required absorption at the UV-laser wavelength is not a very restrictive criterion; it is necessary but not sufficient for a good matrix. A large number of materials have been evaluated, and only a few have been found satisfactory. Compatibility of the solvents required for analyte and matrix, absence of chemical reactivity with analytes, and stability in a vacuum are some of the properties required of a matrix. A number of the compounds that have been tried empirically can work as matrixes, but very few are presently regarded as high-quality matrixes for UV-MALDI. These include sinapic acid and some related cinnamic acid deriva-

tives,[104,105] 2,5-dihydroxybenzoic acid (DHB)[106] often with an admixture of 10% 2-hydroxy-5-methoxybenzoic acid(super DHB),[107] α-cyano-4-hydroxycinnamic acid (4-HCCA),[108] 3-hydroxypicolinic acid (HPA),[109] and di- and triacetophenones.[110] The first three are generally used for proteins and peptides and the latter two for DNA oligomers. A number of other matrixes satisfying the co-solubility requirement have been employed with industrial polymers.[111]

I. IR MALDI

The use of infrared (IR) lasers instead of the UV lasers traditionally employed in MALDI has been studied by several research groups.[112−114] It has been demonstrated that approximately the same performance can be obtained with either laser source with respect to mass resolution, mass accuracy, and analytically useful sensitivity.[114] The major differences observed are that IR generally gives less fragmentation of large molecules, but much more sample is consumed per laser shot. The ionization efficiency is generally several orders of magnitude lower with IR than with UV, but the sensitivity is generally limited by chemical noise from matrix and other impurities, and a much smaller fraction of the sample is desorbed in typical UV measurements. The IR laser also penetrates more deeply into the matrix and allows sample to be successfully analyzed from gels and membranes. On the other hand, UV MALDI detects sample present at or near the surface and does not detect molecules embedded more deeply. The major limitation on the use of IR lasers in MALDI is that the available lasers are rather expensive and somewhat more difficult to use routinely.

J. Delayed Extraction

Electrospray was more rapidly accepted than MALDI for many routine applications primarily because it was readily adapted to the quadrupole and magnetic mass analyzers predominately in use. The pulsed ion beam produced by MALDI was particularly compatible with time-of-flight (TOF) techniques, but the resolution and mass accuracy obtained with MALDI-TOF on larger molecules was not as good as that obtained with electrospray on either quadrupole or magnetic analyzers. The use of reflecting TOF instruments such as that described originally by Mamyrin[115] improved the performance for peptides and other small molecules, but the resolution and accuracy for oligonucleotides and proteins was similar for both linear and reflecting analyzers and clearly inferior to that obtained by electrospray. Early studies showed that the ions produced by MALDI exhibit a rather broad velocity distribution and that the initial velocity of desorbed ions is nearly independent of mass.[116,117] Also, when desorption occurs in a strong electric field, energy is lost presumably by collisions with the neutral plume.

In pioneering work, Wiley and McLaren[118] described a two-field, pulsed ion source for time-of-flight analyzers that provided first-order correction for the initial space distribution of ions. They also described a technique which they named "time-lag energy
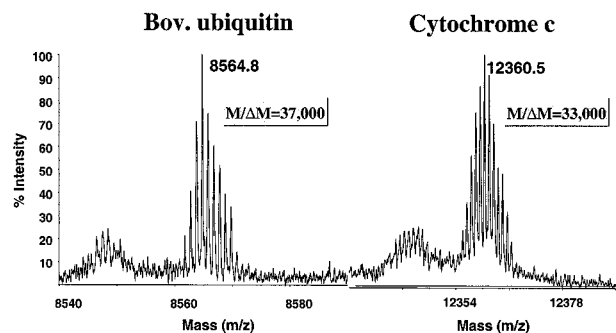
Figure 12. Molecular ion region of the MALDI-TOF mass spectrum of two small proteins, bovine ubiquitin and cytorchrome *c*, obtained using a state-of-the-art reflecting analyzer with a delayed extraction source. Measured resolving powers (fwhm) are indicated on the spectra.

focusing" to correct for initial velocity distributions. The ions are produced in a field-free region, and the accelerating field is turned on by application of a fast pulse at a predetermined delay time. The correct choice of delay time and accelerating field allows the initially slow ions to receive enough additional energy to catch the initially fast ions at the ion detector. In MALDI, the ions are produced from a nearly flat surface; thus, space focusing is not required, but velocity focusing greatly improves performance. The improvement in performance resulting from delayed extraction has been independently demonstrated in a number of laboratories[119−123] and is now used for almost all analytical applications of MALDI. An example of the resolving power presently possible by MALDI-TOF is shown in Figure 12. Mass measurement accuracy with internal calibration is typically better than 10 ppm for such spectra. Higher resolving power can be obtained at low m/z by FT-ICR and double-focusing magnetic mass spectrometry, but for singly charged ions produced by MALDI, the results of Figure 12 represent the current state of the art for any available mass spectrometric technique.[124]

K. New Mass Analyzers

It is difficult to discuss methods of ionization without occasionally straying across the boundary with mass analyzers. The evolution of mass analyzer designs has strongly interacted with that of ionization methods. Also, the potential applications have sometimes driven the development of the instrumentation, but more often, the applications have been determined by the capabilities of commercially available systems. MALDI and electrospray have made mass spectrometry applicable to very large molecules which were not previously accessible. This has driven the development of improved mass analyzers with larger mass range and improved resolving power, mass accuracy, and sensitivity. Since these new ionization techniques often produce molecular ions with little or no fragmentation, development of tandem mass spectrometers compatible with electrospray and MALDI has been essential for obtaining structural information. Most of these developments have been in the areas of TOF,[125,126] quadrupole ion traps,[127] and Fourier transform ion cyclotron resonance (FT-ICR).[128] Dramatic improvements in these

techniques have occurred during the past few years, particularly for biological applications, and coupled with the new ionization techniques these have revolutionized the practice of analytical mass spectrometry for nonvolatile molecules.

V. Present Status and Future Prospects

The methods of ionization presently available for mass spectrometry are briefly summarized above. It is now possible to generate either atomic ions or molecular ions from almost any material and to efficiently introduce these ions into the vacuum of the mass spectrometer for analysis. Except for large, nonpolar organic polymers that are difficult to ionize, there appears to be no limit on the size and complexity of molecules that can be ionized and analyzed. During the past decade no new ionization methods have been introduced, but enormous advances have been made in the sensitivity and utility of existing methods. These developments have occurred in parallel with dramatic improvements in mass analyzers with extended mass range, higher resolution, improved mass accuracy, and vastly improved sensitivity and speed. Applications for these techniques have grown exponentially, particularly in the life sciences. It appears that mass spectrometer technology may be approaching maturity, but the applications are still in their infancy.

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