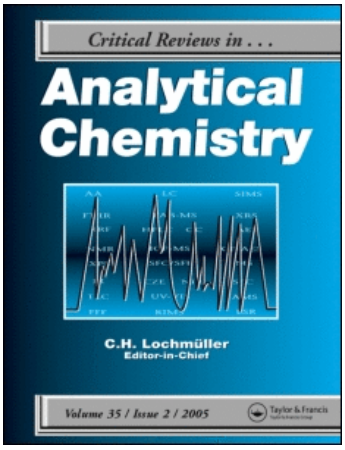

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Modern Ionization Techniques in Mass Spectrometry

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MODERN IONIZATION TECHNIQUES IN MASS SPECTROMETRY

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I. INTRODUCTION

It is widely recognized that electron ionization (EI),* the most common of the methods of molecular ionization used in organic mass spectrometry, has deficiencies of various sorts. Its survival in the face of alternative methods has been facilitated by the even greater problems associated with these other methods, particularly concerning their sensitivity.

However, many workers have devoted their efforts to the development of other ionization techniques during the last decade, and these efforts are beginning to bear fruit. EI is now experiencing some competition from two techniques, viz., field ionization (FI) and chemical ionization (CI).

This article will review the state of development of these two methods and estimate their respective utilities in organic mass spectrometry as compared to that of EI. Two earlier reviews with much the same objectives have been published[1,2] and this article may, in a sense, be regarded as the third in a series.

All pertinent papers that appeared before the end of 1973 have been considered in the preparation of this review. However, results that have been published only as conference reports have been almost totally omitted. Such reports are virtually inaccessible and, in any event, frequently tend to be too superficial to permit a critical reading.

The authors have both worked in EI and CI mass spectrometry for a number of years, but have had no direct experience in FI and Field Desorption (FD) techniques. As a result, a possible CI bias may be present in this review, particularly in the subjective comparisons made in Section V.

II. METHODS OF MOLECULAR IONIZATION

At least thirteen distinct methods of converting an organic molecule to an ion are known. Few of these are currently of more than academic interest and still fewer find much use in organic mass spectrometry where the three most important methods, in the order of the number of users of each, are EI, CI, and FI. All of the thirteen techniques are defined in the section, FI and CI are treated in detail in the next two sections, and a final section contrasts EI, CI, and FI.

A. Electron Ionization

When a molecule is placed in a beam of electrons whose average energy considerably exceeds the ionization potential of the molecule, one of the products of collisions between electrons and the molecules is the odd-electron molecular ion, $M^{\cdot+}$. Most of the mass spectrometers used in organic analysis rely upon this phenomenon to generate positive ions from sample molecules. In the ion sources of these instruments, the electrons used are those spontaneously emitted from an electrically heated filament of tungsten, for example, as shown in Figure 1. The emitted electrons are accelerated, typically to 70 eV, and then

*Abbreviations that are used throughout this article are as follows: EI – electron ionization; FI – field ionization; CI – chemical ionization; FD – field desorption.

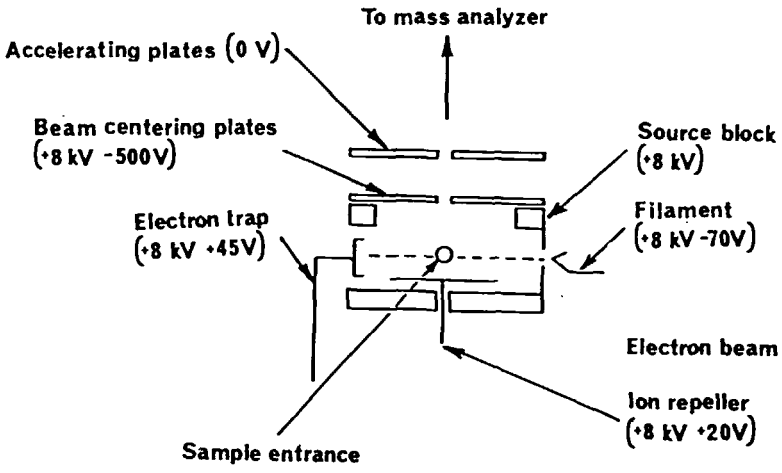


FIGURE 1. Schematic diagram of an EI source. The voltages shown are those used typically in the EI source of the AEI-MS9.

collimated, usually rather crudely, into a "beam" which is allowed to enter the so-called ion chamber. Molecules of the sample are also admitted into this chamber where they are ionized in collisions with the electrons. The resulting positive ions are extracted from the ion chamber by electrostatic fields and focused into an ion beam which enters the analyzer portion of the mass spectrometer. The mechanical and electrical details of the construction of such sources have been treated in the standard texts on mass spectrometry.[3,4]

The "ion gun" which accomplishes this operation is a rather simple device, as can be seen from Figure 1. It must, however, be used in a chamber which is continually pumped to maintain a high vacuum. This is necessary to prevent electrical discharges of the high voltages, destruction of the heated filament, and interparticle collisions which would vitiate ion-beam formation. The pumping systems account for much of the sheer size and apparent complexity of mass spectrometers.

The deficiencies of the electron ionization method stem from energy considerations. It is not easy to produce a beam of monoenergetic electrons; accurate measurements of ionization potentials and appearance potentials are therefore difficult. The maximum sensitivity, in terms of the ion current derived from a given number of molecules, is attained at relatively high electron energies such as 50 eV; the $M^{\cdot+}$ ion formed under these conditions is frequently unstable with respect to fragment ions, and it tends to break up rapidly. These considerations have been dealt with in some detail by Beckey and Comes.[1]

The precise mechanisms of the fragmentation reactions resulting from EI have been the objects of intense theoretical[5] and experimental[6] study; but most organic analysts continue to treat the matter empirically and rely upon precedents, of which there are an increasingly large number.[7] The large numbers of recorded EI mass spectra have, in recent years, prompted the assembly of large, computer-stored files[8] and the development of effective methods of searching through such files.[9]

As a consequence of this history, EI is very well established in organic mass spectrometry. It is the means of ionization used in over 95% of all the mass spectrometers engaged in organic analysis; thus for better or for worse, it is the standard against which any new ionization techniques must be measured.

B. Radioactive Sources

The use of a radioactive element, rather than a hot filament, as a source of electrons has been explored by several workers. The particles emitted during radioactive decay are often of quite high energies and are particularly suited to use in high pressure mass spectrometer sources. Thus, β-emitters such as ^{3}H (0.018 MeV)[10] and ^{63}Ni (0.067 MeV)[11] have been used in high-pressure sources; the latter has also been used in a plasma chromatograph-quadrupole combination operating with source pressures of one atmosphere.[11] There have also been reports[12] of the use of α-particle (He^{++}) emitters such as ^{210}Po.

The chief disadvantage of radioactive sources is

that relatively high amounts (0.01 to 1.0 curies) of isotope are necessary to produce a satisfactory ion current, even at high source pressures; evaporative loss of the isotope at higher temperatures is also a problem that does not appear to have been solved. The main advantage of radioactive isotopes in this role is that they emit particles with well-defined energies at room temperature. One of the major drawbacks of the hot filament is thereby overcome.

C. Photoionization

In place of a beam of electrons, a beam of photons can be used to ionize organic molecules. The energy of electromagnetic radiation is inversely related to its wavelength, 124 nm corresponding to 10 eV. Thus, to be useful for the ionization of organic molecules, whose ionization potentials are generally between 15 and 7 eV, the radiation used must be in the vacuum ultraviolet with wavelengths in the range of 83 to 177 nm. This imposes considerable technical difficulties. Windows, for example, can be used only above 105 nm, and since radiation of these wavelengths is generated in gas discharge tubes at pressures of 0.1 to 1 torr, these must be differentially pumped with respect to the high-vacuum system of the mass spectrometer.

The overall sensitivities of photoionization sources are not high compared to EI sources, but the energy of the photons can be controlled very precisely by optical techniques. Photoionization is therefore an excellent means of measuring ionization potentials. The energy of the electron ejected during photoionization can also be measured, as in photoelectron spectroscopy.

The use of photoionization mass spectrometry in analytical organic chemistry is rather infrequent[13] and the mass spectra that have been published of, for example, alicyclic bromides,[14] *cis-* and *trans-tert*-butyl cyclohexanols Figure 2,[15] tricyclo-octanes,[16] amino acids,[17] and peptides[18,19] have not encouraged many other workers to apply the method in organic analysis.

D. Thermal Ionization

In principle, the energy necessary to remove an electron from an atom or molecule could be provided thermally. This is true in practice for some metals with low ionization potentials (3 to 5 eV) which emit electrons at temperatures below 2,000°C. The ionization potentials of many elements and all covalent compounds are far too high to permit such an ionization method using conventional heating methods; this technique is therefore restricted to a few metals. The pyrolytic decomposition of organic compounds often shows similarities to the EI-induced fragmentation undergone by the same compounds; a great deal of study has been carried out on this putative relationship.[20]

The impact of a focused, pulsed laser beam on a solid may also result in thermal ionization; this technique has shown some interesting results with

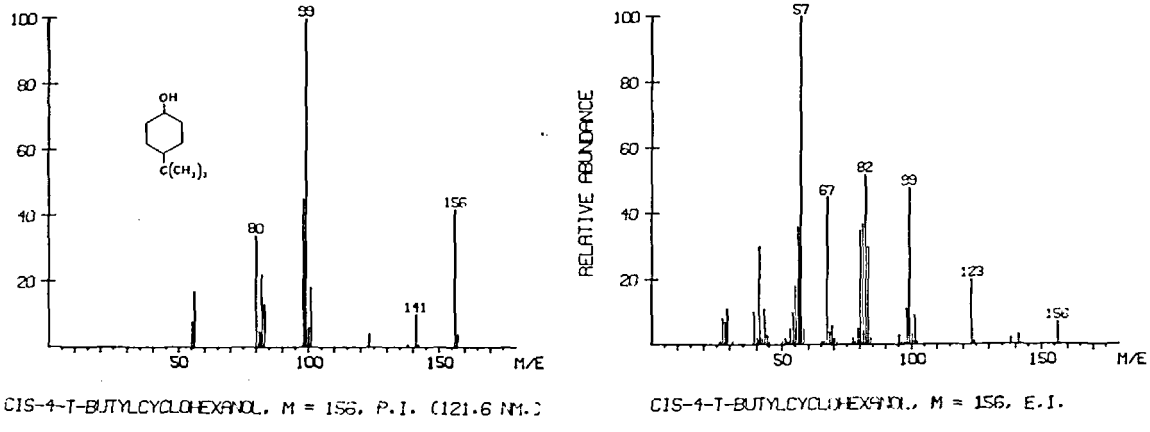


FIGURE 2. Photo-ionization and EI mass spectra of *cis*-4-*tert*-butylcyclohexanol.[15]

organic compounds. Polycyclic aromatic hydrocarbons, for example, give a molecular ion as the base peak in the mass spectra obtained this way.[21] The amino acid leucine also gives a molecular ion as the major ion but, in this case, some intense fragment ions are also formed, as are some unexplained ions of mass greater than the molecular weight.

The laser ionization mass spectra of salts of sulfonic, sulfuric, and thiosulfuric acids have also been reported.[22] In the laser ionization mass spectrum of sodium hexyl sulfate shown in Figure 3, ions containing two or more atoms of the metal (e.g., $C_6H_{13}SO_4Na_2^+$) are observed in these spectra.

E. High Voltage Discharges

An ac potential in excess of 10 kV with a frequency of up to 1 MHz between two electrodes in the source of a mass spectrometer will result in electrical breakdown and formation, in the gap, of ions from the electrode material. The "spark source" depending upon this principle is extensively used for qualitative and quantitative determinations of elements in mixtures such as metal alloys.[23] The sample under investigation is mixed with a relatively large amount of graphite, for example. The resulting mixture is formed into rods, which are mounted in the source of the mass spectrometer as electrodes. During the discharge between the electrodes, ions derived from the sample are formed and can be accelerated into the mass spectrometer where they are mass analyzed in the usual way. Free elements, such as the metals in an alloy, give various ions of the type M_y^{x+}, where x and y are small integers.

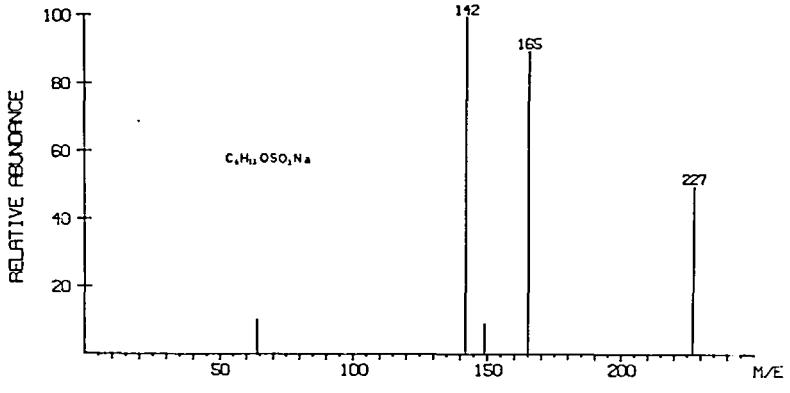
Covalent bonds are easily broken under these conditions; thus, the spark source mass spectra obtained from electrodes into which organic compounds have been mixed have, as expected, many small fragment ions. However, a substantial proportion of the organic molecules are ionized with relatively little bond cleavage. The resulting mass spectra, such as that of anthraquinone, shown in Figure 4, are quite similar to the corresponding EI mass spectra,[24] and it has been suggested that EI may be important in rf discharges. Now that the maior difficulties associated with the mass analysis, such as the wide energy spread of the ions and the short-term instability of the ion beam, have been solved by those interested in inorganic analysis, the technique may have something to offer workers interested in the mass spectral analysis of nonvolatile organic compounds.

F. Flame Ionization

When placed in a flame, organic molecules are converted to ions with considerable efficiency by little known pathways. This is the basis of the flame ionization detector that is widely used in gas chromatography.[25] Mass spectrometry has been used to study the ions formed in flames,[26] but the technical difficulties are formidable. Although interesting kinetic data have been derived from such studies,[27] the applicability of flame ionization to the mass spectrometry of organic compounds appears somewhat limited.

G. Glow Discharges

A potential difference of 10 to 60 kV between electrodes some 50 cm apart will lead to a



SODIUM HEXYL SULFATE (M = 204) LASER IONIZATION

FIGURE 3. Laser ionization mass spectrum of sodium n-hexyl sulfate.[22]

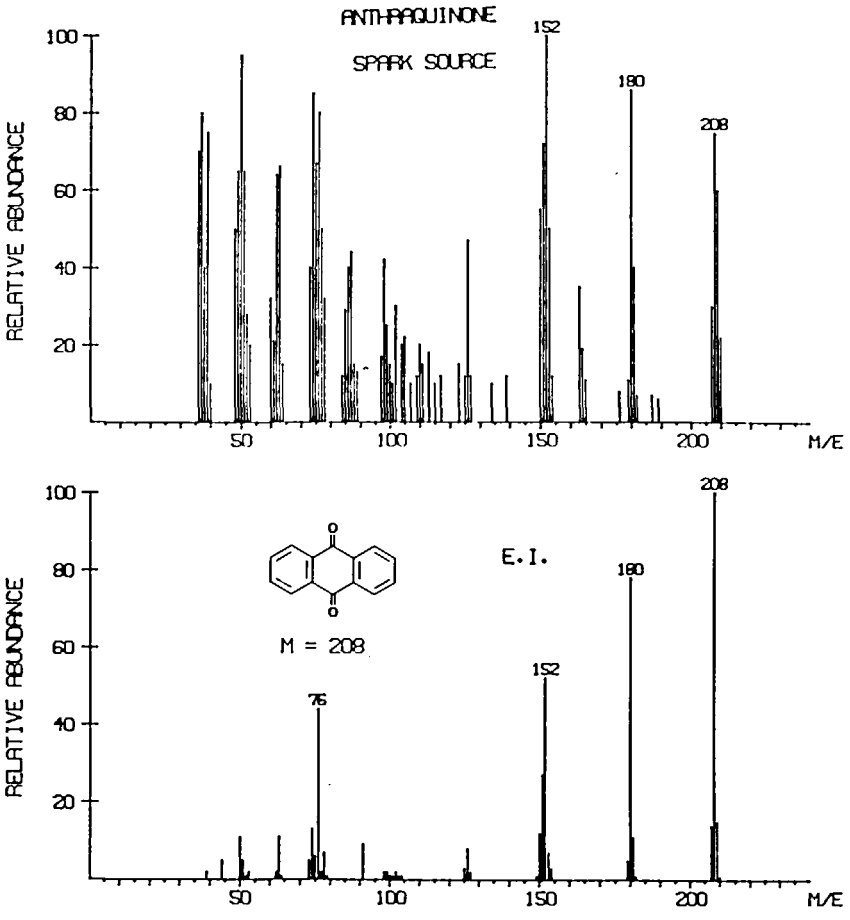


FIGURE 4. High-voltage discharge mass spectrum and EI mass spectrum of anthraquinone.[24]

"high-voltage glow discharge" if the space between the electrodes is filled with a gas at pressures between 10^{-1} and 10^{-3} torr.[28] This is the ionization technique used in the earliest of mass spectrometers, but it has never been used extensively in modern organic mass spectrometry.

A stable glow discharge can be achieved if a steady pressure of a rare gas is supplied to the system. Sample molecules that are then introduced into this system will also be ionized, probably as a result of ion-molecule reactions. The positive ions formed can be electrostatically extracted from the discharge region via a hole in the cathode, and analyzed in a mass spectrometer. Because of the importance of second- and higher-order reactions at such pressures, and mass spectra so obtained are complex and difficult to reproduce.

The "flowing afterglow" method[29] has been used by a number of groups to measure the rate constants of ion-molecule reactions. In this method, a glow discharge is employed as the initial source of ions that are then swept out of the discharge region by an inert gas such as helium. They are then allowed to react with a different compound under controlled conditions, and the ionic products of the reaction are analyzed by mass spectrometry.

The "Townsend discharge" that occurs in gases at pressures of about one torr across potential drops of several hundred volts has been used by Hunt[30] as a source of primary ions in a CI mass spectrometer. This experiment, which permits the operation of a CI source without a heated filament, is discussed in more detail in Section IV-C-2 below.

H. Ion Bombardment

Collision of an accelerated ion with a neutral molecule may result in ionization of the latter. If the neutral atom or molecule is in the solid phase, the process is known as secondary ion emission, or cathodic sputtering. The analytical potential of

this method is currently being explored[31] and may prove to be substantial.

There has been little work on the ionization of organic molecules by this means, but a variation on the method, collision-induced decomposition of ions, has recently received some attention.[32] A beam of $C_6H_6^{\cdot+}$ ions with insufficient internal energy to permit unimolecular decomposition was accelerated into the electrostatic analyzer of a double-focusing mass spectrometer. At analyzer pressures of 10^{-7} torr, the ions are transmitted normally and the mass spectrum of benzene obtained under these conditions consists of only the molecular ion peak at m/e 78. However, if the pressure in the analyzer is permitted to rise to between 10^{-6} and 10^{-4} torr, different ions are observed at the collector of the mass spectrometer. This is because the low-energy ions entering the analyzer acquire internal energy by collision with molecules in this region. These higher-energy ions can then fragment in unimolecular fashion. If this reaction occurs in the analyzer, the product ions will be lost; if it occurs in the field-free region between the electrostatic and magnetic sectors of the mass spectrometer, product ions may be observed in the mass spectrum at some point during the magnet scan. Such reactions are said to be "collision-induced," and the ions formed constitute what might be called a "molecular impact" mass spectrum.

McLafferty and co-workers have studied the same process, which they call "collisional activation."[33] They have confirmed the similarity, previously noted[32] between normal EI mass spectra and collisional activation mass spectra. They have also shown that collision-induced decompositions are largely independent of the internal energy of the original ion, but depend strongly upon the nature and pressure of the target gas.

Collisional activation could be very valuable in dealing with mixtures because it permits the unequivocal identification of the parent ion which is the origin of any given fragment ion. The technique has been used to aid in interpreting the EI mass spectra of mixtures of peptides[34] and is discussed in more detail in Section IV-B-3 below.

I. Negative Ion Formation

Negative ion mass spectrometry has been rather neglected in comparison to positive ion mass spectrometry. This is not entirely unjustified since negative ion formation by EI is relatively inefficient, difficult to reproduce, and very sensitive to the structure of the molecule at hand. A large amount of exploratory work has been done on the subject,[35-38] but the method is rarely used in analytical problems. The interesting possibilities of negative ions in CI mass spectrometry have recently been explored.[39]

J. Penning Ionization

Species such as the rare gases possess neutral metastable excited states. Helium, for example, at energies below its ionization potential (*ca.* 24.6 eV), has two metastable states with excess energies of 19.81 and 20.61 eV. Such energies are more than sufficient to ionize an organic molecule by the process known as Penning ionization:[40]

$$He^* + M \rightarrow He + M^+ + e^-$$

In practice, the metastable species such as He* are generated by electron bombardment of the appropriate gas at electron energies just below the ionization potential of the gas. Any ions formed can be removed electrically; the metastable species are then allowed to collide with the molecule under study, whereupon Penning ionization occurs.

The few Penning-ionization mass spectra of organic molecules that have been published[41,42] are rather similar to the corresponding EI mass spectra. Penning-type ions are an inevitable component of the charge-exchange spectra that are discussed in Section IV-B-4 below.

K. Chemi-ionization

Very closely related to Penning ionization is the process known as chemi-ionization:

$$He^* + M \rightarrow HeM^{+\cdot} + e^-$$

This technique has been applied to organic compounds even less than has Penning ionization. The two methods differ from most other ionization techniques in that both particles in the collision are neutral and collision complexes may be relatively long-lived.[40]

L. Chemical Ionization

A molecule can be ionized by reaction with an ion in an "ion-molecule reaction." The exact mechanism of the ionization and the nature of the

product ion depend upon the reactants and the conditions. The use of this technique in organic analysis was first demonstrated by Munson and Field[43] in 1966. Since then, increasing numbers of workers have investigated the method; now it ranks as probably the second most commonly used ionization technique in organic mass spectrometry. It is discussed in detail in Section IV.

M. Field Ionization

The ionization of a molecule by strong electrostatic fields is known as field ionization. The method was developed during the fifties and sixties from earlier experiments in field-ion microscopy and owes its present position of importance in organic mass spectrometry largely to the pioneering work of Beckey and his colleagues.[44] This technique is dealt with in some detail in Section III.

III. FIELD IONIZATION

A. Introduction

When an atom or molecule in the gas phase is subjected to the influence of an external electrical field, comparable to the nuclear field normally experienced by the outer electron of an atom, then the possibility exists that an electron may be removed from its atomic or molecular orbital by the influence of this external field. This process, field ionization, was described theoretically more than forty years ago[45,46] and first demonstrated experimentally in 1951.[47] In the past twenty years, it has been gradually developed by a small number of workers until it now stands as the third most commonly used method of ionization of organic molecules in the gas phase.

The area of FI mass spectrometry has been reviewed many times in the last five years.[1,2,44,48-56] Of these articles, two[1,2] are reviews of ionization methods in general; the present article constitutes a sequel to these. Of the other reviews, several[44,48-51,55] have concentrated on the physical problems of FI, while others[52-54,56] have stressed applications of FI mass spectrometry to problems in organic chemistry. The article by Block[48] is an excellent introduction to the principles of FI, and the monograph by Beckey[44] stands as the most thorough and exhaustive treatment of FI, although it is marred by typographical errors.

The technique of FI as applied to organic molecules has, in the past, acquired the reputation of being insensitive in comparison to EI, and also of being technically difficult. These difficulties have to some extent been ameliorated in recent years; the development in 1969 of field desorption mass spectrometry,[57] whereby solid samples can be ionized without first being vaporized, promises to give FI a unique advantage over other methods of ionization.

B. Theory

1. Energy Considerations

The attractive force between an orbiting electron and the corresponding nucleus depends on the type of orbit and on the nucleus. However, for the most loosely bound electrons of organic molecules, the binding energy is about 10 eV. This is the magnitude of the potential barrier (Figure 5A) that must be overcome if the electron is to be dissociated from the molecule.

2. Quantum-mechanical Tunneling

It is possible to subject the electron to a higher, external electrical field such as that in the vicinity of a metal electrode at a high positive voltage. In such a situation, the potential well of the electron will be distorted (Figure 5B) to an extent proportional to the magnitude of the external field.

The potential barrier that must be negotiated by an electron migrating from the molecule to a vacant orbital in the metal is now greatly reduced. Furthermore, the electron can pass this barrier without the molecule's acquiring any substantial internal energy. For this to happen, the electron must pass through a region of space in which its total energy is less than its potential energy. This process, which is forbidden by classical mechanics but permitted by quantum mechanics, is known as quantum-mechanical tunneling.

3. "Minimum Distance" Criterion for FI

For this process of FI to be energetically possible, the energy of the migrating electron must be at least that of the lowest unfilled orbital in the metal. This latter quantity is determined by the work function (ϕ) of the metal. If the ionization potential of the molecule is I, the external field F, and the separation of the electron from the anode d, it follows that for FI to take place at a flat surface (neglecting image and polarization terms),

$$-I + eFd \geq -\phi$$

or

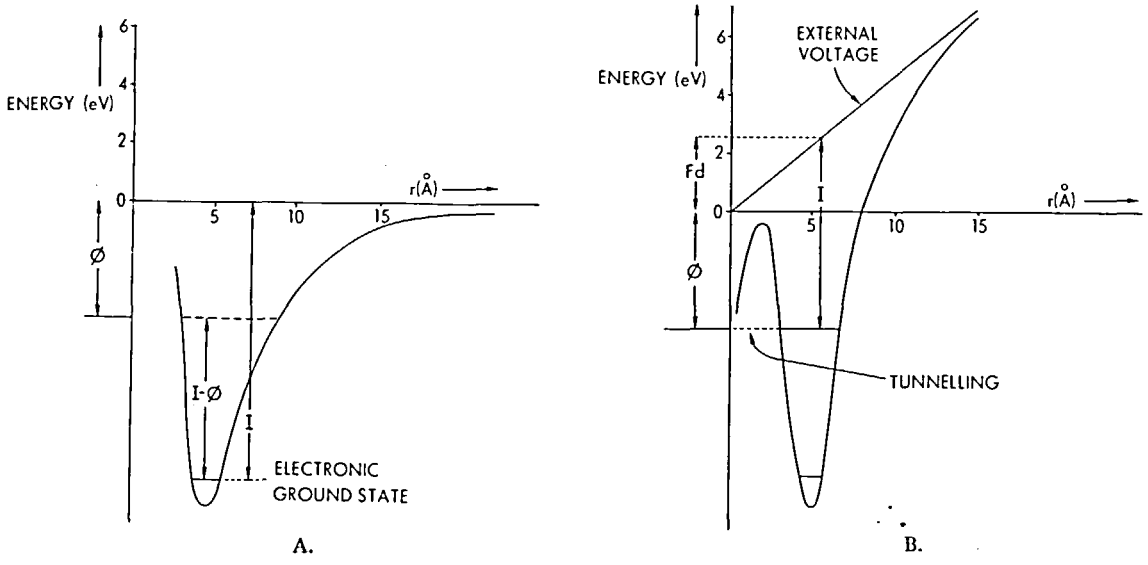


FIGURE 5. (A) Potential energy diagram for an atom near a metal anode at ground potential. (B) Potential energy diagram for an atom near a metal anode at a high positive potential. The diagonal line represents the field due to the external voltage; if this is added to the curve in Figure 5A, the potential energy curve shown in Figure 5B results.

$$d \geq (I - \phi)/ef$$

For typical values of I, ϕ, and F, this minimum value of d is about 5 Å, and the implications of this criterion can be largely ignored from the point of view of organic mass spectrometry; even if a typical organic molecule were to touch the anode, some parts of the molecule would be more than 5 Å away from the anode.

4. Probability of FI

It can be calculated[48,58] that the probability that a molecule in the vicinity of the anode will be ionized increases exponentially with the field strength, which itself depends on the voltage at the anode.* The probability can also be shown to decrease exponentially with some power of the ionization potential and to decrease exponentially with increasing values of the work function of the metal. Of these three quantities, only the field strength can be readily varied in a given experiment.

Calculations of FI probabilities have not been completed precisely for even moderately complex organic molecules.[59,60] Accurate descriptions of the rate of FI of very simple systems such as the hydrogen atom have been derived[61,62] and while they agree with the dependences cited above, they further suggest that when a molecule is in the vincity of the anode, the probability of its being ionized approaches unity if the electrical field is sufficiently high, of the order of 10^8 V/cm (1 V/Å). The production of such high fields is discussed in Section C.

5. Supply of Molecules to the FI Anode

Molecules can arrive in the ionization region, either in the gas phase or in the liquid phase, by diffusion over the surface of the FI anode. In the latter case, the minimum distance criterion will be satisfied if the length of the molecule itself is greater than this minimum distance. At fields in excess of 10^8 V/cm, the critical distance is about 5 Å. Most molecules are longer than this and if adsorbed on the FI anode will satisfy the minimum distance criterion and be ionized.

However, in conventional FI gas sources the major contribution to the ion current is thought to be from the FI of molecules arriving at the areas of the FI anode from the gas phase. The number of molecules arriving in the ionization region in a given time at a given source pressure may be calculated.[63,64] This number will be increased by a "multiplication factor" (σ)[63] based on the fact that molecules with a permanent or an induced

*The positively charged electrode in FI is often called the emitter. This term, which was used by earlier workers i connection with field emission, is confusing when used in connection with FI. Therefore, it will not be used here; th electrode in question will be referred to as the "FI anode," or simply "anode."

dipole will be attracted into the high-field region.[47] This factor can be calculated if the necessary polarizability and dipole moment data are available, and it can be as high[63] as 10^3. If the FI probability is assumed to be unity, this leads to a figure of $10^{-10} \times \sigma$ amp as the ion current generated by a point emitter of surface area 10^{-8} cm^2 with an electrostatic field of 5×10^7 V/cm and a source pressure of 10^{-3} torr.

Such calculations suggest that FI is not inherently insensitive; it seems likely that the unquestioned inefficiency of the early FI mass spectrometers was related to two separate but manageable problems. First, the generation of high fields is not straightforward and many of the sources were operated at fields that are too low. Second, the focusing of ions generated in an FI source is peculiarly difficult. Unless they are designed with some care, FI mass spectrometers often fail to collect more than a very small fraction of the ions formed in the source.

Both of these problems have received a large amount of attention in recent years and, as is discussed below, have been largely solved, with the result that FI mass spectrometers are beginning to escape their reputation for insensitivity.

C. Generation of High Fields

The fields necessary for efficient FI are of the order of 10^8 V/cm. If anode-cathode gaps of 0.1 to 3.0 mm are used, as is common, the potential differences between the two will have to be on the order of 10^6 to 10^7 V. Such dc voltages are too high to be practically employed and recourse must be made to the fact that the field generated at an electrode at a given voltage depends upon the shape of that electrode.

In the ideal case of a sphere, the field generated at the surface is equal to the voltage applied divided by the radius of the sphere. In the general case, however, of a noncircular conic section, the field, F, is related to the voltage, V, and the radius, r, by the equation:

$$F = V/kr$$

in which k is a "geometry factor." For a sphere $k = 1$, but for all other surfaces $k > 1$. For a parabolic tip, for example,[65] $k = 5$.

The smaller the radius, the higher the field generated at the extremity by a given voltage. This has prompted workers in the field to explore the utilities in FI of fine points, thin wires, and blades. Each of these is discussed separately below.

1. Points

The use of fine points produced by chemical or electrochemical etching of wires as FI anodes was first reported in the late fifties.[66-68] The geometry factor, k, for a fine point is typically about 5, and the radius of curvature of the tip of the point can be as low as 10^{-5} cm. Application of 10 kV to such a point will therefore produce a field of 2×10^8 V/cm which is quite adequate for FI. Points are favored as FI anodes by those studying the physics of FI[69] because they are geometrically well-defined and they usually provide higher fields than either wires of blades.

However, to the analytical chemist, a serious limitation of the single point anode is that its effective area is very small; the sensitivity of an FI source using such an FI anode is low. Some interesting experiments have been conducted with arrays of points in an attempt to deal with this difficulty. Wanless[50] used between 4 and 20 tungsten tips, each with a diameter of 0.015 to 0.020 mm; they were spot-welded to a support wire 1 mm in length. An array of two such wires, then electrolytically etched, was found to produce about 10 times as much ion current under standard conditions as a 2.5 μm platinum wire used as the FI anode. As with wires,[70] the juxtaposition of the cathode slit and the support wire of the array affects both the magnitude of the ion current and also the mechanical stress imposed on the tips by the high electrostatic fields. The increase in sensitivity is clearly of value although, as was pointed out by Wanless,[71] this probably stems from the "backup" of new points which can take over when the tip in action is broken. It is unlikely that several tips are functioning simultaneously as repulsive effects can combine at any given time to favor one particular tip over the others.

A very interesting tip array has been fabricated by Spindt[72] using vacuum deposition techniques. Molybdenum is evaporated onto a base of the same metal using a template of alumina, which is subsequently removed by etching. The resulting multipoint array, shown in Figure 6, contains between 500 and 600 points/mm^2; when assembled into an FI source as shown in Figure 7, it is capable of efficient FI at anode-cathode potentials as low as 3 kV.[73]

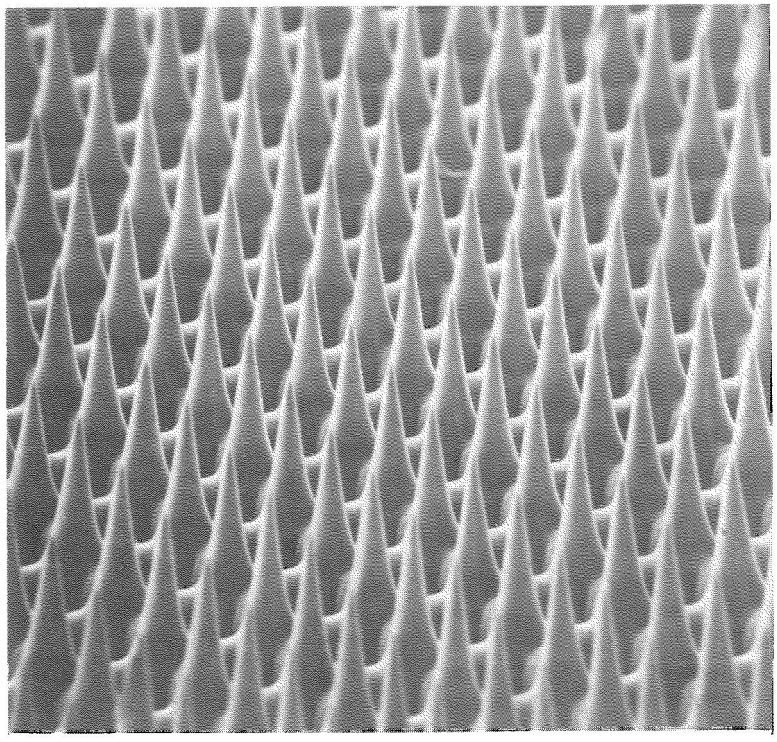


FIGURE 6. Scanning electron micrograph of Spindt's multipoint array.[72]

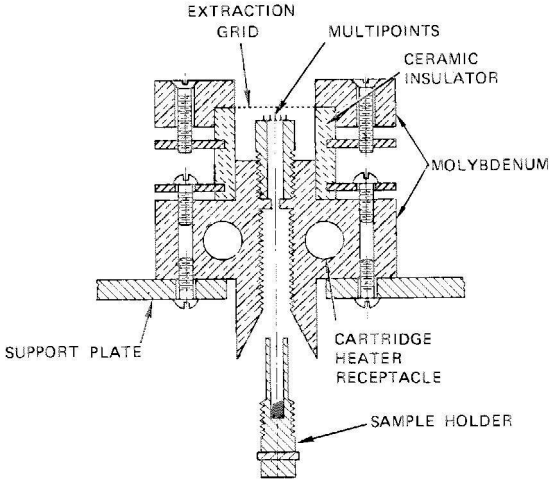


FIGURE 7. Schematic of a multipoint FI source.[73]

A somewhat similar FI anode has been described by Gentsch and Prüller,[74] who constructed a thin-film diode system with which FI is feasible at very low voltages, such as 0.1 kV. No practical application of this system has been reported.

2. Wires

A radius of less than 10^{-4} cm is not feasible in a wire since the tensile strength would be vanishingly small. The wires most commonly used as anodes in FI have radii between 10^{-4} and 10^{-3} cm. With a given applied voltage, such wires provide a lower field than the equivalent fine point. Nevertheless, the wires are useful in FI because they have a finite length (e.g., 3 to 5 mm) and the irregularities on the surface can be regarded as an assembly of point sources. With potentials of 10 kV, wires of 10^{-3} cm radius will produce fields of 10^{7} V/cm and can be used in FI sources. They are, however, marginal in their performance and are rarely used without prior conditioning. This pretreatment of wires, which greatly improves their efficiency as FI anodes, is dealt with in Section D.

Very thin wires cannot be formed by drawing techniques; instead, most methods rely upon an etching process. Wollaston wires are drawn, for example, from a relatively coarse silver wire containing a fine central core of the anode material, which may be platinum. After drawing, the outer cover of silver, which has a diameter of 1

$\times 10^{-2}$ cm, is etched away to expose the inner platinum wire, which with a diameter of perhaps 2.5×10^{-4} cm is ready for conditioning and use as an FI anode.

Taylor wires are slightly stronger than Wollaston wires; they are similar to Wollaston wires except that their outer covering is glass or quartz. Only a few metals are suitable for fabrication of Taylor wires because the inner core must be a material whose melting point is below that of the glass outer cover. The wires are drawn by melting both inner core and outer cover, the latter removed by etching with hydrofluoric acid.

Tungsten wires with a diameter of 10×10^{-4} cm are more useful than either Taylor or Wollaston wires in the fabrication of FI anodes. They possess about 160 times the tensile strength of platinum wires of diameter 2.5×10^{-4} cm and can be obtained either commercially or made by etching larger wires. They can be activated very successfully with benzonitrile as is discussed in Section III-D below. The high potentials used in FI can create considerable mechanical stress in the anode and very thin wires tend to break too easily under these conditions. Beckey[49,75] has shown that a workable compromise between the small radii that are required for high field strength and the larger radii that are necessary for mechanical strength can be achieved with the use of 2.5×10^{-4} cm diameter Wollaston Ag/Pt wires at potentials up to 12 kV.

The use of a second cathode on the side of the wire opposite the main cathode has been shown[49] to cancel out much of the mechanical stress experienced by a wire FI anode. Presumably the second cathode must also decrease the ion current transmitted towards the collector, but no data bearing on this point have been made available. The use of FI anodes containing several wires has been described[76] but, since only small gains in sensitivity were observed, little further work has been done on such systems.

The properties of wires as FI anodes can be greatly improved by activation, or conditioning,[49] a process which promotes the growth or microneedles on the relatively smooth surface of the wire. This technique is discussed below.

3. Blades

The use of sharp edges, or blades, as FI anodes has been investigated by Robertson et al.[77] Edges have some important advantages over wires in that they are very robust and moreover, as the common razor blade, are very readily available. They possess two serious disadvantages: First, they can be heated resistively only by large currents, of the order of amps; the convenience of milliampere currents, adequate to heat wires,[78] is lost. Second, the radius of curvature at the edge of a razor blade is about 10^{-5} cm;[79] this, together with the geometry factor k, which is highest for tips and intermediate for wires, but lowest for edges,[80] leads to a requirement for very high potentials when using blades as FI anodes. Insulation problems prevent the use of voltages in excess of 15 kV; at voltages below this, calculations suggest and experiments confirm[78] that the ion current from a razor blade is about 10% of that from a wire of radius 5×10^{-4} cm.

Edges can, however, be activated[79] much as can wires and activated blades, because of their mechanical strength, are very useful. To those more interested in producing large numbers of FI mass spectra from a single FI anode than in accurate measurements of ion current, edges are especially useful.

The use of thin etched-metal foils as FI anodes has been reported [81,82] These give useful ion currents, but they are rather too fragile for routine use.

A major improvement in the convenience of FI mass spectrometry was made by Chait et al.[83] who showed that a blade anode could be incorporated into a direct insertion probe, permitting its facile replacement when necessary. Anodes of all types are now generally handled this way rather than being permanently mounted within the source as was common in earlier designs.

Multibladed emitters have been used for FI[84,85] but as is similarly true of multipoint and multiwire anodes, only limited increases in ion current were observed. It was found that increasing the number of blades beyond three led to little further gain in sensitivity.[85]

D. Conditioning of Anodes for Use in Field Ionization

An observation made frequently[49] by workers in FI mass spectrometry was that the efficiency of an FI anode – that is, the ion current produced under standard conditions – tended to increase with the working life of the anode. This is because there is a steady growth of "whiskers" on the surface of the anode as it is used. These whiskers, or micro-

needles, can be clearly seen by electron microscopy;[49,86,87] they frequently terminate in tips whose radii are far below that of the "host" edge. There are, moreover, very many of these microneedles formed on a small length of the original edge and the result is a very effective multipoint array, which is responsible for the enhanced FI efficiency.

Beckey et al. were quick to recognize that FI anodes could be submitted to this microneedle-forming process prior to their use in a mass spectrometer, and they devised a process[49] for anode activation. In this method, wires were maintained at 10 kV in a chamber containing acetone, at pressures between 0.004 and 0.1 torr, for periods of several hours each. After a full day's activation, the high voltage was switched off and the acetone removed until the following day when the process was repeated. The temperature, pressure, voltage, time, and chemical nature of the gas are all critical. Optimum conditions were established[49] with which microneedles could be reliably produced on a Wollaston wire; the efficiency of the wire as an FI anode was thereby increased by between 1 and 3 orders of magnitude. The activation of anodes in a separate apparatus has the incidental but important advantage of increasing the working life between cleanings of the mass spectrometer.

Derrick and Robertson[88,89] demonstrated that stainless steel razor blades can be activated in the same way as wires; comparable gains in FI efficiency are obtained. The use of benzonitrile,[78,90] rather than acetone, leads to a far higher degree of activation; wires activated in this way are some three orders of magnitude more efficient as FI anodes than those activated with acetone. Trimethylacetonitrile[88] is said to give an increase of FI efficiency similar to that obtained with benzonitrile, although this result was not reproduced elsewhere.[91]

Much systematic study of the activation process[44,90,91] suggests that nitriles are indeed the best compounds for this use. The degree of activation depends on the voltage, temperature, pressure, and the radius of the starting anode. Optimum values for each of these parameters have been established.[91]

In 1972, Schulten and Beckey[87] demonstrated that activation with benzonitrile at anode temperatures high enough to cause pyrolysis (typically 800 to 1,000°) results in the formation of far larger numbers of microneedles on a given length of anode than does the room-temperature activation process. This high-temperature process provides microneedles that are mechanically very strong and very useful for field desorption.

Both the low-temperature and the high-temperature activation processes produce microneedles that are semiconductors. The microneedles produced by the low-temperature activation process are neither pure carbon nor pure metal; neither are they mixtures of carbon and metal. The exact chemical nature of the microneedles has not been established, but the concensus of opinion[86,90,92,93] is that they are composed of organic polymers with a high carbon content. The microneedles produced by the high-temperature activation process, on the other hand, appear to be composed of a high-temperature modification of carbon.[87]

Low-temperature activation results in microneedles that are chemically unreactive and have moderate mechanical strength.[91] The chemical reactivities are much smaller, and the mechanical strengths are much greater, for microneedles that result from high-temperature activation,[94] and only those anodes activated at high temperatures are suitable for the measurement of FI mass spectra of materials such as the mass standard, perfluorokerosene,[87] that are chemically reactive under these conditions.

E. Field Desorption

In 1969, Beckey showed[57] that, if a wire FI anode was activated with benzonitrile, the mass spectrum of a compound could be measured simply by coating the compound on this anode which may then be used in the normal FI mode. When such a coated anode is inserted via a vacuum lock into the high field of an FI source, ionization may occur at relatively low temperatures and the result is the field desorption (FD) mass spectrum. With compounds of lower vapor pressure, some heating of the anode may be necessary. If the anode were originally activated at high temperature, it could be re-used many times.

Compared to the corresponding FI mass spectra, FD mass spectra show a vastly reduced degree of fragmentation.[95] The reason is that, although ionization does not involve major transfer of internal energy to the molecule in either case, conventional FI has the disadvantage that vaporization of the sample is necessary prior

to ionization. The energy needed to overcome crystal lattice forces is sufficient to cause cleavage of the weaker covalent bonds in many molecules giving pyrolysis products. The "fragment ions" observed in normal FI mass spectra may, in some cases, be more accurately regarded as ions formed from pyrolysis products.

An early published example of the power of FD mass spectrometry[96] involved glutamic acid. This acid loses water so readily upon heating, giving pyroglutamic acid whose molecular weight is 129, that the ion of this mass is the highest mass ion in the EI mass spectrum shown in Figure 8. In the FI mass spectrum, the $(M + H)^+$ ion at m/e 148 is present, but with a relative abundance of only about 10% of the ion at m/e 130. In the FD mass spectrum, however, the only ion seen is at m/e 148.

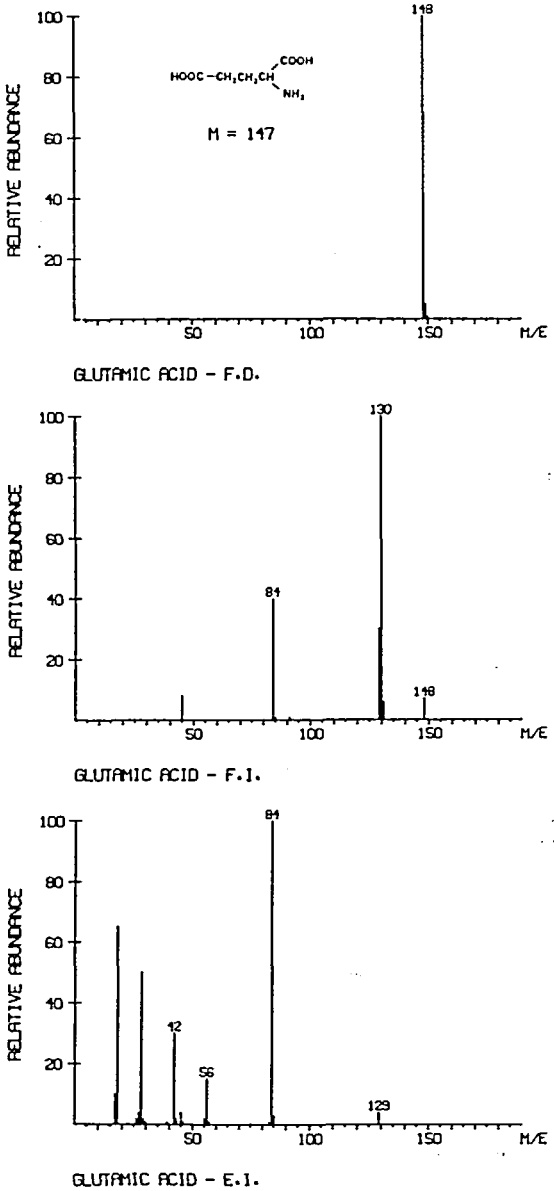


FIGURE 8. EI, FI and FD mass spectra of glutamic acid (molecular weight 147).[96]

Field desorption mass spectra are far more easily obtained and are more reproducible if high temperature activated anodes are used.[87] The main difficulty with the FD method, at its present stage of development, is that the range of anode temperatures at which good FD mass spectra may be obtained with polar compounds can be very small.[97] If electrical heating of the anode is employed and only small amounts of the sample are available, some integrating technique, such as photoplate detection or computer time-averaging of multiplier output signals, is necessary. Irradiative heating of the anode has been used in FD experiments[98] and may prove to be some help with this problem.

The possibilities of FD are very exciting and the appearance in the literature of FD mass spectra of the potassium salt of guanosine[99] in which, as is shown in Figure 9, the highest mass ion (relative intensity 85%) corresponds to $(C_{10}H_{13}N_5O_5K)^+$ makes it clear that much new ground will be broken by this method.

F. Effect of Temperature Upon Field Ionization

Two detailed studies have been carried out, by Beckey[100] and Knöppel,[101] on the effect of temperature on FI. Using a knife-edged platinum anode, Knöppel[101] showed that the degree of fragmentation of a field-ionized molecule increases with source temperature. For aliphatic compounds, this increase is about one order of magnitude for an increase in anode temperature of 800°C. The FI mass spectra of aromatic compounds are less sensitive to changes in temperature.

The effect of temperature upon ionization efficiency is more subtle, and emerges most clearly from theoretical considerations such as those of Pokrovsky et al.[102] who have calculated the way FI probabilities may be expected to increase with temperature.

At higher anode temperatures, the reactions that follow FI will proceed more rapidly than at lower temperatures; under these conditions, rearrangements normally not observable in FI may be seen.[49,83]

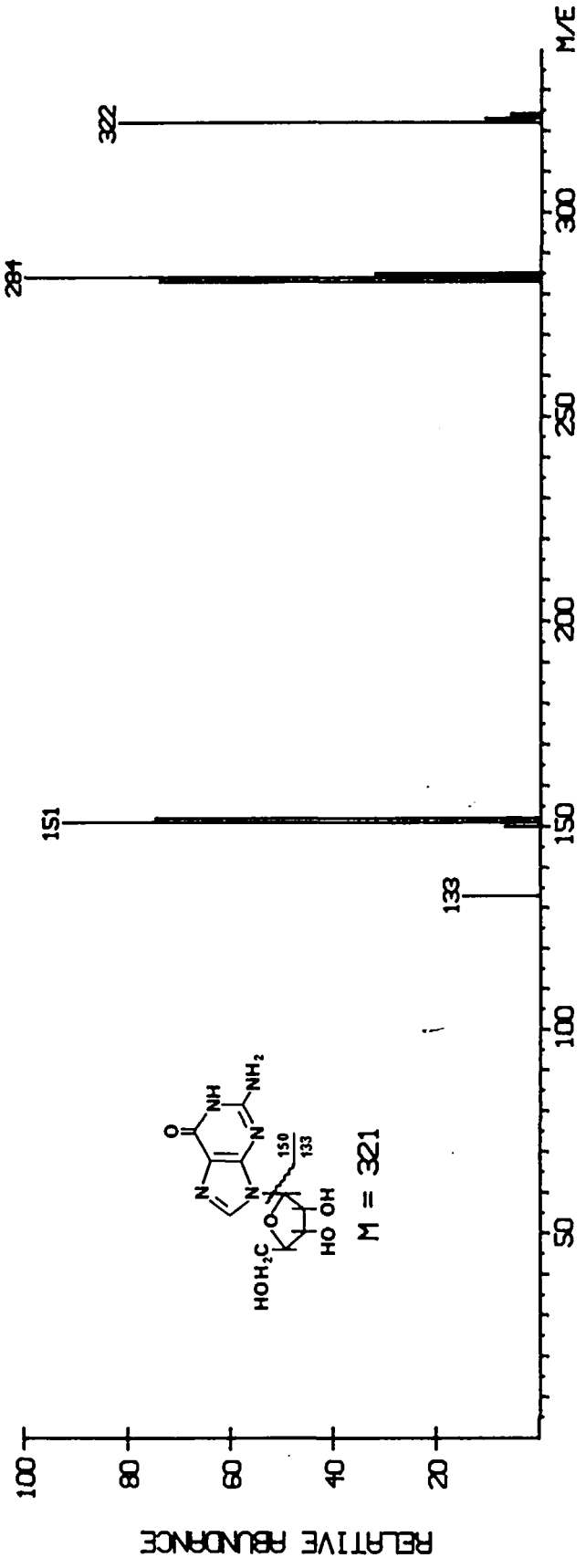


FIGURE 9. FD mass spectrum of the potassium salt of guanosine (molecular weight 321).[99]

Temperature effects on FD are not at all well understood. This is because at high anode temperatures pyrolysis of the neutral molecule on the heated anode has far larger effects than, and precedes, ionization by FD; the relatively subtle effects of temperature changes on the latter will therefore be difficult to detect.[87]

G. Metastable Ions Formed by Field Ionization

1. Types of Metastable Transition

An important difference between FI and EI is that in the former, full acceleration of ions is complete within 10^{-10} sec after their formation, as against 10^{-6} sec in EI. Thus the only fragment ions that will be seen as normal peaks are those formed during the FI process, or very rapidly after ionization.

Normal, symmetrical peaks will appear in the FI mass spectrum for $M^{+\cdot}$ or $(M + 1)^+$ ions and also for these rapidly formed fragment ions.[103] Some of the peaks at integral m/e values in the FI mass spectrum tail markedly towards lower mass values; these tails represent ions which are decomposing in the short time between formation and full acceleration. These ions are sometimes known as "fast metastables."

Such a tail may extend into the third type of peak, which is the broad metastable peak well-known from EI mass spectra. The contributors to this are ions formed after acceleration but before mass analysis; the peak appears on the mass scale at m/e (daughter^2/parent).

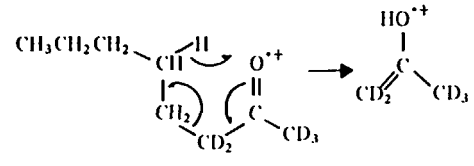
A fourth type of ion in FI mass specta, reported by Tou,[104] appears at an m/e value slightly below that of the daughter ion, and may be seen to tail towards lower mass values. Thus, in the loss of $C_2H_5^{\cdot}$ from the molecular ion $CH_3COC_2H_5^{+\cdot}$, $(72^{+\cdot} \rightarrow 43^+)$, there is a normal metastable peak at m/e $43^2/72 = 25.7$, but there is also a broad, skewed peak at m/e 42.4. Metastable peaks of this latter type are thought[104] to result from the decompositions of ions in the field-free region of the cylindrical focusing electrode.

A fifth type of peak, observed by Block,[105] appears at m/e values lower than the molecular weight of the material. Such peaks are thought to result from the formation of molecular ions in the source at positions remote from the anode.

Finally, the metastable peaks arising from the doubly charged ions formed in FI have been discussed.[106] Collapse of a doubly charged ion (M^{++}) to two singly charged ions $(M - m)^+$ and m^+ after acceleration and prior to mass analysis can give two metastables, at $2(M - m)^2/M$ and $2m^2/M$, respectively.[107] In addition to these there is observed,[106] when $m > M/2$, a tailing of the normal daughter ion towards higher mass values due to "fast metastable" formation.

2. Defocusing of Metastable Ions in FI Mass Spectrometry

A technique of metastable defocusing in an FI double focusing mass spectrometer has been developed.[108,109] If the magnet current is held at the appropriate value for a given fragment ion, and the voltage on the FI anode is swept, a plot of ion current against anode potential can be obtained. This can be mathematically transformed into a plot of ion current against parent-ion lifetime; it has been suggested that the technique as a whole be referred to as a field ionization kinetic (FIK) study. Using this method, detailed studies have been carried out[108,109] on some of the very rapid rearrangement reactions that occur in an FI source. Many of these reactions had previously been extensively studied by Beckey's group[100,110-114] using a single-focusing FI mass spectrometer. With a double-focusing mass spectrometer, the time scale of the reactions that can be studied is resolved further. Thus the FIK method permits study of ions with lifetimes of about 10^{-10} sec. Ions with still longer lifetimes can be studied via first field-free region metastables (10^{-7} to 10^{-6} sec) or second field-free region metastables (10^{-5} sec). By these methods, it was shown[108] that the McLafferty rearrangement of the labeled octanone (1) is very fast, producing the fragment ion of m/e 63. Deuterium



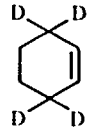
1 m/e 63

scrambling, followed by a McLafferty rearrangement, will lead to a fragment ion of m/e 62, but this is a slow process: it is not observed in ions whose lifetimes are less than 10^{-7} sec. Similarly, while loss of $CD_3^{\cdot}$ from 1 is rapid, loss of $CD_2H^{\cdot}$, which requires prior deuterium randomization, is not observed in times less than 10^{-7} sec.

The relative slowness of randomization implies that ions in which scrambling has occurred will not

be observed in normal FI mass spectra; this is indeed the case in the FI mass spectra of specifically deuterium-labeled heptanals.[115]

Randomization of the deuterium atoms in cyclohexene-3,3,6,6-d_4 (2), on the other hand, is quite fast[116] occurring in 10^{-9} to 10^{-11} sec, and may be postulated to occur by a series of allylic rearrangements. The similarity between the FI and the 12-eV EI mass spectra of the cyclohexenes is noted and



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is discussed further in Section V below.

In n-hexanol-3,3,-d_2 and n-hexanol-4,4-d_2, randomization is not observed at lifetimes of less than 10^{-9} sec.[117] Elimination of water is very much more rapid[118] and involves C_3 in a 5-membered ring transition state and C_4 in a 6-membered ring transition state with roughly equal probabilities.

These results help to clarify some of the earlier misunderstandings about rearrangement processes in FI. The hydrogen rearrangements that are observed in FI, such as the McLafferty rearrangement,[119] appear to be intermediate in rate between direct bond cleavage (10^{14}–10^{12} sec^{-1}) and skeletal rearrangements (10^{8}–10^{5} sec^{-1}).[103, 120] It has also been shown[121] that there are two types of McLafferty rearrangement in FI. The faster of the two (10^{11}–10^{9} sec^{-1}) is a concerted process and is entropy-preferred, while the slower (10^{6} sec^{-1}) proceeds by steps and is favored energetically.

H. Design of Field Ionization Mass Spectrometers

Focusing the ion beam is essential in a mass spectrometer and so the unfocused FI sources used in FI microscopy[122] will not be discussed here.

The problems of beam focusing in EI and CI sources are much simpler than those in a conventional FI source. As has been seen, the anode-cathode potential difference must be high for FI to be efficient. Since this is usually the primary accelerating voltage of the system, the kinetic energy of ions leaving the ion gun will be very high. This problem is further aggravated by the fact that ions leave a tip anode throughout a solid angle of some 120°[70] and it is extremely difficult to focus more than a small proportion of these ions into a useful beam.

In fact, the discrepancies in early sources between the theoretical ion current and that measured by monitoring the ion beam[123] revealed that loss of ions during transmission was the reason for much of the poor sensitivity attributed to FI. Goldenfeld and Nazarenko[124] have been able to account for the angular distribution of ions observed in an FI source using a platinum tip anode. They assumed that molecules arriving in the ionization region do so only from the gas phase,[125] although the supply of molecules diffusing along the surface of the anode may also be appreciable.[126] This supply by diffusion is invoked, for example, to explain the fact that maximum ion currents from compounds such as n-hexane are observed[70] at angles of about 40° to the optical axis of the FI source.

Fairly effective focusing of such a high-energy, divergent ion beam can be achieved by an electrostatic lens system. In the source designed by Beckey's group[127] for a single-focusing machine, the potential of the FI anode is about +5 kV, and the cathode is held at about −5 kV. Before reaching the entrance slit of the mass spectrometer, the beam is focused in the plane perpendicular to the magnetic field by a spherical lens. A second spherical lens, placed after the entrance slit, focuses the beam in the plane parallel to the magnetic field.

The focusing difficulties are more easily dealt with in a double-focusing mass spectrometer; McLafferty's group took advantage of this in designing an FI source for the CEC 21-110B.[83] In this case, the blade anode was at +10 kV, the cathode was at between −0.5 and −1.0 kV, and the one focusing slit was placed after the cathode and held at about +5 kV. This machine was clearly well focused as it delivered ion currents of 10^{-7} A/torr (measured at the total ion beam monitor), and a resolving power of 1 part in 20,000 (10% valley).

Other designs of FI sources for the 21-110B have been completed.[85,128] A computer simulation of the fields prevailing in one of these sources has also been published.[129]

Perhaps because of the focusing power available in a double-focusing mass spectrometer, FI sources have been designed for several other such machines, and design and/or performance details are available for FI sources designed for the AEI

MS-9,[130] the AEI MS-7,[131] and the Varian MAT SM-1.[132]

The single-focusing mass spectrometers for which FI sources have been built include the Varian-MAT CH4[76,132-134] and, in the Soviet Union, the MI-1305.[135]

Modification of the source of an EI mass spectrometer for FI seems in all cases to lead to a degradation of the resolving power of the mass spectrometer. The same phenomenon (stemming from different causes) has been observed after modification of an EI mass spectrometer for operation in the CI mode; this point will be discussed later. In both FI and CI modification, the loss of resolving power is not critical in double focusing machines, but it may render a single focusing mass spectrometer barely usable. Therefore, source focusing in such machines should be approached with some care.

In the earlier designs of FI sources,[131-134] the FI anode was mounted within the source and the sample could be admitted on a direct insertion probe. In recent years it has become clear that the ion current developed by an FI source depends upon the anode-cathode separation and, when the anode is an edge or a wire, upon its orientation with respect to the cathode slit.[83] Further, since anodes have a limited lifetime, easy replacement is an advantage.

For these reasons, it is now common practice[83,128,132] to introduce the FI anode into the source on a direct insertion probe and to adjust its position and angle to the cathode slit from outside the vacuum chamber.

Some of the sources mentioned above have been designed for exclusive use as FI sources[128, 131,134,135] while others have been designed as dual EI/FI sources[83,130,132] with varying degrees of success.

Details of the design, construction, and performance of an FD/FI source for use with the CEC 21-110B have been published,[96,99,136] and a coupled GC-FI mass spectrometer using a membrane interface has been described.[137]

I. Field Ionization and Field Desorption Mass Spectra

Field ionization has been promoted as a method of obtaining mass spectra in which the molecular ion, M^+ (or $(MH)^+$) is very likely to be present. In fact, this is by far the best known property of FI mass spectra. If a molecule can be vaporized unchanged, it can then be ionized by FI with an increase of less than 2 eV in its internal energy. As a consequence, the complete breakdown of a molecule by FI alone is very rare. Many groups have sought to exploit this feature of FI, which promises to lead to an infallible method of molecular-weight and molecular-formula determination. The nature of the reactions that take place following FI has received less attention until quite recently, when detailed studies of the kinetics of FI-induced fragmentation began to appear in the literature. These were referred to in the preceding Section. In this Section some of the FI and FD mass spectra that have been published recently will be discussed.

1. Hydrocarbons

Normal alkanes all give $M^{+\cdot}$ as the base peak in their FI mass spectra.[138] In all such spectra, the only fragment ion is $C_2H_5^+$, the intensity of which is approximately 90% of the base peak, and which is thought[139] to be formed by field-induced dissociation of the C_2-C_3 bond of the alkane aligned along lines of force near the FI anode. Support for this view is found in the work of Goldenfeld and Korostyshevsky,[140] who showed that, in n-alkanes labeled with ^2H and ^{13}C, FI led to C-C bond cleavage without any observable scrambling. Clearly, the breaking of the C-C bond by field-induced dissociation is very rapid compared to hydrogen or skeletal reorganization.

Branched hydrocarbons are more susceptible to C-C bond cleavage,[51,134] but even for neopentane[151] there is a clearly detectable $M^{+\cdot}$ ion (relative intensity 8%). The FD mass spectrum of neopentane, if run under carefully controlled conditions, has the molecular ion as the base peak.[95] Analysis of hydrocarbon mixtures by FI mass spectrometry has been attempted[130,141] with some success.

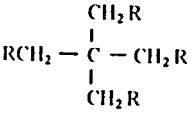
One of the earliest comparisons of FI and CI was that carried out by Beckey[142,143] who, using the isomeric decanes, found the relative intensity for the $M^{+\cdot}$ ion formed in the FI mass spectrum to be generally greater than that for the $(M-H)^+$ ion in the CI mass spectrum. This result is valid for hydrocarbons but, as in discussed later, is probably not generally true, particularly if CI mass spectra with, for example, isobutane reagent gas are considered.

2. Esters

Simple esters give intense $M^{+\cdot}$ ions upon FI.[85] It has also been observed[144] that the C-terminal methyl ester of peptide methyl esters survives FI, and Tou[145] has shown the $M^{+\cdot}$ ion in the FI mass spectra of esters of phthalic acid to be very intense. This last observation stands in contrast to the EI mass spectra of phthalates[145] in which the relative abundance of the $M^{+\cdot}$ ion is generally less than 1% of the base peak at m/e 149. An ion at m/e $(M + 1)^+$ is also found in the FI mass spectra of the phthalate esters. This is thought to be formed by an intermolecular proton transfer in the condensed phase on the surface of the FI anode. Ions of m/e 148 and/or 149 are found in the FI mass spectra of all the phthalate esters studied, including, interestingly, those of two terephthalate esters.

3. Polyhydric Alcohols

Pentaerythritol (3, R = OH) and its various corresponding alkyl bromides (3, R = OH or Br) all give FI mass spectra consisting almost entirely of an intense $M^{+\cdot}$ or $(M + H)^+$ ion,[148] and an ion of m/e 31. The high explosive pentaerythritol tetranitrate



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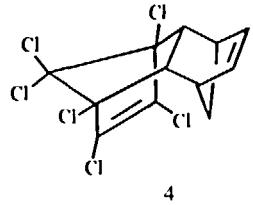
(PETN, 3, R = ONO_2), on the other hand, gives an $M^{+\cdot}$ ion of very low abundance in its FI mass spectrum[132] while somewhat surprisingly, the $(M + H)^+$ ion in its CI(H_2) mass spectrum[147] has a relative intensity of 75%.

4. Pesticides

Chlorinated insecticides such as aldrin (4), dieldrin (5), and other related compounds give $M^{+\cdot}$ ions in their EI mass spectra,[148] but these are of limited intensity. Field ionization,[149] a far superior method for the ionization of such compounds, gives the $M^{+\cdot}$ ions as the base peaks in the spectra of aldrin and dieldrin. In either case, the only important fragment ion is that corresponding to $(M - HCl)^+$. The intensity of this fragment ion is considerably higher for dieldrin (5) and its stereoisomer endrin than it is for aldrin (4) and its stereoisomer isodrin.



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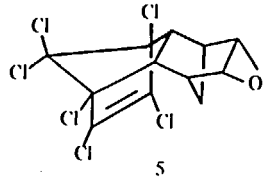

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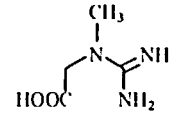
The FD mass spectra of these compounds and some of their biodegradation products consist, for the most part, of just the $M^{+\cdot}$ ion.[97,150] The *cis* and *trans* diols from aldrin lose water to different extents upon FD and thus may be distinguishable from each other.

5. Amino Acids

The EI mass spectra of free amino acids do not contain intense molecular ions.[151] This problem can be overcome for most of the natural amino acids by the use of CI,[152,153] which gives intense protonated molecular ions from all amino acids except cystine and arginine. No systematic study of the FI mass spectra of amino acids has been published, although data for glutamic acid[96] and proline, serine, and aspartic acid[154] reveal that, of these, only the last has no $(M + 1)^+$ ion in its FI mass spectrum, the ion of m/e (M - 17) being the most intense ion in the spectrum.

Of the ten amino acids whose FD mass spectra have been studied,[154] eight give the $(M + H)^+$ ion as the base peak in the spectrum; the only fragment ion in all cases is formed by loss of $COOH_2$ from the $(M + H)^+$ ion. The remaining two compounds, arginine and cystine, both give intense $(M + H)^+$ ions upon FD. The ion from arginine (relative intensity 27%) collapses by loss of NH_3 or the complete guanidine unit. The $(M + H)^+$ ion from cystine, as shown in Figure 10, loses H_2O, or $COOH_2$, or breaks down by cleavage of the S-S bond.

The FD mass spectrum of creatine (6) has been reported.[98] With electrical heating of the anode, as is normal in FD work, the $(M + H)^+$ ion appears with a relative abundance of 38%. Use of infrared heating of the anode, on the other hand, gives an FD mass spectrum in which the $(M + H)^+$ ion is the



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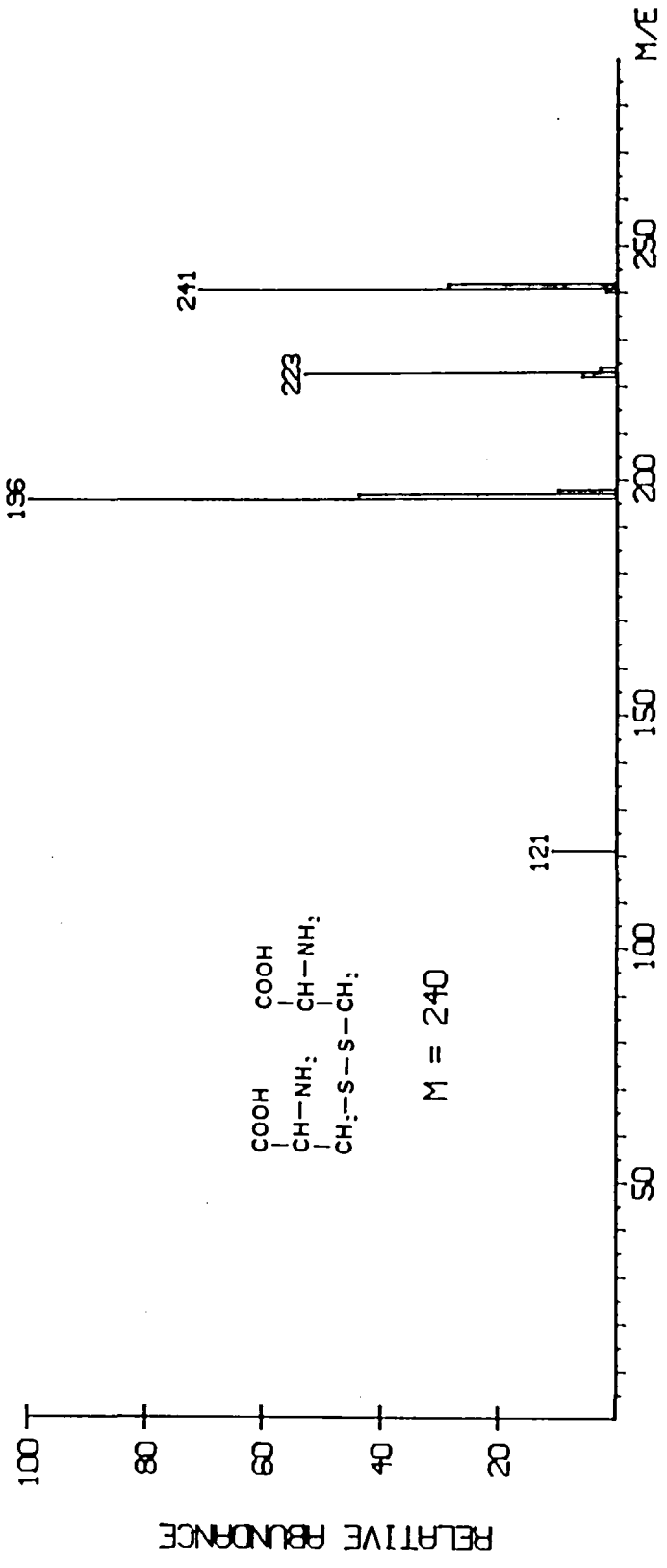
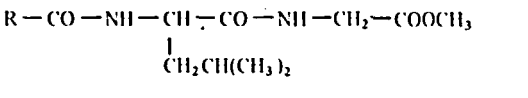


FIGURE 10. FD mass spectrum of cystine (molecular weight 240).[154]

base peak. Presumably, this method of heating would be very useful for blade anodes, which are difficult to heat resistively.

6. Peptides

The FI mass spectra of some benzyloxy- and *tert* butyloxy-carbonyl peptide methyl esters (e.g., 7 and 8) were reported in 1970 by Brown and Pettit.[144] Seven spectra were published, three of



7. R = $C_6H_5CH_2O$ (Z-Leu-Gly-OMe)

8. R = $(CH_3)_3C-O$ (tBOC-Leu-Gly-OMe)

dipeptides, two of tripeptides, and one each of a tetra- and a pentapeptide. Amino acids known to cause difficulties in mass spectrometric sequencing work were, with the exception of serine and threonine, absent from the peptides studied. In the FI mass spectra, the $M^{+\cdot}$ ions appear with far greater intensity than in the corresponding EI mass spectra. On the other hand, the intensities of the fragment ions containing sequence information are generally higher in the EI mass spectra. Similar conclusions can be drawn from the FI mass spectral data on peptides published by Winkler and Beckey.[155]

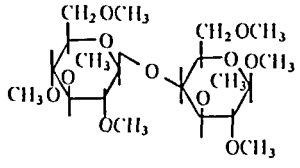
In peptide FD mass spectra[155] the amount of sequence information is decreased still further, but the intensity of the molecular ion is very high and, most importantly, appears to be rather independent of the identity of the constituent amino acids. A further major point is that derivatization of the polar termini appears to be unnecessary if FD is used.

These two observations are very significant and the lack of sequence ions might be corrected by collisional activation[34] of the $M^{+\cdot}$ ion so formed.

7. Carbohydrates

A natural target for techniques such as FI and FD is the carbohydrate molecule which, in general, gives unsatisfactory EI mass spectra. In an early report from Beckey's group,[156] monosaccharides such as fructose, xylose, and glucose were shown to give FI mass spectra in which the base peak is at m/e (M + 1). The hydrogen transfer takes place on the surface of the FI anode and is a particularly important process in polar molecules such as these.

Underivatized disaccharides fail to give intense $M^{+\cdot}$ or $(M + 1)^+$ ions, but permethylated compounds such as octamethyl cellobiose (9) give FI mass



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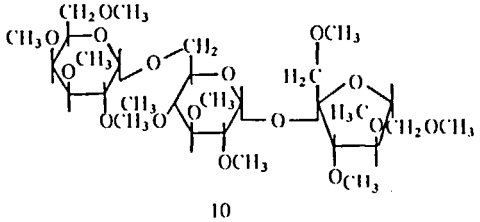
spectra in which the $M^{+\cdot}$ (m/e 454) is very intense (90% of the base peak). In the case of 9, the base peak is at m/e 235. The identity of the monosaccharide moieties can be deduced from such spectra.

The FI mass spectra of permethylated and peracetylated monosaccharides[157] usually contain the $M^{+\cdot}$ ion as the base peak although the ion at m/e (M + 1) is often still quite intense in spite of the absence of hydroxyl groups. Permethylated trisaccharides such as raffinose (10) give FI mass



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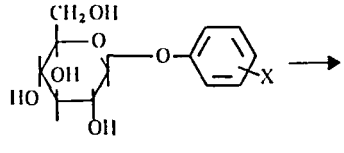
spectra (Figure 11) with prominent (relative intensity 15%) $M^{+\cdot}$ ions and some useful, but incomplete, sequence information.

Aryl-O-glucosides such as 11 fragment upon FI to give 12 and/or 13 in proportions which



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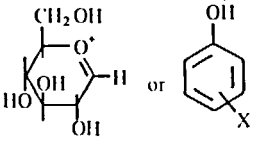


12 13

depend[158] upon the nature of the substituent X in a Hammett relationship. Details of the Hammett correlation were provided in a subsequent paper.[159]

The aglycone of the antibiotic tuliposide B (14)

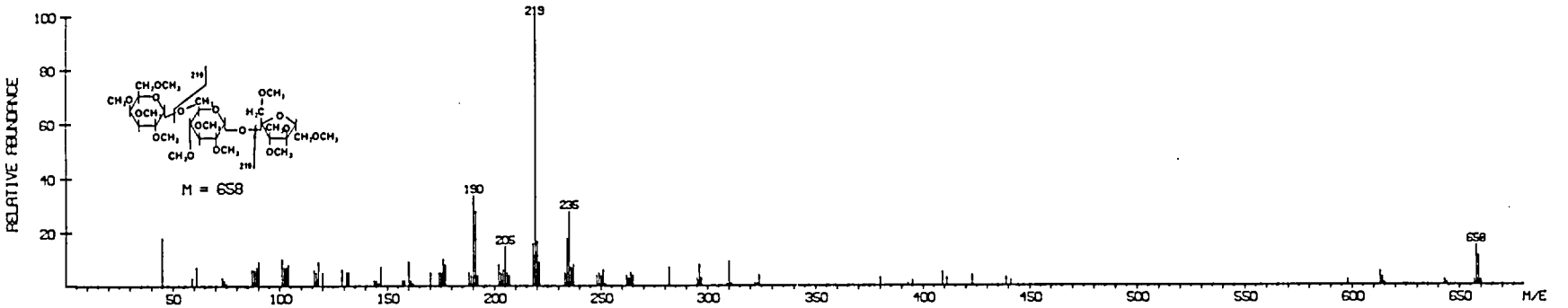
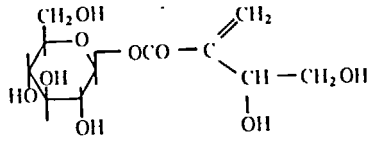


FIGURE 11. FI mass spectrum of permethylated raffinose (molecular weight 658).[157]



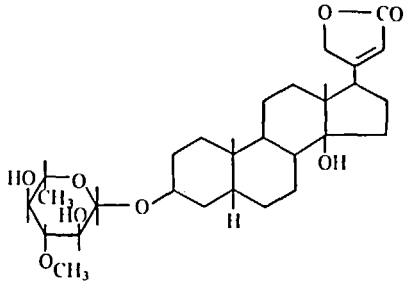
14

was found by FI mass spectrometry[160] to have a molecular weight of 114, rather than of 96 as had been concluded from the EI mass spectrum.

The FD mass spectra of sugars are very interesting. In contrast to its FI and especially its EI mass spectrum, the FD mass spectrum of free d-glucose[57] (Figure 12) shows almost no fragmentation; the base peak is at m/e (M + 1) and the only two fragment ions, at m/e (M + 1 - 18) and (M + 1 - 36), both have relative abundances less than 5%. The FD mass spectrum of free cellobiose[157] has a base peak at m/e (M + 1) and rather more fragmentation.

8. Carbohydrate Derivatives

A systematic study of the FI mass spectra of the naturally occurring steroidal glycosides or cardenolides has been completed.[161,162] The simpler compounds in this series, such as neriifolin (15), give satisfactory $M^{+\cdot}$ ions, but as the number



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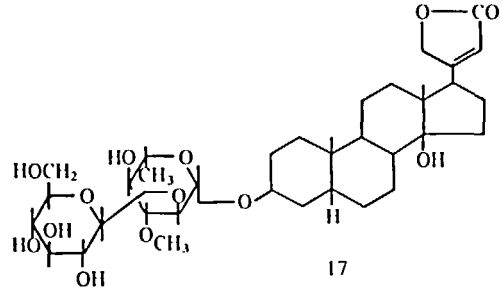
of sugar residues increases, the intensity of the molecular ion drops sharply. Compounds such as cerberoside (16) appear to have no molecular ion.

A further difficulty is that some of these compounds give $M^{+\cdot}$ ions while others, unpredictably, give $(M + 1)^+$ ions instead. In an analytical situation, if nitrogen is known to be absent from the molecule, the molecular weight will have to be an even number; distinguishing between the $M^{+\cdot}$ and $(M + 1)^+$ ions will therefore be simple. In many cases, however, confusion as to the molecular weight of an unknown may still be possible; Beckey's rule,[163] that ions of m/e (M - 1) are always absent, clearly cannot be applied with complete success to the FI mass spectra of the cardenolides.[162]

These difficulties notwithstanding, the FI mass spectra of cardenolides can only be described as very successful. As the FI mass spectrum of thevebioside shows (17, Figure 13), the correct

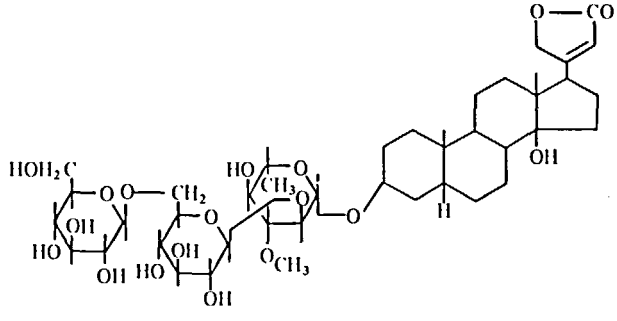


17

molecular weights of these compounds are frequently provided by the method, which would also appear to be the only one by which the sequence of the carbohydrate residues can be determined in small amounts of such compounds.

Two problems in carbohydrate chemistry that EI and CI mass spectrometry have so far failed to solve are (i) identification of stereoisomers; and (ii) determination and identification of sugar phosphates.

With respect to the first of these, Lehmann et al.[164] have reported that there are quantitative differences between the FD mass spectra of α- and β-d-glucopyranosides.



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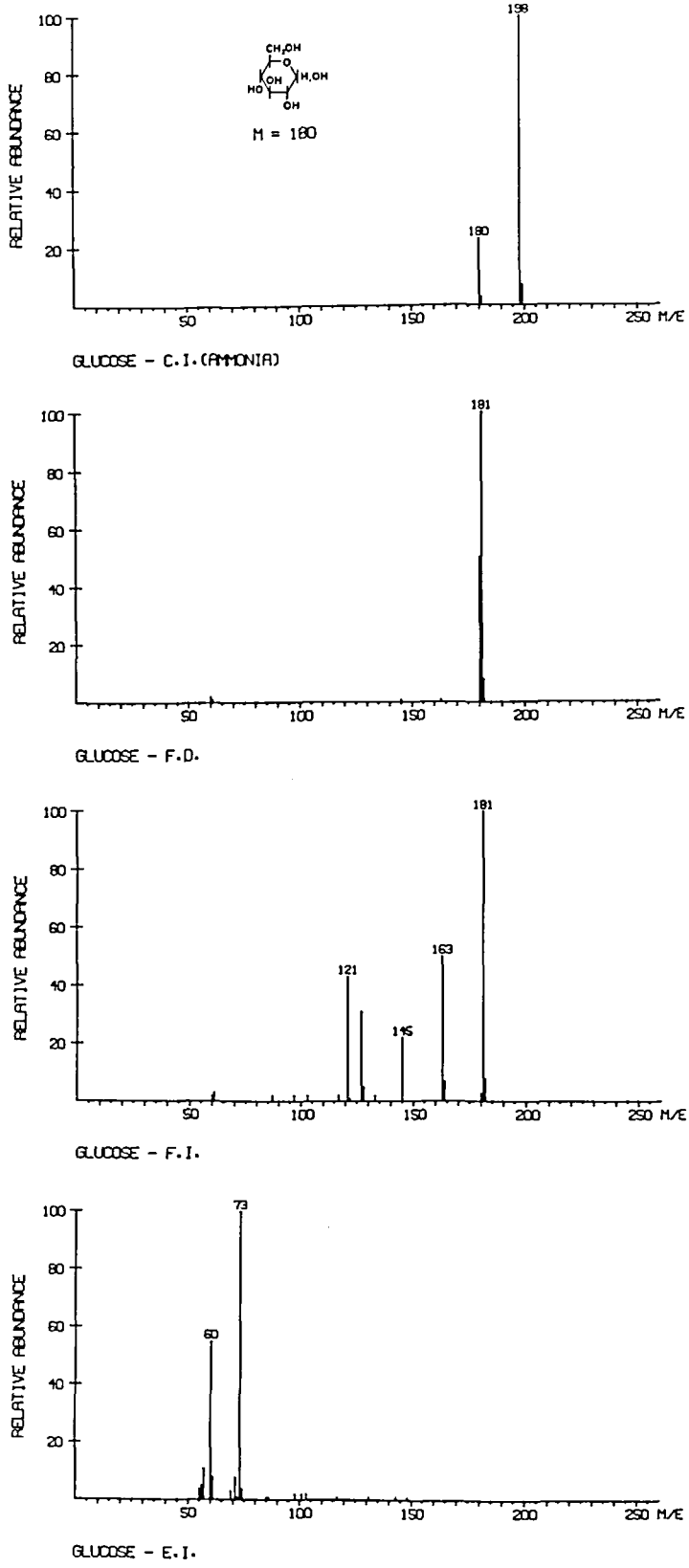


FIGURE 12. EI, FI, FD and CI(NH_4) mass spectra of *d*-Glucose (molecular weight 180).[57,284]

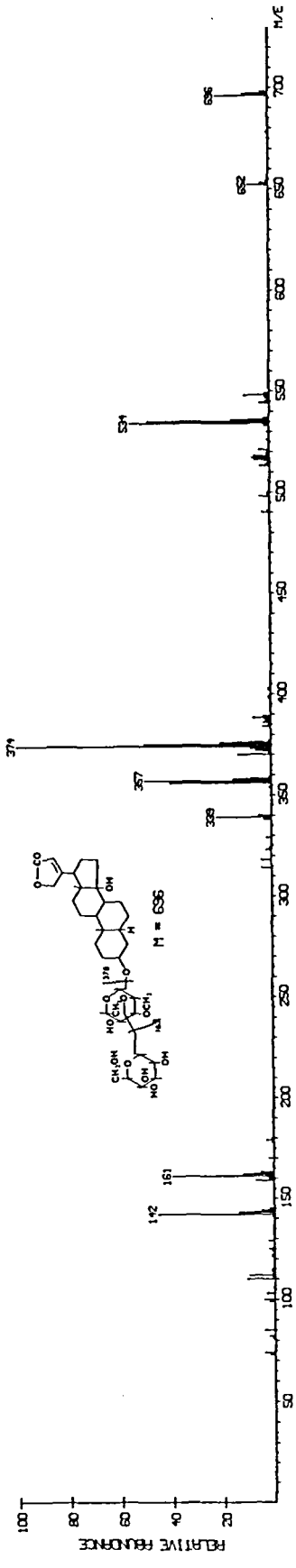
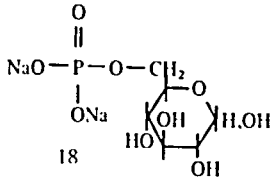


FIGURE 13. FI mass spectrum of thevebioside (molecular weight 696).[162]

Perhaps of more importance to biochemistry is the demonstration[165] that compounds such as D-glucose-6-phosphate disodium salt (18) give, as



18

the base peak in the FD mass spectrum, the intact (protonated) molecule, $C_6H_{12}Na_2O_9P^+$, at m/e 305.

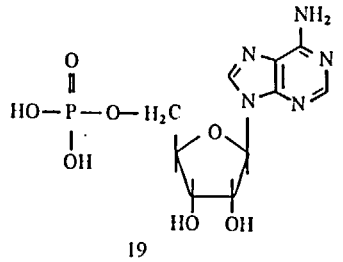
9. Nucleosides and Nucleotides

Like free carbohydrates, nucleosides frequently fail to give intense molecular-ion peaks in their EI mass spectra. This is due mainly to the instability of the bond between C_1 of the sugar and the base. Brown et al.,[166] in a study of the FI mass spectra of nucleosides, showed that of the ten compounds studied, only guanosine fails to give an $M^{+\cdot}$ or an $(M + 1)^+$ ion. All nucleosides give two major fragment ions in their FI mass spectra: one corresponds to $(\text{sugar} - OH)^+$ and occurs, for example, at m/e 133 for ribose or 117 for 2′-deoxyribose; the other corresponds to $(\text{base} + nH)^+$, where n = 1 or 2. The base peak of the spectrum is usually either the molecular ion or the fragment ion associated with the base. It was suggested that guanosine could not be vaporized unchanged and, in support of this, it was shown that *N,N*-dimethyl guanosine, which can be vaporized at 230° as against 250° for guanosine, gives an FI mass spectrum with an $(M + 1)^+$ ion of relative intensity 10%.

The FD mass spectra of nucleosides[99] are similar to the FI mass spectra, but they involve less nonspecific (presumably thermal) fragmentation. The molecular ions have a higher relative abundance. Under these conditions, guanosine gives a molecular ion with relative intensity of 20%.

The free bases themselves give only molecular ions ($M^{+\cdot}$) upon FI.[87] Both the free bases and the nucleosides, if adsorbed onto the activated anode from a KOH solution,[99] give, upon FD, a molecular ion of the protonated potassium salt [that is, at m/e (M + 39)].

With nucleotides, FD is quite unrivaled as an ionization technique for mass spectrometry; in an area like this the power of FD in biochemical analysis becomes very clear. Adenosine-5′-monophosphate (AMP, 19) gives an FD mass spectrum (Figure 14) whose base peak corresponds to $(\text{base} + H)^+$ at m/e 136. The ion at m/e (M + 1) has a relative intensity of about 75%. Loss from this ion of the phosphate residue gives an ion of relative abundance 30%, corresponding to the protonated nucleoside. The only other ions in the mass spectrum are at m/e 99 ($H_4PO_4^+$, 35%) and m/e 60 and 61 [$(CHOH)_2^+$ and $H(CHOH)_2^+$, respectively].



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As a logical continuation of this work Schulten and Beckey, in collaboration with Boerboom and Meuzelaar,[167] have studied the pyrolysis products of DNA by FD mass spectrometry. In this work the DNA was pyrolyzed in the mass spectrometer on an FI anode previously activated at high temperatures. The ions produced during pyrolysis and FD at increasing anode temperatures were recorded on a photoplate at high mass spectrometer resolving power. Essentially, no ions with m/e < 112 were observed, but some intense peaks appeared between m/e 200 and m/e 400. The bases adenine, guanine, cytosine, thymine, and methylcytosine were all detected as their $(M + 1)^+$ ions; twelve phosphorus-containing ions were found in which there was no carbon. The appropriate nucleoside $(M + 1)^+$ ions were present; most interestingly, there were detected ions corresponding to mononucleotides and a few dinucleotides.

The data reported are complex and as they stand will probably lead to no more than limited structural information. The importance of the experiment is, however, clear; this is the first instance in which the power of mass spectrometry has been effectively brought to bear upon the nettlesome problem of primary structure determination of polynucleotides.

Some related work involves the pyrolysis products of bacteria.[168] Many attempts have been made[169-171] to classify bacteria on the basis of their pyrolysis products, but gas chromatography, with or without coupled EI mass spectrometry,

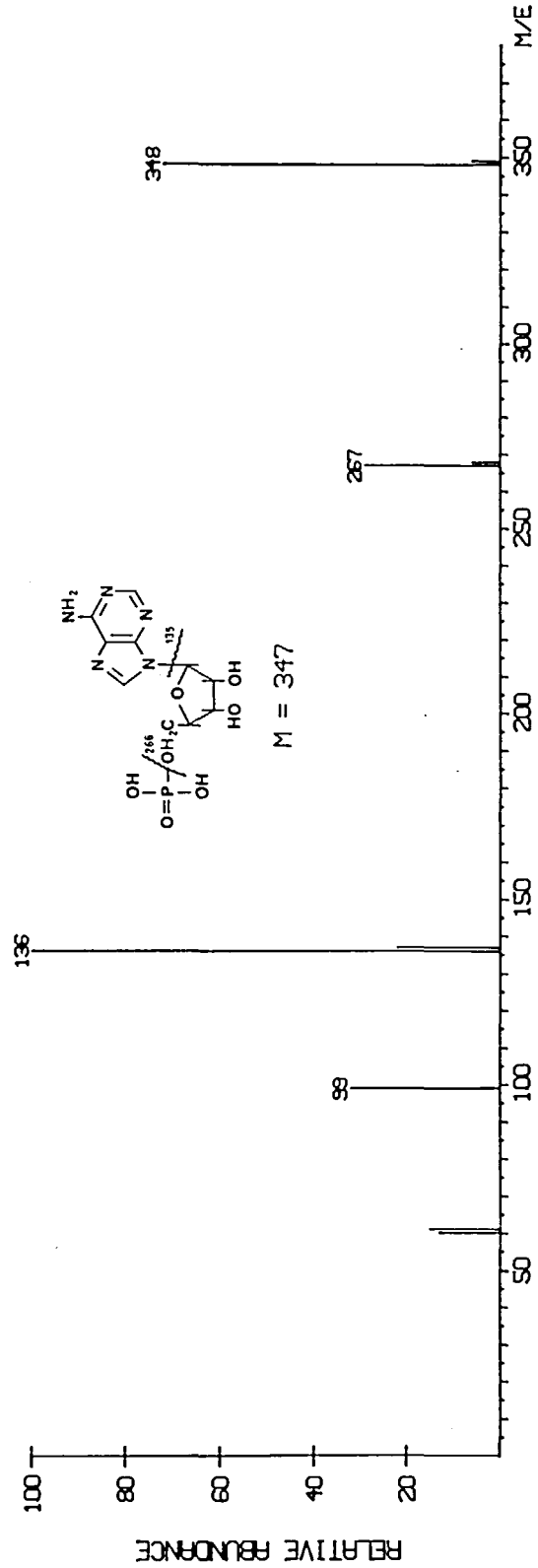

FIGURE 14. FD mass spectrum of adenosine-5'-monophosphate (molecular weight 347).[99]

gave such complicated results that classification was not possible. The volatile pyrolysis products of *Pseudomonas putida* were collected and examined by high-resolution FI mass spectrometry. Some 200 ions were identified and almost all were found to correspond to ions previously observed[172,173] in the pyrolysis-gas chromatography-mass spectrometry system devised by Simmonds.[173]

IV. CHEMICAL IONIZATION

A. Introduction

Much earlier work had been done on various aspects of ion-molecule reactions, but the work of Field and Munson during the sixties[174] is generally regarded as having laid the basis upon which chemical ionization (CI) mass spectrometry now rests.

While the bulk of the developmental work in FI was carried out in Europe, CI can fairly be regarded as a largely American development. An immediate consequence of this is that there are few references beyond this point to journals such as *Zeitschrift für Naturforschung*. In CI, many of the major papers are instead found in *Analytical Chemistry* and the *Journal of the American Chemical Society*.

A number of excellent reviews of CI mass spectrometry are available.[1,2,174-182] Almost all of them are concerned with the application of CI mass spectrometry to organic analysis, but the articles written by Field reflect a physicochemical view of the process of CI.

b. Ion-molecule Reactions

1. General Considerations

The reaction of an ion with a neutral molecule is, in principle, a very efficient method of ionizing the latter. Gas-phase ion-molecule reactions are among the fastest of elementary processes;[183] rate constants are frequently so large (e.g., 10^{-9} cm^3 mol^{-1} sec^{-1}) that there is a high probability of a reaction at each collision. This is considered a consequence of the formation of long-lived collision complexes, as a result of long-range dipolar interactions and possible orbiting trajectories of the reactants at low relative translational energies.[183,184]

2. Ion-molecule Reactions Under EI and FI Conditions

Significant ion-molecule reactions occur with some compounds at the low pressures of an EI source. In some of the earliest mass spectra ever recorded,[185] an ion at m/e 3 was observed and it was decided by a process of elimination that it must have been H_3^+. In retrospect, it seems likely that it was formed in an ion-molecule reaction. In a low pressure EI source, there is a small possibility that an ion may collide and react with a neutral molecule during the maximum residence time of the ion in the source (about 10^{-6} sec). Protonated molecular ions, $(M + H)^+$, are invariably the only secondary ions of significant abundance; they can usually be distinguished from the normal ions formed by EI in that their relative intensity is pressure dependent.

The even-electron $(M + H)^+$ ions are nearly always more resistant to fragmentation than odd-electron $M^{+\cdot}$ ions. McLafferty,[186] Beynon,[187] and Biemann[188] pointed out more than ten years ago that valuable molecular weight information could be obtained by either increasing sample pressure in the source or increasing source residence times by electrical adjustments. Ions whose relative intensities are increased by either of these measures are characterized as $(M + H)^+$ ions; a reliable value for M follows from this.

Ion-molecule reactions can be much more important in an FI source than in an EI source despite the much shorter source residence times in the former case. Thus, polar molecules containing labile hydrogen atoms can give rise to abundant protonated molecular ions, $(M + H)^+$, as well as, or instead of, molecular ions, $M^{+\cdot}$. The protonated molecular ions are formed by reactions in the adsorbed layers on the anode surface rather than in the gas phase. Hydride abstraction only occasionally occurs in the condensed phase or by field-induced fragmentation. So $(M - H)^+$ ions are not often observed. Beckey has proposed as a general rule[163] that the lower in mass of two peaks in the molecular ion region of an FI mass spectrum may be assumed to be the $M^{+\cdot}$ ion. Therefore, the other peak, 1 amu higher in mass, is the $(M + H)^+$ ion; there is no ambiguity as to the molecular weight. When only one ion is observed in this region, it will not be immediately apparent whether it is the $M^{+\cdot}$ or the $(M + H)^+$ species (cf. Section III-I-8).

3. Collision-induced Ion Reactions

If ions are accelerated from an EI source into a pressurized drift region, at 10^{-6} to 10^{-4} torr, ion decompositions resulting from collisions can be observed in the field-free regions of the mass spectrometer. Mass analysis of the products of these ion-molecule reactions can assist in structural differentiation, provide new information on ion structures and fragmentation pathways, and help in the analysis of the mass spectra of mixtures.

Some of the high kinetic energy of the ion may be converted to internal energy, and dissociation of the ion may result. Mass analysis of the fragment ions formed in this way provides the collisional activation (CA) mass spectra referred to in Section II-H above. These spectra are virtually independent of the internal energy of the precursor ion.[33,189,190] They are unlike unimolecular, metastable ion mass spectra[190] and are more reliable in distinguishing ion structures.

Collisional activation mass spectra have been obtained of protonated molecular ions formed by increasing the sample pressure in an EI source.[190] It is quite possible that the $(M + H)^+$ ions formed in a CI source are equally suitable for such studies.[191]

If the magnetic sector of a double focusing mass spectrometer is placed before, rather than after, the electrostatic analyzer, then the metastable ion spectra of selected precursor ions decomposing between the two sectors can be readily obtained.[192] Collisional activation spectra measured on such an instrument have been used[190] for the differentiation of the isomeric ions $C_nH_{2n+1}O^+$ found in the mass spectra of aliphatic alcohols and others.

Other ion-molecule reactions occurring in field-free drift regions have been used by Beynon et al.[193-195] to generate new types of mass spectra. These spectra may also have considerable potential in ion structure determination, particularly for doubly charged and negative ions.

A magnetic sector scan at half the normal electrostatic sector voltage produces a spectrum of doubly charged ions formed from singly charged ions of the same mass in collisions with neutral molecules in the first field-free region.[193] Alternatively, if the electrostatic sector voltage is held at twice its normal value, then a scan of the magnetic sector will give a spectrum of singly charged ions that have been formed from doubly charged ions by a similar process.[194] Finally, the mass spectrum obtained by scanning of the magnet, after reversing the polarity of the electrostatic sector plates and the magnet, is that of the negative ions formed by collision of positive ions with neutral molecules in the first field-free region.[195]

4. Charge Exchange

Electron transfer from a sample molecule to a reagent ion leads to ionization of the former by the process known as charge exchange. The excess internal energy of a molecular ion $M^{+\cdot}$ so formed at low relative translational energies is determined by the difference between the recombination energy of the reagent ion and the ionization potential of the sample molecule.[196,197] Suitable reagent ions are those derived from inert species: gases, such as nitrogen, carbon monoxide, and the noble gases. Inspection of their recombination energies[196,197] reveals that the amount of fragmentation of a molecular ion formed in this way should decrease in the order:

$$He^{+\cdot} > Ne^{+\cdot} > Ar^{+\cdot}, N_2^{+\cdot} > Kr^{+\cdot} > Xe^{+\cdot}$$

If the reactions are carried out in a high-pressure source at about one torr, then the reagent ions should have near-thermal energies.[196] This cannot be easily achieved in beam experiments. Results obtained under these conditions by various groups have confirmed that common fragmentation modes are followed by the molecular ions formed in charge exchange and in EI. However, the degrees of fragmentation observed in the former case depend on the choice of reagent gas. In practice, the spectra obtained using nitrogen and argon (which have recombination energies of approximately 15 to 16 eV) as charge-exchange gases are quite similar to those obtained by 70-eV EI in which the total energy transfer is about 15 eV.[198] The appearance in charge-exchange mass spectra of $(M + H)^+$ rather than $M^{+\cdot}$ ions[2] can be satisfactorily explained on the basis of reactions between sample ($M^{+\cdot}$) ions formed by charge exchange and sample molecules. This process should become more important with increasing partial pressure of the sample, as is in fact observed.

5. Chemical Ionization

Reactions between sample molecules and low velocity reagent ions that result in the ionization

of the former by transfer of a charged species other than an electron are generally referred to as chemical ionization (CI). They are discussed in detail in Section IV-C below. By far the most commonly used charged species is the proton, but others, such as NO^+[199] and $(CH_3)_3Si^+$,[200] have been used.

The CI mass spectrum is determined then by the fate of the primary reaction products over a defined time interval. The primary reactions between reagent ions and sample molecules can lead to transfer of relatively massive particles such as H^+, H^-, or carbonium ions. If the reagent ions are even-electron ions, as is usually the case, then the ions produced by these CI processes will also be even-electron in character. As a result, they tend to fragment by routes different from those followed by the odd-electron ions formed in EI and FI mass spectrometry.

C. Chemical Ionization

1. Chemical Ionization at Low Pressures

Ion residence times in an ion cyclotron resonance (ICR) mass spectrometer are of milliseconds; the ion-molecule reactions that constitute CI can be carried out quite efficiently at relatively low pressures in such a system. At such low pressures (about 10^{-4} torr) there is a wider choice of reagent ions than at higher pressures. This permits greater latitude in the application of selective ion-molecule reactions to reveal subtle differences in the structures of sample molecules.[201]

Reaction pathways of selected reagent ions with molecules can be determined by the techniques of double resonance and ion ejection in an ICR mass spectrometer. The double resonance technique depends upon the principle that changes in the abundance of a daughter ion will occur if the appropriate parent ion is irradiated at its cyclotron resonance frequency. This is because the rates of ion-molecule reactions are proportional to the kinetic energy of the reactants and such rates can be increased by irradiation. Alternatively, the putative parent ion can be ejected from the ICR cell by increasing the amplitude of the irradiating field. Any other ions whose intensity decreases as the ion is ejected may be identified as its daughter ions.

Ion-molecule reactions in the ICR mass spectrometer have been used[202] to distinguish between isomeric hydrocarbons which give nearly identical EI mass spectra. Bursey and Hoffman have shown[203] that acetyl ions have a greater reactivity with the *exo*- than with the *endo*-isomer of norborneol, as is the case in solution. Acetyl-transfer reactions are also of use[204] in the selective CI of functional groups.

ICR studies of the CI mass spectra of various C_6 hydrocarbons with the reagent ions derived from methane have been compared[205] with the CI mass spectra of the same compounds obtained at high pressures (one torr) of methane. A few of the product ions in these CI mass spectra are formed almost exclusively by CI with CH_5^+, but most of the products arise from reaction of the sample molecules with either CH_5^+ or $C_2H_5^+$.

A second method of studying CI at low pressures is by the use of a tandem mass spectrometer. The first of the two mass spectrometers is used to generate a beam of a given type of ion, such as H_3O^+. This beam is decelerated and allowed to enter the source chamber of the second mass spectrometer, where ion-molecule reactions with a sample molecule can take place. There is thus considerable control possible over the identity and the kinetic energy of the reagent ions; it has been shown[206] that, at low kinetic energies, the mass spectra derived from isomeric hydrocarbons show significant differences. The degree of fragmentation observed depends upon the amount of energy available in the reagent ions. Friedman's group[207] has shown that the amount of energy associated with the hydrated proton depends upon the number of molecules of water incorporated into the species. With a tandem mass spectrometer it is a simple matter to select a particular reagent ion and use it to produce the required amount of fragmentation of the sample molecule.

A third method of conducting CI at low pressures is by use of ion trapping in an EI source. In the system described by Blair and Harrison,[208] positive ions produced by EI in a pulsed source are trapped in the negative space charge of a continuous electron beam. Measurement of the delay times between ionizing and withdrawing pulses give the reaction times. Alternatively, in the method described by Bonner et al.,[209] ions are formed by EI in a three-dimensional quadrupole storage trap. After a predetermined time, on the order of milliseconds, the resultant ions are ejected into a mass filter and analyzed, giving CI mass spectra at source pressures of 2×10^{-4} torr[209] similar to those obtained by other means at source pressures of one torr (cf. following section).

2. Chemical Ionization at High Pressures

In 1966, Munson and Field[43] showed that, with the appropriate design, a high-pressure source could be incorporated into a conventional low-pressure mass spectrometer and used to generate CI mass spectra. This approach, which is simpler than the tandem mass spectrometers or cyclotron devices, has in subsequent years become by far the most popular means of obtaining CI mass spectra. As is discussed later, it is already the basis of several successful commercial designs.

In high-pressure CI mass spectrometry, ion-molecule reactions in a reagent gas at pressures of about one torr produce a set of "reagent ions." These reagent ions are essentially inert toward reagent molecules under the conditions of temperature and pressure that pertain in the source. They are, however, frequently extremely reactive towards molecules of the sample, which is admitted to the same source with a partial pressure less than 1% of that of the reagent gas. The reagent gas is ionized by EI, but at these partial pressures, EI of the sample molecule can, on a statistical basis, be ignored.

These are five essential requirements for conversion of a standard EI source to a source capable of operation at high pressures, as shown in Figure 15:

1. An ion chamber must be constructed that can be operated at 1 torr while the pressure in the remainder of the source is at about 10^{-4} torr. For this chamber to be sufficiently "gas-tight," the two holes in its walls, the electron entrance aperture and the ion exit slit, must be very small; their combined area should be 1 to 2 mm^2.
2. A high capacity vacuum pump coupled with a large manifold to the source housing is necessary to keep the pressure in this chamber below 10^{-4} torr. At pressures above this, discharges become a problem and ion-beam formation is made difficult by scattering.
3. The analyzer of the mass spectrometer should be pumped differentially with respect to the source.
4. The filament, which is in the low-pressure region outside the ion chamber, is usually operated at relatively high voltages (that is, above 100 V) to ensure sufficient electron penetration

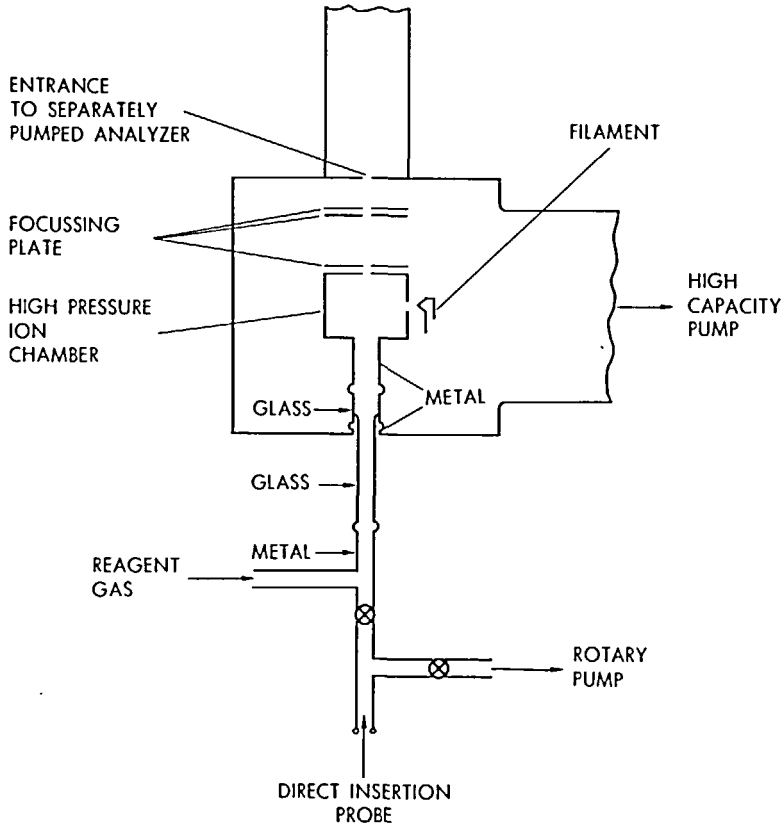

FIGURE 15. Schematic diagram of a CI source.

into the high-pressure region. As very few electrons penetrate very far into the source, the filament current is usually total-emission controlled. The replacement of a heated filament with radioactive isotopes that emit electrons with very high energies has been most successful with high-pressure sources.[11]

5. The gas inlet lines to the source will, at the appropriate gas pressures, be efficient electrical conductors. Care must be taken to prevent discharging along the lines of the various high voltages available in the source. This is one of the more troublesome items in the construction of a CI source, and the absence of a high accelerating voltage, as in quadrupoles, makes the overall task of building a CI source considerably easier.

Technical details have been published of dual EI/CI sources designed for many of the commercially available magnetic sector mass spectrometers, including the CEC 21-110B,[210-214] the AEI MS-902,[215,216] the AEI MS-12,[217] and the Dupont 21-490.[218]

Three quadrupole mass spectrometers have been equipped with CI sources. These are the EAI Quad-300,[219] the Finnigan 1015,[220] and the Extranuclear 011-1.[221] A CI source has very recently been described for the Hewlett-Packard HP 5930A dodecapole mass spectrometer.[222] This is a novel system consisting of a standard EI source which can essentially be replaced by a CI source without breaking the vacuum.

All these mass filters operate with very low accelerating voltages, of the order of tens of volts. As pointed out above, this alleviates the problem of high-voltage discharges; it considerably simplifes the problem of coupling a gas chromatograph directly to the CI mass spectrometer, without the molecular separator necessary with an EI source. The carrier gas is not removed; it is admitted to the source where it is used as the reagent gas. This technique is discussed further in Section IV-C-6.

The CI source built for the AEI MS-902[215] has been successfully used[39] for the generation of beams of negative ions. Such negative ion CI mass spectrometry appears to be particularly useful with highly halogenated compounds, such as some of the insecticides (see Section IV-D-8).

Primary ionization of the reagent gas is usually achieved by EI using electrons emitted from a heated filament situated in the low-pressure region. It is, however, possible to avoid using a filament and employ a low pressure Corona or Townsend discharge for the formation of the primary ions in a CI source. Shahin[28] has discussed the use of gas discharge in the study of ion-molecule reactions. Hunt[30] demonstrated that stable Townsend discharges can be sustained in a CI source at reagent-gas pressures of one torr. In the "cathode mode" of operation, the insulated gas inlet line is maintained as a positive electrode and a molybdenum screen at source potential is the negative electrode; reversal of these polarities permits "anode mode" operation, which is somewhat more reliable. Use of this discharge system in place of a heated filament leads to intense negative or positive ion CI mass spectra. Since the current dissipated in a Townsend discharge is only between 10^{-6} and 10^{-9} A, the source can be operated at ambient temperature.

3. Plasma Chromatography

The plasma chromatograph[223,224] is essentially a CI source operating at 760 torr. It is not possible to use a filament in such a system; isotopes such as ^{63}Ni are employed as the primary source of electrons.[11] The plasma generated in the CI source at 760 torr will reflect the fact that very large numbers of collisions are taking place; as a result, considerable numbers of ions will be formed from even the trace constituents of the gas in the source. The ions formed are permitted to leave the source through a very small slit into a mass analyzer. The mass spectra, or "plasma chromatograms," produced in this way are very simple because they contain only those ions that can survive the many collisions taking place during the long residence times. The plasma chromatograph is a very sensitive mass spectrometer, capable of detecting as little as 25 picograms (25×10^{-12} g) of a compound.[11]

4. Proton Affinities

Most commonly, the species transferred from the reagent ion to the sample molecule in CI mass spectrometry is a proton. The initial product of the transfer is a protonated molecular ion, $(M + H)^+$, whose excess energy depends upon the relative proton affinities of the molecule and of the conjugate base of the reagent ion, e.g., CH_4 in the case of CH_5^+. The proton affinity of a molecule M is defined as equal to, but of the opposite sign from, the heat of formation of the ion MH^+.

If the acid strength of the reagent ion is increased (so that the proton affinity of its conjugate base is decreased), then the excess energy available during proton transfer to a sample molecule M will also be increased. Much of this energy will become internal energy in the product ion MH^+, and as this internal energy increases, the probability that MH^+ will fragment increases. Thus the degree of fragmentation in a CI mass spectrum can be controlled to some extent by the choice of reagent gas. It is possible in fact to arrive at an estimate of this excess energy and also of the specificity of protonation in polyfunctional compounds by using information pertaining to gas-phase proton affinities.[225,226] If the proton affinity of the sample molecule is much lower than that of the conjugate base of the reagent ion, as would be the case for example with NH_4^+ as a reagent ion and CH_4 as the sample molecule, then proton transfer will not take place. Thus, if enough proton affinities are known, it should be possible, by judicious choice of a reagent gas, to protonate selected compounds in mixtures, or alternatively to gain chemical information about the sample molecule from a knowledge of which ions will protonate it and which will not. This interesting branch of CI mass spectrometry will be discussed below.

Proton affinities can be determined qualitatively by "bracketing" methods. Upper and lower limits of the proton affinity are obtained by observing whether or not proton-transfer reactions occur with a series of reference reagent ions. This method rests on the assumption[227] that the nonoccurrence of a simple proton-transfer reaction is prima facie evidence that it is endothermic.

An absolute value of gas-phase proton affinities can be calculated[227] from the appearance potential, as measured from EI mass spectra, of the protonated molecular ion MH^+, but this method depends upon the coincidental occurrence of the ion in question in an EI mass spectrum. Such a possibility, that is the formation of an ROH_2^+ ion in the EI mass spectrum of an alcohol, is somewhat rare; because of this, the method is difficult to implement.

A more general, if less accurate, method used by Haney and Franklin[227,228] is based on their observation that the translational energy of the products of a hydrogen-transfer reaction such as $NH_3^{+\cdot} + NH_3 \rightarrow NH_4^+ + NH_2^{\cdot}$ is a nearly constant fraction, 20%, of the enthalpy change for the exothermic reaction.

Accurate determinations of relative proton affinities can be obtained[229] from gas-phase ion equilibria. The equilibrium constant of the reaction

$$AH^+ + B \rightleftharpoons A + BH^+$$

can be measured and used to calculate the free-energy change for either reaction. Since temperature studies have confirmed[230] that the entropy change is negligible for transfer of a proton between relatively large molecules such as A and B, the standard free-energy change, ΔG°, may be equated to the enthalpy change, ΔH°, for the reaction. This enthalpy change represents the difference between the proton affinity of A and that of B and can be measured to within about 0.2 kcal $mole^{-1}$.

The gas-phase proton affinities of several simple organic compounds are summarized in Figures 16 and 17, where it is readily apparent that, with few exceptions, the highest proton affinities are possessed by nitrogenous compounds. Oxygen-containing compounds have lower proton affinities, and the lowest are possessed by compounds such as alkyl halides, methane, and hydrogen. The inductive effect of methyl groups can be seen in several series[231] and in some of the simple oxygenated compounds, it is clear[226] that this inductive effect is attenuated rapidly as the methyl group is moved further from the oxygen atom. A proton will, in general, be transferred from any compound in the Figures to any compound on its right. This fact should be useful in the identification of the functional groups, and indeed Hunt[232] has demonstrated that NH_3 can be used as a reagent gas in this way. The NH_4^+ ion will protonate only compounds of relatively high proton affinity, such as amides, amines, and (as McCloskey and Dzidic have shown[233]) α,β-unsaturated ketones, all of which have MH^+ and $(M + NH_4)^+$ ions in their CI(NH_3) mass spectra. Aldehydes, acids, and unconjugated ketones give only the $(M + NH_4)^+$ ion; no ionization at all is observed from less basic sample molecules such as ethers, phenols, nitro compounds, and aliphatic or aromatic hydrocarbons.

The proton affinities of α,ω-diamines are much larger than those of monoamines of similar polarizability[234,235] and the substantial (negative) entropy changes accompanying reactions in which

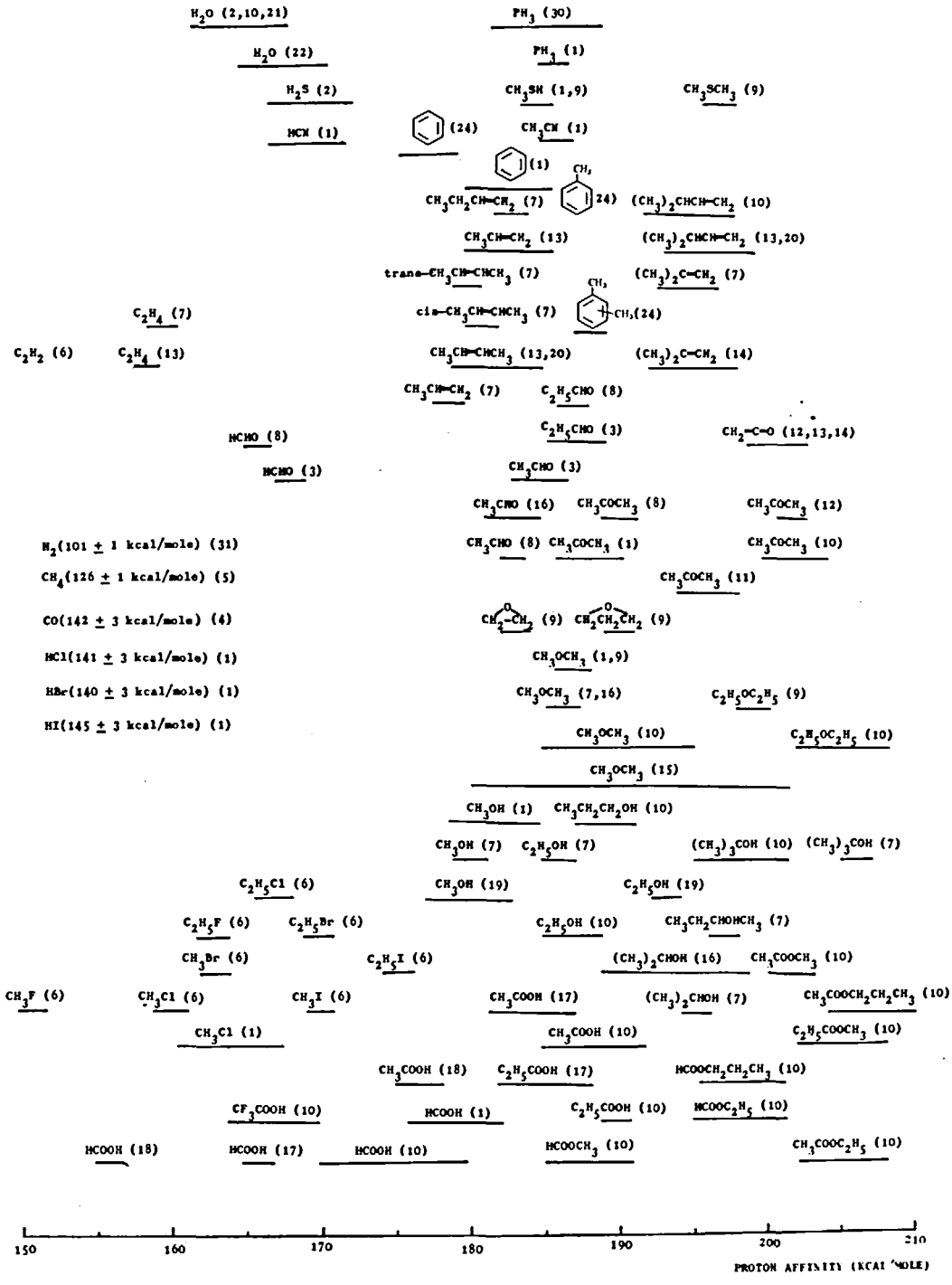

FIGURE 16. Proton affinities of selected organic molecules. The center of each horizontal line represents the measured proton affinity; the length of the line provides a measure of the experimental error. If the proton affinity relative to a standard was measured accurately, or if no experimental error was given in the original paper, an arbitrary length of ± 1 kcal mole^{-1} has been given to the entry. The number in parentheses for each entry refers to the original paper. (See following references.)

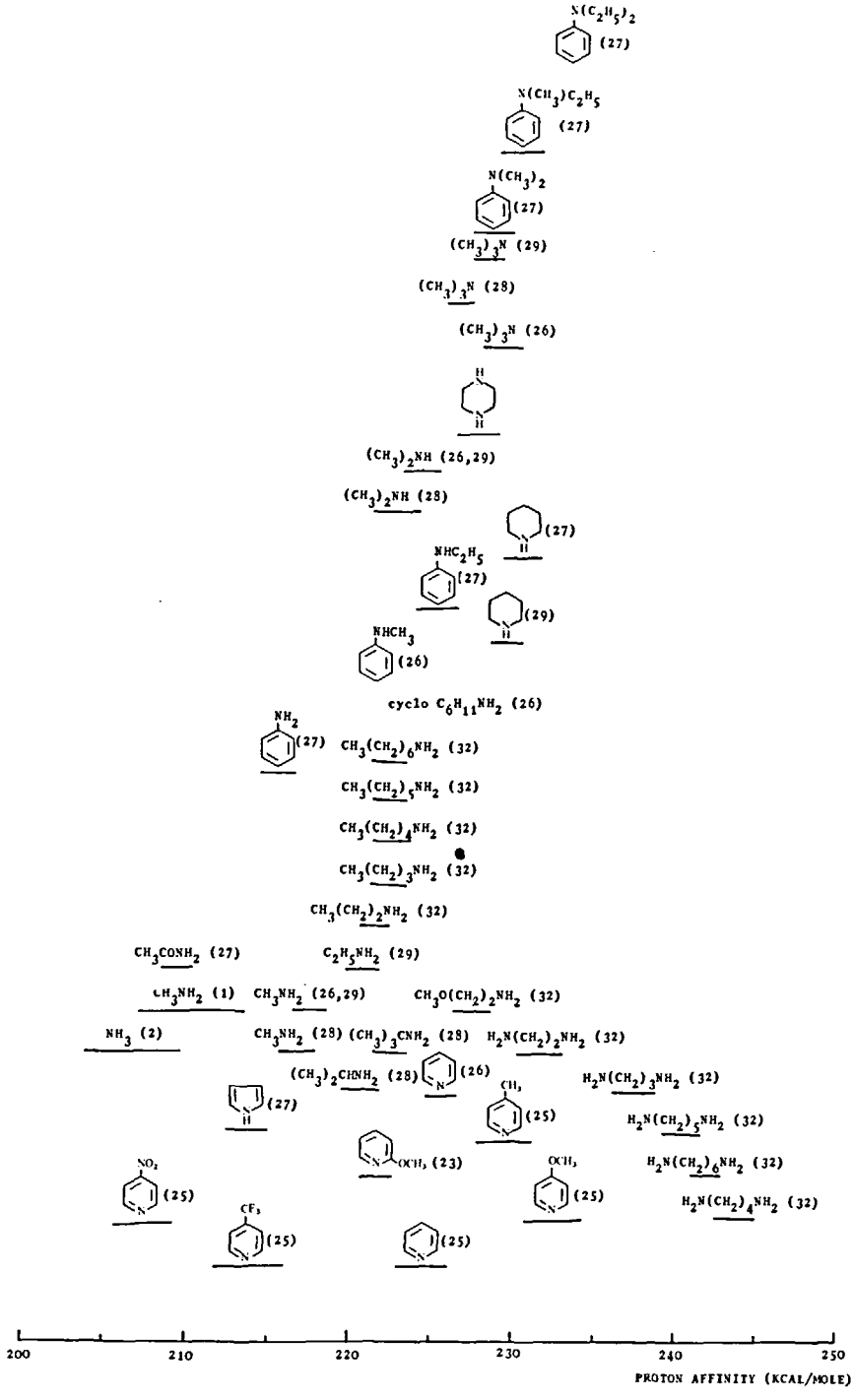

FIGURE 17. Proton affinities of selected organic molecules. The center of each horizontal line represents the measured proton affinity; the length of the line provides a measure of the experimental error. If the proton affinity relative to a standard was measured accurately, or if no experimental error was given in the original paper, an arbitrary length of ± 1 kcal mole^{-1} has been given to the entry. The number in parentheses for each entry refers to the original paper. (See following references.)

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protons are transferred to the diamines are consistent with the formation of proton-bound cyclic structures.

The differences between the basicities of compounds in gas phase and in solution may permit the distinction of "intrinsic" basicities from solvation effects. For example, pyridine is a much weaker base than ammonia in aqueous solution, but a much stronger base than ammonia in the gas phase,[225,230,235,236] where solvation effects are absent. Similarly, phenyl-group substitution of an amine increases its gas-phase proton affinity relative to hydrogen, but decreases it relative to cyclohexyl.[225,230]

5. Reagent Gases

The choice of reagent gas for CI mass spectrometry is now fairly wide. It may be governed in particular cases by the degree of fragmentation sought in the mass spectrum, the chemical and physical properties of the reagent gas, the proton affinity of the sample molecule, and the method of sample introduction.

The most commonly used reagent ions are, in order of their acid strengths (i.e., the reverse order of the proton affinities of their conjugate bases shown in Figures 16 and 17):

$$H_3^+ > CH_5^+ > C_2H_5^+ > (CH_3)_2CH^+ > H_3O^+ > (CH_3)_3C^+ > NH_4^+ > (CH_3)_3NH^+$$

Hydrogen and the aliphatic hydrocarbons have been used very extensively as reagent gases in CI because they are (i) chemically inert, (ii) readily available, and (iii) the most convenient gases with

very low proton affinity. They have a drawback common to all gases with proton affinity lower than that of water; this is that H_2O molecules present in the system will be converted to H_3O^+ ions, which can compete quite effectively for sample molecules with the major reagent ions. This can lead to difficulties in reproduction of the intensities of CI mass spectra[237] and in labeling work.[238] It might be noted that water cannot be protonated by $C_4H_9^+$ under CI conditions and isobutane CI is therefore not subject to these difficulties. Because of this, isobutane CI has been used successfully in the quantitative analysis of $^{14}NH_3/^{15}NH_3$ mixtures.[239] The ammonia, which is isolated from larger molecules by the Kjeldahl method, is contaminated with water, but since water cannot be protonated by the t-butyl ion, the ion H_3O^+, which would interfere with $^{15}NH_4^+$ at m/e 19, is never formed.

The problems created by residual water can also be circumvented by the use of the fully deuterated reagent gases, D_2O[240] and ND_3.[241] Use of isotopically labeled reagent gases leads to complete replacement of all active hydrogens in the molecule; hence active hydrogen determinations are easily accomplished in this way. The species transferred in the CI will also of course be D^+ rather than H^+.

At a given source temperature, the fragmentation that takes place subsequent to CI may be minimized by a reagent ion with a conjugate base having a proton affinity only slightly less than that of the sample molecule. As is seen in Section IV-D, isobutane has been used very extensively as a reagent gas for precisely this reason.

Electrophilic addition to sample molecules of cations other than H^+ has received a little attention. This often seems to give rise to very little fragmentation and may be useful as the basis of methods of molecular weight determination. Tetramethylsilane, for example, gives essentially a single reagent ion, $(CH_3)_3Si^+$, which undergoes addition to a very wide variety of compounds giving intense ions at m/e (M + 73).[200] Nitric oxide also appears to have interesting possibilities as a reagent gas[196,199] since it gives intense ions at m/e (M + 30) from compounds such as ketones, esters, and carboxylic acids. Aldehydes, however, when treated with NO^+ give exclusively ions of m/e (M − 1), while alcohols tend to fragment.

Gas mixtures have been used as reagent gases. The addition of a small amount (<10%) of a second gas to a reagent gas plasma can lead to the formation of a completely new group of reagent ions with properties different from those of the original reagent ions.[242]

The second reagent gas can be added to the effluent from a gas chromatograph prior to its entry into the CI source;[243] if mixtures of, for example, water and helium are produced in this way, the CI mass spectra that result contain many features of both the water CI and the "helium charge-exchange" mass spectra.[243] Argon-water mixtures have been used with similar results.[244] The mass spectrum so obtained is effectively the combined EI/CI(CH_4) mass spectrum,[245] and will generally contain more structural information than either of its two components. As an example, the argon-water CI mass spectrum[244] of amobarbital is shown in Figure 18.

6. Sample Introduction

The techniques for sample introduction that are usually employed in EI mass spectrometry are also suitable for CI work. However, extra precautions must be taken when a magnetic sector machine is being used for CI, because of the problems created by the high voltage used in such machines. In most of these sources, the direct insertion probe is at the source potential while the machine is being operated, and it must be shielded to protect the operator. Liquid-inlet systems must also be somehow raised to, or insulated from, the accelerating voltage. This is more difficult, although it has been done.[210] Many designs simply rely on a direct insertion probe and omit a liquid-inlet system.

An important advantage of the CI source is that it can usually tolerate the gas-flow rates common in gas chromatography. A gas chromatograph can therefore be coupled directly to the CI source, without the molecular separator that is necessary with EI sources, and the carrier gas used as a reagent gas.[219,246-248] The advantages of using CI in gas chromatography-mass spectrometry are further enhanced by its application to detection systems that monitor a small number of characteristic ions rather than scanning through the entire spectrum. The technique of single or multiple ion monitoring, often known as mass fragmentography,[249] has attracted some attention in recent years as a result of its power in the detection of very low levels of specific compounds. Since much, if not all, of the ion current in CI mass spectra is carried by the protonated

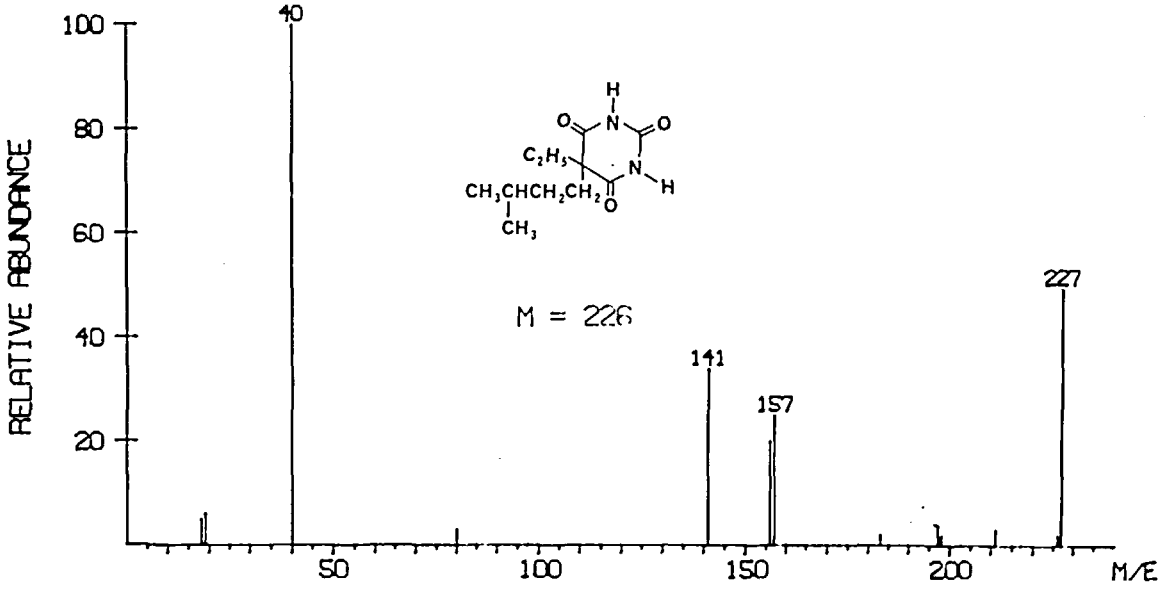


FIGURE 18. CI(Ar/H_2O) mass spectrum of amobarbital (molecular weight 226).[244]

molecular ion, it would seem that the two techniques of mass fragmentography and CI mass spectrometry should complement one another well.

Evidence has been presented[250,251] to suggest that compounds can sometimes be made to give CI mass spectra at lower temperatures if the sample is exposed directly to the plasma of the CI source. Thus, a compound such as the free tetrapeptide Leu-Leu-Val-Tyr, inserted into the source in a sample holder on a direct insertion probe, failed to give a satisfactory CI or EI mass spectrum at temperatures up to 340°.[251] However, when the compound was coated on the outer surface of the probe, in a direct contact with the plasma in the source, a protonated molecular ion was observed at temperatures as low as 150°.

The flash evaporation in a CI source of solutions of compounds has received a little attention.[250] This may well provide another means of introducing nonvolatile samples into a mass spectrometer and should also permit the direct interfacing of a high-pressure liquid chromatograph with an EI[252] or a CI[251] mass spectrometer.

D. Structural Applications of Chemical Ionization Mass Spectrometry

1. Rationalization of Fragmentation Reactions in CI Mass Spectra

The vast majority of the ions observed in CI mass spectra can be considered as arising from localized processes in the sample molecule. It is widely felt that many of the primary fragmentation pathways can be rationalized *formally* in terms of proton transfer to specific sites, commonly heteroatoms, followed by loss of HX from the protonated molecule ion:

$$AX + [H^+] \rightarrow A\text{–}\overset{+}{X}\text{–}H \rightarrow A^+ + HX$$

Field[180] has suggested that, for this reaction, the leaving ability of the group X, for a given A, is inversely related to the proton affinity of HX. Thus, the leaving tendencies of some common functional groups would be:

$$NH_2 < CH_3S, C_6H_5, CH_3O, COOH <$$
$$CN, SH < OH < I, Cl, Br$$

Conversely, the relative tendency of the moiety A to form A^+ follows from the conventional concepts of ion stability. This view of the CI-induced fragmentation of monofunctional compounds is eminently reasonable and finds a wealth of support in the growing literature of CI mass spectra.

Hydride abstraction, as opposed to protonation, may be regarded as a special case in which X = H and $A^+ = (M - H)^+$. It is, however, a moot point whether $(M + H)^+$ ions are precursors to the $(M - H)^+$ ions that are so intense in molecules with low proton affinities, such as alkanes. Field and Munson[254] have shown that in the CI(CH_4) mass spectrum of cyclohexane-d_{12}, the only ion in the

region of the molecular ion is $C_6D_{11}^+$. This, coupled with the subsequent observation[253] that $C_{10}H_{21}^+$ is the major ion in the CI(CD_4) mass spectrum of n-decane, strongly suggests that these $(M - H)^+$ ions are not formed by protonation and loss of H_2, but rather by direct abstraction of H^- from the molecule.

With molecules other than alkanes, most of the common reagent ions appear to behave predominantly as Brønsted acids, as opposed to Lewis acids. In the case of alcohols, abstraction of a hydride ion from the hydroxyl-bearing carbon might be expected to be energetically favored in the CI mass spectra of primary and secondary alcohols.[255] This is indeed an important process in the CI mass spectrum of cyclohexanol-1-d_1,[232] but labeling experiments indicate[256] that this is a much less specific process in the case of long chain primary alcohols. Similarly, α-hydride abstraction, as expected, does not appear to be important in the CI mass spectra of ketones.[237] Abstraction of D^- is however, an important process in the CI mass spectra of toluene-α-d_3 and cycloheptatriene 7-d_1.[257]

Cleavage of carbon-carbon bonds during CI may be regarded[258] as an electrophilic attack by a proton on the carbon-carbon bond followed by or concomitant with dissociation. It is apparent that fragment alkyl ions in the CI mass spectra of n-alkanes may also be formed by secondary decomposition of rearranged $(M - H)^+$ ions.[253] In general, however, the primary fragmentation of protonated molecular ions in CI mass spectrometry occurs mainly by localized charge transfer without rearrangement. Rearrangement processes appear to be much less important primary fragmentation processes in CI than in EI, as is discussed in the next Section.

2. *Hydrogen Rearrangements in Chemical Ionization Mass Spectra*

The paucity of hydrogen rearrangements in protonated molecular ions may be explained in terms of the much reduced capacity of a protonated functional group to participate in additional bonding as compared to that of the same functional group in a molecule ionized by EI.[180] Hydrogen rearrangements may occur when interactions are possible between the functional groups of a protonated molecular ion, or when a functional group contains more than one nucleophilic center. For example, the CI mass spectra of many carboxylic esters contain an intense ion corresponding to the appropriate protonated carboxylic acid, $RCOOH_2^+$. This ion is much less intense in esters such as those of benzyl alcohol in which the alcohol moiety does not have a hydrogen atom on the β-carbon. It is therefore considered[259] that this particular fragment ion is formed by a hydrogen rearrangement.

Hydrogen rearrangements are also seen in the CI mass spectra of polyfunctional esters,[260] alkyl benzenes,[261] and aryl alkyl ketones.[237,262] It is noteworthy that the loss of H_2O from the protonated molecular ion of steroidal ketones[263] does not appear to involve hydrogens on the α-carbon to any significant extent.

Some hydrogen rearrangement may be involved in the CI mass spectra of peptides[264] and nucleosides.[265] There is, however, no evidence for hydrogen rearrangements in negative CI mass spectra.[39]

3. *Functional Group Interactions*

When possible, interactions between functional groups in the same molecule can be quite important in their effect upon the CI mass spectrum. Such interactions often serve to stabilize the protonated molecular ion by proton bridging between functional groups. The stabilitity of the protonated molecular ions derived from compounds that are not rigid and that contain widely separated functional groups (such as decane-1,10-diol) has been observed[256,266] and attributed to this proton-bridging effect. The same effect in rigid systems such as steroidal 1,2- and 1,3-amino alcohols has been found[267] to prevent loss of H_2O from the protonated molecular ions of those isomers in which a bridge is possible but not from the other isomers.

In contrast to this, some fragmentation reactions may be enhanced if intramolecular nucleophilic attack is a possibility. Thus backside-assisted decompositions of this sort are observed in the CI mass spectra of some alkyl polyamines,[198] amino acids,[152] aliphatic and aromatic diesters,[268] ortho-substituted aromatic compounds,[269] and macrolide antibiotics[270] and other drugs.[271] Anchimeric effects of these types seem to be more pronounced in CI mass spectrometry than in EI mass spectrometry.[269]

4. Characterization of Isomers

Fine structural differences between isomers may be reflected in their CI mass spectra. Quantitative differences in the CI fragmentation patterns of stereoisomers have been reported with respect to *cis*- and *trans*-cyclohexane-1,2-diol[176], geometrical isomers such as the esters of maleic and fumaric acids,[268] epimeric steroids,[263,267,272] and alkaloids.[273]

Stereochemical influences on EI mass spectra are not significant in most cases, although some notable exceptions have been recorded.[274] The EI mass spectra of the C_7H_8 isomers toluene, cycloheptatriene, and norbornadiene are very similar; much evidence has been adduced in support of the theory that common intermediates are involved in their ion fragmentations. In contrast to this, the CI(CH_4) mass spectra of these compounds are quite distinctive.[257] The isomeric photodimers of α,β-unsaturated ketones give virtually identical EI mass spectra but can easily be distinguished from their CI(CH_4) mass spectra.[275]

Characterization of isomeric mono-alkenes by conversion to the corresponding dideuteroalkanes and CI mass spectrometry is unsuccessful[253] because of the hydrogen scrambling that takes place in the $(M - H)^+$ ions. The CI mass spectra of the corresponding *vic*-diols, as their trimethylsilyl derivatives, can be used to identify the position of the double bond.[276]

Functional-group interaction (*vide supra*) can lead to major differences between the CI mass spectra of *o*-disubstituted aromatic compounds and the corresponding *m*- and *p*-isomers.[269] This is true for only a small number of functional groups and is not likely to lead to a general method for structure determination in this area.

Isomers which differ in their active hydrogen content (such as diethylamine and *n*-butylamine) can be distinguished from one another by measuring the number of deuterium atoms incorporated into the deuterated molecular ion from D_2O[240] or ND_3.[241] This technique can be used in EI mass spectrometry but the CI method is particularly useful when the compounds fail to give a molecular ion in their EI mass spectra.

Isomer differentiation is also possible if there is a significant difference in the basicities of the isomers. In such a case, it may be possible to select a reagent ion that will protonate one isomer but not the other.[225] For example, there is a much greater degree of proton transfer from NH_4^+ to α,β-unsaturated ketones than to their β,γ-isomers, which add the entire NH_4^+ ion giving an $(M + 18)^+$ ion.[233]

5. Characterization of Functional Groups

Some functional groups, such as hydroxyl groups, are easily identified in most cases from their CI mass spectra. Others, such as the carbonyl group, are not so easily characterized and have been the objects of some study.

Hunt and Ryan[199] have investigated the use of NO as a reagent gas in problems of this sort. With NO^+ reagent ions, ketones, esters, and carboxylic acids all give intense ions at m/e (M + 30). Aldehydes give ions of m/e (M + 30) and (M - 1), while esters give only $(M - 1)^+$ ions. All alcohols give ions at m/e (M - 17), but only primary and secondary alcohols give ions at m/e (M - 1) and (M + 30 - 2) in addition to this. The loss of 2 amu, as in the last of these ions, is evidence of oxidation by the reagent gas NO, of the alcohol to an aldehyde or a ketone; this is why ions of this sort are not found in the CI(NO) mass spectra of tertiary alcohols.

E. Chemical Ionization Mass Spectra

Chemical ionization mass spectrometry has found its widest application in the area of complex natural products.[248] Competition between localized CI-induced reactions at various sites in a molecule can provide structural information that is often absent from the EI mass spectrum. Molecular weight information is frequently available from the CI but not the EI mass spectrum.

On the other hand, the general absence of carbon-carbon cleavage reactions from the CI mass spectra means that, unlike the EI mass spectra, they will provide little skeletal information. Consequently, EI and CI mass spectra will, in a sense, be complementary, a fact that has been noted by many workers in the field.

This Section deals with the published CI mass spectra of a variety of fairly complex organic molecules much as Section III-I above treated their FI mass spectra. Unless otherwise stated, the CI mass spectra discussed in this Section were all measured at source pressures of the order of one torr.

1. Amino Acids

Intense $(M + H)^+$ ions are observed in the CI(CH_4) mass spectra of most of the natural

amino acids.[152,153] This stands in marked contrast to their EI spectra[277,278] in which the molecular ions are of very low relative abundance.

The CI(CH_4)[152] and EI[278] mass spectra of proline are shown in Figure 19. The fragmentation in the CI mass spectrum is typical of an aliphatic amino acid and is very simple. Metastable transitions indicate that the ions at m/e (M - 17) and (M - 45) are formed directly from the MH^+ ions; they represent the loss of H_2O and $COOH_2$, respectively, from these ions. Isobaric ions could, it has been suggested,[153] be formed at m/e (M - 17) by the loss of $COOH_2$ from the adduct ion, $(M + C_2H_5)^+$, which is not shown in Figure 19.

The CI(isobutane) mass spectra of several of the amino acids have been measured by Meot-Ner and Field[279] who point out that the CI(CH_4) mass spectrum of a compound is essentially identical to the CI(isobutane) mass spectrum of the same compound run at source temperatures 167 ± 10°C higher.

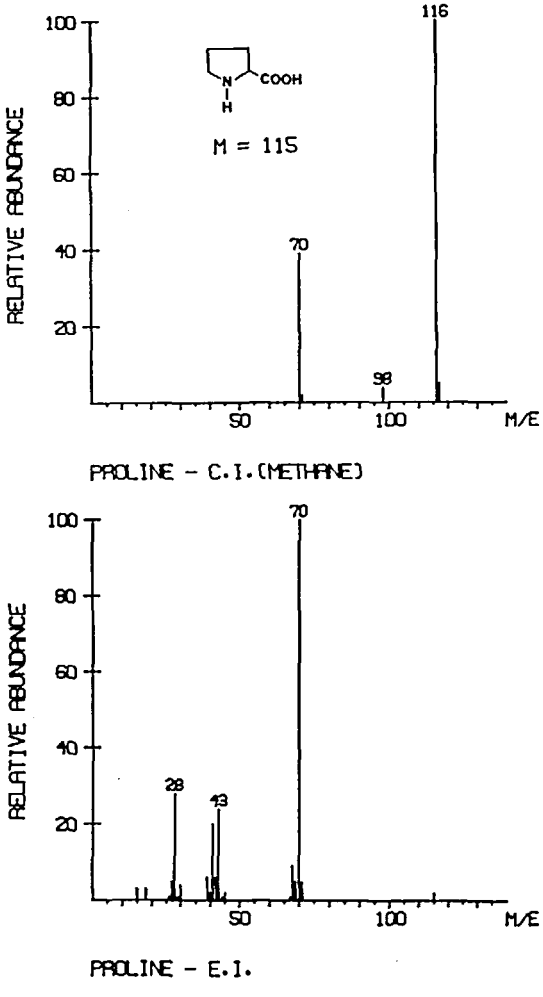
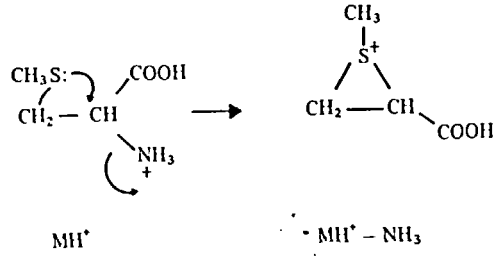


FIGURE 19. EI and CI(CH_4) mass spectra of proline (molecular weight 115).[152,278]

Although protonation of the amino group of amino acids is undoubtedly an important process, subsequent loss of NH_3 is only observed in cases where internal nucleophilic assistance is possible, as with S-methyl cysteine:[152]



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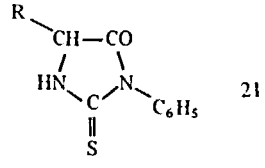
The phenylthiohydantoin derivatives of amino acids (e.g., 21), which are released in the successive steps of



21

the Edman degradation (e.g., 21), of a polypeptide may be analyzed quantitatively by various methods based on thin-layer chromatography, gas-liquid chromatography, and EI mass spectrometry. The CI(isobutane) mass spectra of these compounds are very simple indeed.[280] Most of these spectra contain only one ion, usually the MH^+ ion, and lend themselves to identification and quantitation of the compound at hand. The CI method of analysis is rapid and may offer some advantages of sensitivity higher than that possible in methods based on EI mass spectrometry.[281]

2. Peptides

Groups working in CI mass spectrometry were quick to examine the possibilities of the method in the problem of peptides sequencing; two early papers dealt with this subject.[264,282] Both pointed out that the CI method has some distinct advantages over EI mass spectrometry: (1) There is a much greater intensity associated with the MH^+ ion in CI than with the $M^{+\cdot}$ ion in EI. (2) Cleavage of side-chains, which tends to complicate the spectra without adding to the sequence informa-

tion available, occurs to a much smaller extent in CI than in EI. (3) The relative intensities of the sequence-determining ions, particularly those of the type H_3N^+-CH(R)-$COOCH_3$, are generally greater in the CI than in the EI mass spectrum.

Both EI and CI are useful in peptide sequencing, then, and it may be that CI is the more useful of the two. However, neither method enjoys much acceptance by peptide chemists, and it seems that, when they are viewed from a longer perspective than that available in some mass spectrometry laboratories, the methods that are provided by these laboratories simply do not compete with other techniques available to the biochemist.

Chemical or enzymatic breakdown of proteins almost always gives mixtures of peptides of varying sizes; there is at present no really satisfactory method for identifying the components of these mixtures. McLafferty's group has explored the possibilities of mass spectrometric methods and contrasted them with the chemical methods available.[191] They showed that a combination of mass spectrometric methods, including CI, could provide complementary sequence information of high reliability. There is general agreement that useful CI mass spectrometric data can be obtained from a peptide at the 10 to 100 nanomole level, which represents a substantial improvement over EI mass spectrometry.

The ionization of peptides by single ion impact in a tandem mass spectrometer has been investigated.[207] The spectra of N-acetyltrialanine with solvated protons $[H(H_2O)_n]^+ (0<n<5)$ show increasing yields of the MH^+ ion with increasing values of n. Protonation of the ester function seemed to be favored.

The reaction of dipeptides with $(CH_3)_2NH_2^+$ ions has been examined[225] to assess the value of selective CI in complex molecules. Pro-Ala gives an intense MH^+ ion under these conditions, which is taken as a reliable indication of the presence in the molecule of a secondary amine (Pro). When such a unit is absent, as in Phe-Phe, the major ion is the adduct ion Phe-Phe + $(CH_3)_2NH_2^+$ at m/e (M + 46).

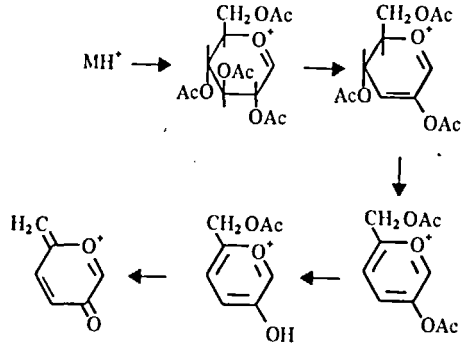
3. Carbohydrates

Electron ionization mass spectrometry has had rather limited success in carbohydrate chemistry. This is mainly because EI mass spectra of sugars typically fail to show molecular ions; the large fragment ions observed are often of low intensity. Furthermore, fragmentations undergone by these compounds during EI appear to be particularly complicated.[283] Hogg[284] has shown that the first of these problems can be overcome in CI mass spectrometry by choosing a reagent gas with basicity comparable to that of the carbohydrate molecule. Thus the $(M + NH_4)^+$ ion is the most intense, and often the only ion in the $CI(NH_3)$ mass spectra of free pentoses and hexoses, simple glycosides of monosaccharides, acetylated monosaccharides, and some disaccharides. This permits the facile determination of molecular weights and molecular formulae of these compounds. There is less fragmentation in the $CI(NH_3)$ mass spectrum of d-glucose than in its FI mass spectrum[57] as can be seen from Figure 12.

It has also been shown[285] that intense $(M + NH_4)^+$ ions are given similarly by peracetylated oligosaccharides. Sequence information on these compounds is often available from the fragments formed pyrolytically and then converted to the corresponding $(M^1 + NH_4)^+$ ions. Metastable evidence also indicated that some of the fragment ions are formed from the $(M + NH_4)^+$ ions.

More extensive fragmentation is observed when the more common reagent ions are used. Thus the $(M + H)^+$ ions formed in the CI(isobutane) mass spectrum of the pentaacetate of 2-amino-d-glucose are of very low intensity.[286]

The details of the fragmentation in the $CI(CH_4)$ mass spectrum of D-glucose pentaacetate have been elucidated by chemical and isotopic labeling[284] and found to conform to Scheme 1 below. With this information, it should be possible



SCHEME 1

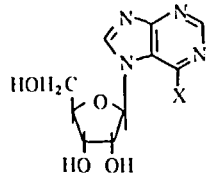
to use the $CI(CH_4)$ mass spectra to determine the ring substituents in monosaccharides. The specificity of the loss of the substituent from C_1

of the protonated molecular ion is quite high[284] and it has been suggested[286] that the proton originally transferred to any one of the several oxygenated sites in the molecule proceeds to migrate rapidly between these sites. Whenever the proton migrates to the oxygen on C_1, the very weak C_1-O bond breaks.

4. Nucleosides

McCloskey and his co-workers have shown that the CI(CH_4) mass spectra given by nucleosides can be used for their identification, because they give intense $(M + H)^+$ ions.[265] For a nucleoside S-B, composed of a sugar S and a base B, the most abundant fragment ion in the CI(CH_4) mass spectrum invariably corresponds to the protonated free base, BH_2^+. Less intense ions corresponding to the sugar, less a hydroxide ion, S^+, are also seen. These two ions, together with the protonated molecular ion, are the major ions in the CI(CH_4) spectra and are the only ions in the CI(NH_3) spectrum.

It has been shown that the glycosidic bond in 7-β-D-ribofuranosyl purines (22) is less stable during ammonia CI than the corresponding bond in the 9-β-D-ribofuranosyl purines (23).[287] This result is parallel to the behavior of these compounds during acid-catalyzed hydrolysis in solution.



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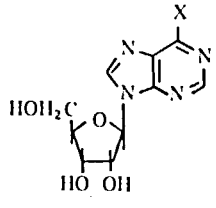

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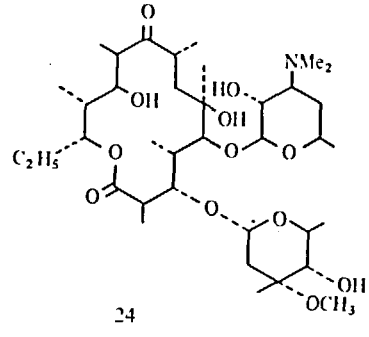
5. Macrolide Antibiotics

The EI mass spectra of macrolide antibiotics such as erythromycin B(24) are very complex with most of the intense ions in the low mass region.



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Foltz and co-workers[248,270,288] have demonstrated that the CI(isobutane) mass spectra of the macrolide antibiotics containing 14- and 16-membered rings are more structurally informative. As is seen in Figure 20, these spectra have intense MH^+ ions and the fragmentation is principally by cleavage of glycosidic bonds, as is true of their solution acid-catalyzed reactions.

6. Steroids

The CI mass spectra of various steroids have been studied by two groups[263,272,289] both finding that, in general, the base peak is in the vicinity of the molecular weight, and the number of fragmentation modes is considerably fewer than in the corresponding EI mass spectra. Munson's group has shown, moreover, that the CI(CH_4) and the CI(isobutane) mass spectra of steroidal alcohols[272] or ketones[263] are fairly sensitive to the position of the functional group in question.

The steroid skeleton is sufficiently rigid that estimates can be made of the relative extents to which specific functional groups are protonated. These estimates are based on the relative intensities of the appropriate fragment ions; the intensity of an ion corresponding to $(MH - H_2O)^+$ is considered a measure of the proportion of the protonated molecular ions that are protonated on the hydroxyl group lost as H_2O.[263,272] In this way, the proton affinity of the functional groups can be measured.

If hydrogen is used as a reagent gas, the CI mass spectra of some pairs of epimeric steroids show quantitative differences.[263,272]

7. Drugs

Many of the drugs commonly encountered in emergency toxicology undergo extensive breakdown upon EI. The resulting mass spectra are usually quite complex; identification of a specific drug from a group of perhaps a hundred for which EI mass spectra are at hand may be done manually or with computer assistance.[290] Gas chromatographic separation of the components of a mixture prior to measurement of the mass spectra is essential because the EI mass spectra of mixtures are prohibitively complex. The CI(CH_4)[291] and CI(isobutane)[271] mass spectra of most drugs are very simple, consisting very often of only the protonated molecular ion. Therefore, the mass spectra of mixtures can be handled successfully without prior chromatography, which is very slow.

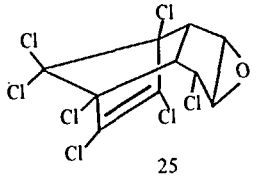
This technique has been used[292] for the analysis of illicit preparations of heroin.

CI mass spectrometry has been used effectively in the analysis of drug metabolites[248,293,294] and of the constituents of marijuana smoke condensate.[295] Plasma chromatography has also been applied in this area.[11]

8. Pesticides

Electron ionization mass spectrometry has been widely used in the analysis of the chlorine-rich pesticides such as DDT, aldrin (4), and dieldrin (5), and of the breakdown products of these compounds. The usefulness of this method is compromised to some extent by the complexity of the fragmentation patterns obtained; this is also true of the EI mass spectra of the polychlorinated biphenyls (PCBs).

Dougherty and co-workers have shown that the CI(CH_4) mass spectra of the polycyclic chlorinated pesticides are fairly simple.[296] The base peak is most commonly at m/e (M - Cl), although the MH^+ ion is occasionally intense, as in heptachlor epoxide (25), and, predictably, the base peak in the spectrum of 1-hydroxychlordene (26) is at m/e (M - OH). This CI(CH_4) mass spectrum of aldrin (4) is shown in Figure 21.



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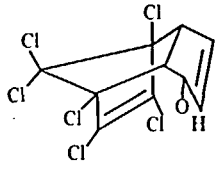

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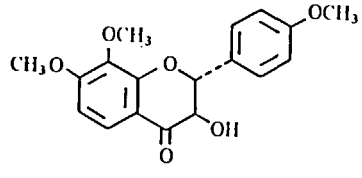
The same group has also extended the CI technique to the observation of the negative ions formed from these compounds under CI conditions.[39] Large numbers of electrons with near thermal energies are present in a CI source at one torr. Resonance capture of these low energy electrons can lead to beams of negative ions several times as intense as the positive ion beams obtained under identical conditions with the polychlorinated insecticides, and CH_4, CH_2Cl_2 or CCl_4 as reagent gas. These polychlorinated molecules all give molecular anions ($M^{-\cdot}$) with intensities between 6% and 39%; in every case the base peak of the spectrum corresponds to the adduct $(M + Cl)^-$. What little fragmentation there was consisted mainly of the loss from $M^{-\cdot}$ of neutrals, such as $H^\cdot$, $Cl^\cdot$, and HCl. An ion at m/e (M - 19) is considered to result from the displacement of $Cl^\cdot$ in the molecule by O^-.

These examples indicate that negative ion formation under CI conditions may complement and extend the study of negative ions formed (usually inefficiently) at low pressures. In principle at least, both positive and negative CI mass spectra may be obtained on the same sample in the same mass spectrometer by reversing the appropriate fields. In this way, two sets of information, to some extent complementary, may be obtained. It may also be possible to gain information about the structures of the stable anions formed at high pressures by collision-activated decomposition, as Bowie has demonstrated[297] for anions formed at low pressures in an EI source.

9. Other Complex Molecules

The NIH group has examined the CI(CH_4) mass spectra of alkaloids from nine of the eighteen major alkaloid families.[273] The fragmentation in these spectra was very much less extensive than in the EI mass spectra of the same compounds. Almost all the alkaloids studied provided an intense MH^+ ion, usually as the base peak of the spectrum. It seems clear that this is because the nitrogen atom, which is as easily protonated as any site in the molecule, is commonly integrated into a fused ring system in the alkaloids. Therefore, the protonated molecular ion formed is very stable.

Shannon and co-workers in Australia[238] have used the tricyclic flavanoids (e.g., 27) as models in attempts to correlate mechanism and structure in



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CI mass spectrometry. It was concluded that most of the fragment ions in the CI(H_2) mass spectra of these compounds arise from isomeric progenitors protonated at different sites in the heterocyclic ring.

This same group has also examined the CI(H_2) mass spectra of various polynitro compounds important in the explosives industry. In these spectra, the fragmentation patterns can be rationalized from known concepts of ion stabilities.[147]

The Baylor group has investigated CI mass spectrometry as a method of identifying the various biologically important prostaglandins at

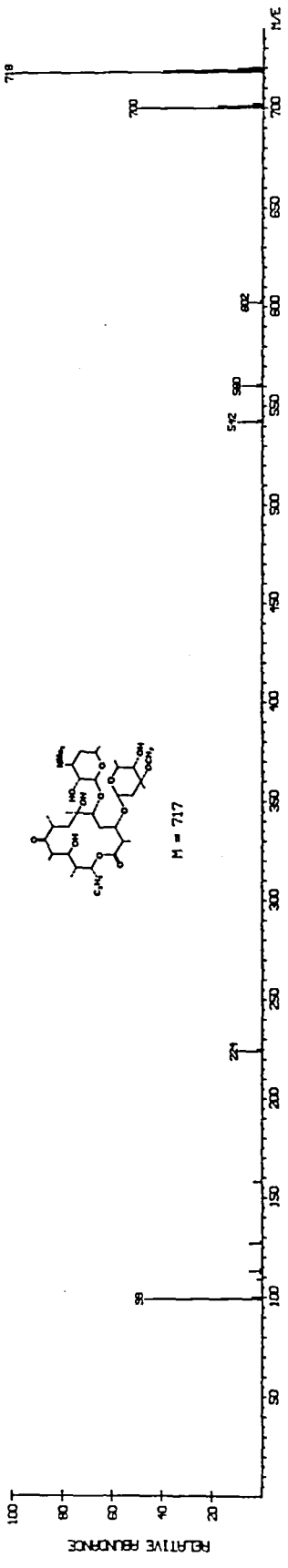


FIGURE 20. CI(isobutane) mass spectrum of erythromycin B (molecular weight 717).[248]

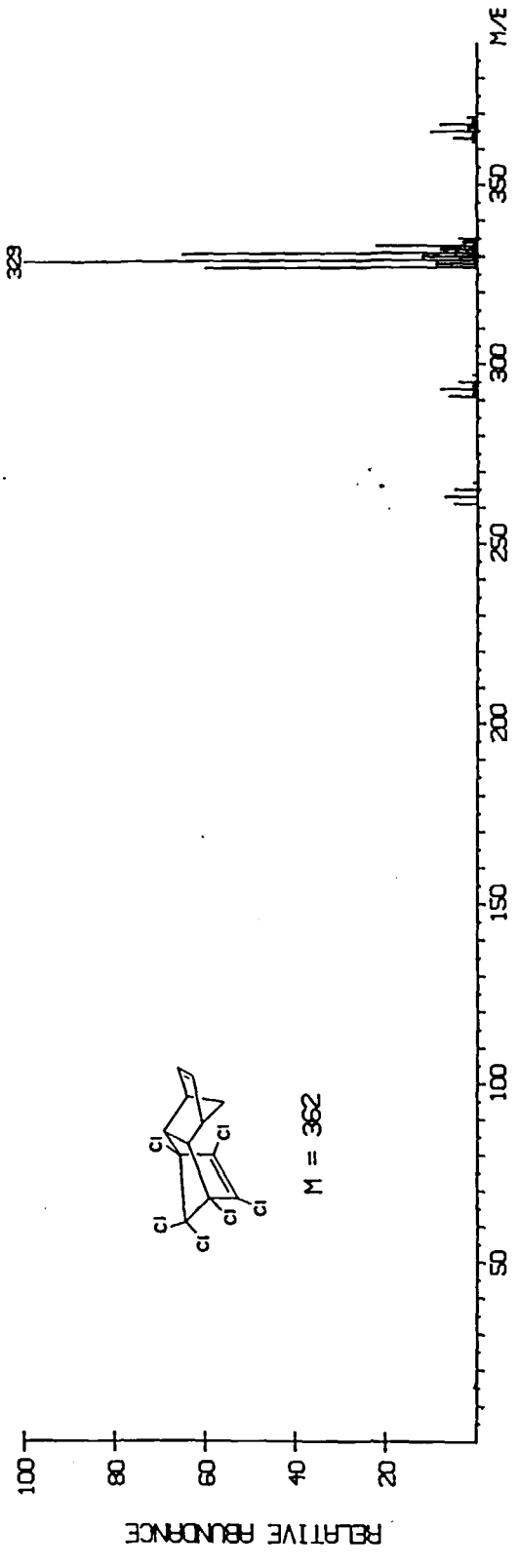
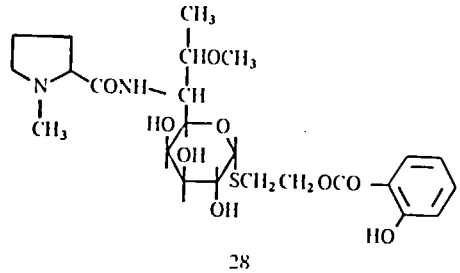


FIGURE 21. CI(CH_4) mass spectrum of aldrin (molecular weight 362).[296]

very low concentrations.[298] The CI(CH_4) mass spectra of these compounds show less fragmentation than the corresponding EI mass spectra, and are, in general, quite straightforward.

CI mass spectrometry has also been used to considerable advantage in structural studies on the antibiotic celesticetin (28),[299] the minor components of the antimycin A complex,[300] and holacurtine (29) and its derivatives.[301]



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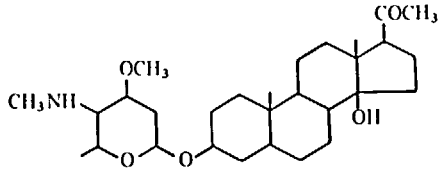

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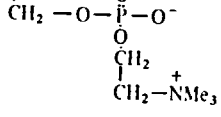
The phospholipid, dioleoyl phosphatidyl choline (30), representative of a family of



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compounds that has been notoriously difficult to handle by EI mass spectrometry, gives the CI(isobutane) mass spectrum shown in Figure 22. Surprisingly, in this spectrum the MH^+ ion is present with moderate intensity.[248] In this case, the molecule seems to rearrange upon heating to give the volatile methyl ester of the phosphate via an internal methylation reaction.

10. Inorganic Compounds

Porter and co-workers have established the proton affinity of borazine (31) by bracketing

31

techniques[302,303] as 203 ± 7 kcal $mole^{-1}$. This is much higher than the value of 183 ± 3 kcal $mole^{-1}$ accepted for benzene, which is isoelectronic with borazine.

The same group has found that the CI(CH_4) mass spectra of the boron hydrides are much simpler than their EI mass spectra, which are complicated by the successive losses from the molecular ion of H or H_2. Furthermore, hydrides (such as B_2H_6, B_4H_{10}, and B_5H_{11}) that have terminal BH_2 groups give rise to $(M - H)^+$ ions in their CI mass spectra, while those with only terminal BH groups (B_5H_9 and B_6H_{10}) give $(M + H)^+$ ions.[304,305]

A survey of the CI(CH_4) mass spectra of some organometallic compounds has been carried out by Hunt and co-workers[306] and reveals that the MH^+ ions are generally less prone to fragment than are the corresponding $M^{+\cdot}$ ions in the EI mass spectra. Complexes that have low basicities and low ionization potentials undergo charge exchange readily under CI(CH_4) conditions. As a result, the $M^{+\cdot}$ ions formed from such compounds may have an appreciable intensity.

F. Physicochemical Studies of Chemical Ionization

1. Mechanisms of Ion Fragmentation Under CI Conditions

The internal energies of the ions formed in an EI source at low pressures do not have a Maxwell-Boltzmann distribution; therefore, it is not possible to define the mass spectrum in terms of a "temperature." On the other hand, a sufficient number of ion-molecule collisions may take place in a CI source at higher pressures to give such a distribution. The typical equations of reaction kinetics at thermal equilibrium would then be applicable, and correlations could be made between reactivity and structure of ions. Also, the chemistry of ions in the gas phase can, in principle, be compared to that of ions in solution; thus, the importance of solvation effects in the latter case can be assessed.

Field and co-workers have undertaken a number of studies of reaction kinetics under CI conditions.[180,181] They have found that certain CI mass spectra, such as the CI(isobutane) mass spectra of some esters, are very temperature dependent and that the rate constants obey the Arrhenius equation. Therefore, they postulate that a sufficient number of collisions occur after formation of the MH^+ ions to establish an equi-

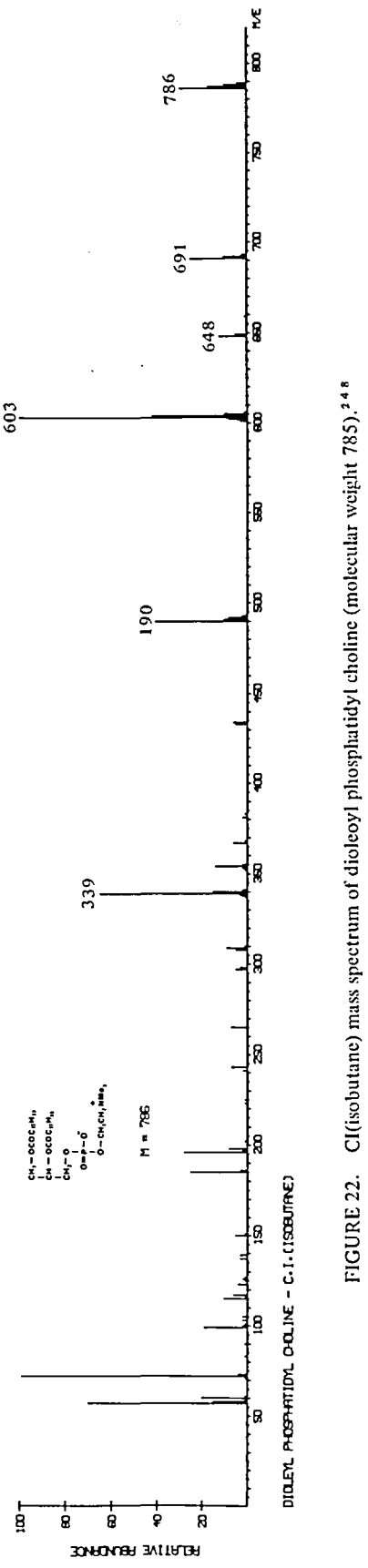

FIGURE 22. CI(isobutane) mass spectrum of dioleoyl phosphatidyl choline (molecular weight 785).[248]

librium distribution of energies prior to unimolecular decomposition.

In support of this proposal, it has been shown that the rate constant for the decomposition of the MH^+ ions derived from *tert*-amyl acetate is independent of the source pressure over the range 0.5 to 4 torr.[307] This implies that meaningful activation energies and frequency factors can be derived from the Arrhenius plots.

Kinetic analysis[308] of the formation of benzyl and *tert*-amyl ions in the CI(isobutane) mass spectra of benzyl acetate and *tert*-amyl acetate reveals that the frequency factor is lower for the former reaction. Restricted rotation in the transition state is thought to be responsible for this. Moreover, the rate constants for the total reaction of ions from methane and isobutane with the two esters exhibited extremely high values ($>10^{-8}$ cm^3 $mole^{-1}$ sec^{-1}) and an unexpected negative temperature coefficient for the isobutane-benzyl acetate system. These latter data, however, could not be reproduced by Futrell et al. using another CI source.[309]

Field has also obtained good Hammett correlations for the formation of benzyl ions in the CI(isobutane) mass spectra of *p*-substituted benzyl acetates. The value of ρ that is obtained is similar to that found for solution reactions in which the rate-determining step is the formation of a carbonium ion.[310] However, Michnowicz and Munson have found[211] that the substituent effects for benzophenones appear to correlate with the formation of the neutral products in the fragmentation of the MH^+ ions to benzoyl ions.

In the other systems, the rates of unimolecular fragmentation in CI also correlate well with the rates of acid-catalyzed solvolysis despite the absence of solvation in the gas phase reactions. For example, the relative rates of formation of the methoxymethyl ion in the $CI(CH_4)$ mass spectra of methoxymethyl acetate and formate are similar to the relative rates of acid-catalyzed hydrolysis in solution.[311] No methoxymethyl ions are formed in the CI(isobutane) mass spectra of these compounds; however, the analogous methyl thiomethyl ions are observed in the CI(isobutane) mass spectra of methyl thiomethyl acetate and propionate,[312] contrary to expectations based on the results of condensed-phase solvolyses.

A similar study of the kinetics of formation of t-alkyl ions from *tert*-alkyl acetates in the CI(isobutane) and CI(isopentane) mass spectra has shown[313] that the rate constants are susceptible to rather small structural changes, suggesting that steric effects are influential in the precursor MH^+ ions. The influence of β-substitution on the rates of these reactions is the same as that observed in condensed-phase solvolyses.[313] Furthermore, these rates are independent of the chain length of the acid portion of the ester, as might be expected from entropy considerations.[314]

Kinetic data have been obtained from a study of the fragmentation modes of protonated amino acids and peptides.[279] The low frequency factors and activation energies observed indicate that the loss from the MH^+ ions of $COOH_2$ or H_2O involves hydrogen rearrangements of ammonium ions, rather than dissociative proton transfer processes as had been previously suggested.[152]

2. *Studies of Ion Equilibria in the Gas Phase*

Ion-clustering reactions in the gas phase may be considered as the equivalent of solvation processes in the condensed phase; studies of proton-transfer equilibria can be carried out as a means of determining relative proton affinities, as is discussed in Section IV-C-4. However, much debate continues as to whether thermodynamic equilibrium is established in high-pressure ion sources and whether the measured ion intensities represent equilibrium ion concentrations. For instance, studies by Field's group[308,315,316] of the hydrated proton equilibria

$$H^+(H_2O)_{n-1} + H_2O + M \rightleftharpoons H^+(H_2O)_n + M$$

generated under CI conditions agree with the results obtained by Kebarle et al.[317,318] for the equilibria in which n = 4 or 5. There is serious disagreement, however, between the two sets of results for the lower equilibria, for which n = 2 or 3. Bennett and Field[319] have used source pressures up to 4 torr in an attempt to demonstrate that thermodynamic equilibrium had been achieved under CI conditions. Kebarle,[318] on the other hand, has suggested that the discrepancies arise because the probable residence times in the source used by Field et al. are insufficient to establish thermodynamic equilibria in the cases where n = 2 or 3.

Meisels and co-workers[320] have used a pulsed high-pressure source in conjunction with a time-of-flight mass analyzer to demonstrate that mean

ion residence times in a CI source (e.g., 4.1×10^{-5} sec for CH_5^+ at 0.7 torr pressure of methane) are substantially less than those calculated from Langevin drift theory. The measured delays between the electron and the detected ion pulses were used to give arrival time distributions of the selected ions and the residence times were calculated from these curves. Meisels[321] has also shown that calculated and observed residence times can be brought into significant agreement by taking into account various effects of the geometry of the ion source. The calculations also suggest that repeller fields in a CI source are inadvisable since ions formed along the electron beam will drift to the side walls rather than to the exit slit.

Field has also studied the reversible reactions of ions at subambient temperatures in a CI source[315,322-325] and has identified some novel adduct ions. While CH_5^+ and $C_2H_5^+$ appear inert toward CH_4 at ambient temperatures, this is not true at much lower temperatures,[323] where the following reactions may be observed:

$$CH_5^+ + CH_4 \rightarrow C_2H_9^+$$
$$C_2H_5^+ + CH_4 \rightarrow C_3H_9^+$$
$$C_2H_9 + CH_4 \rightarrow C_3H_{13}^+$$

In addition to these reactions, collision-induced metastable transitions corresponding to the reverse reactions may also be observed.

V. COMPARISON OF ELECTRON IONIZATION, FIELD IONIZATION, FIELD DESORPTION, AND CHEMICAL IONIZATION

A. Introduction

The four methods of ionization that have been considered in this review are EI, FI, FD, and CI. Of these, the last three have been described in detail in Sections III and IV above; this section will compare these techniques with one another, and draw some general conclusions about their respective utilities in analytical organic chemistry.

Technical aspects of the various methods will be considered first. These are possibly of secondary importance to the analytical chemist, but they should not be neglected because they determine the feasibility of each technique. More significantly, it is in this area that most of the future tactical gains may be expected.

Of immediate interest to the organic chemist is the question of which method will be most useful for a given type of compound. None of these methods is universally applicable and it may serve a purpose to try to identify families of compounds for which one of the techniques is clearly superior or inferior. The advantages and disadvantages of each of the methods can now be seen fairly clearly from the point of view of analytical organic chemistry; delineating these may facilitate choices among the available methods.

Finally, it may be profitable at this point to speculate briefly upon the future directions that may be taken by mass spectrometry in general and how these ionization techniques may impinge upon this.

B. Technical Aspects of Ionization Techniques

1. Mass Spectrometer Performance

For many practicing mass spectrometrists, the overall sensitivity of a mass spectrometer may be defined as the amount of compound that is necessary to obtain a useful mass spectrum. This is a very important parameter; it is unlikely that any ionization method that is less sensitive than EI by more than one or two orders of magnitude will enjoy wide acceptance, particularly by those involved in organic and biochemical analysis.

Using the above definition, it is reasonable to conclude that FI, FD, and CI have sensitivities that are roughly comparable to that of EI. Comparisons of this sort represent an area where angels fear to tread and the data are not available for a more precise statement. However, more precision is not necessary for our present purposes; all of these techniques can be employed in a routine way at the microgram level. With the ionization methods as they now stand, losses during sample introduction are probably a major factor in determining the overall sensitivity of each method. Furthermore, with any of these ionization techniques the proportion of ions formed that are successfully transmitted to the collector is very small and improvement in this area could be very helpful.

It is difficult to see how a more efficient EI source might be contrived, but this is less true in the case of FI, FD, and CI. In FI and FD, improved design of anodes, particularly multipoint arrays,[50,73] and further research into the

conditioning process[87,92,94] may be expected to improve sensitivities considerably. Likewise, in CI, the number of ion-molecule collisions occurring in the source could be greatly increased; a gain in sensitivity should result. The use of atmospheric pressures of reagent gas, as in the plasma chromatograph,[11] leads to precisely this result.

Most EI mass spectrometers are designed optimally with respect to their (EI) sources and the modification of EI mass spectrometers to CI or FI operation may be attended by a degradation in EI performance. This raises the question of the feasibility of so-called "dual" sources. Two sources for little more than the price of one is certainly a very tempting proposition, but a superficial inspection of those laboratories possessing such dual EI/CI mass spectrometers reveals that few of them measure EI mass spectra with these machines. Dual EI/FI mass spectrometers similarly are almost all operated in the FI mode. If a trend can be perceived, it is that laboratories often tend to maintain one mass spectrometer for use in the EI mode and purchase or modify a second machine for use in the alternative mode. The economics of such an approach are debatable and other alternatives may be successful in the future. Rather than trying to obtain EI mass spectra from a CI source, it may prove more feasible to measure the charge-exchange [i.e., the CI(N_2) or CI(Ar)] spectrum[196,197] in the same source. Another promising development is that of a mass spectrometer which uses separate EI and CI sources with one mass analyzer rather than a compromise dual source.[219,222]

Another interesting question relates to data acquisition methods. The manual counting of EI, FI, and CI mass spectra is, in most cases, a straightforward if tedious procedure. It is not clear whether or not this is true of FD mass spectra. A troubling feature of all published work in FI and CI is the paucity of mass spectra of truly unknown compounds of high molecular weight, i.e., of mass spectra that have to be counted.

Automatic data acquisition systems, for either low- or high-resolution mass spectra, require, with few exceptions, a satisfactory internal standard compound. To be useful in high resolution data acquisition, a reference mass spectrum should have moderately intense ions at intervals of about 12 amu. This interval can be much larger in low-resolution mass spectra. Perfluorokerosene (PFK), which has been used for many years as an internal standard in EI mass spectrometry, fails to give a satisfactory CI mass spectrum with many reagent gases;[213] it gives an acceptable FI mass spectrum only when an FI anode activated at high temperatures is used.[87] In CI(CH_4) mass spectrometry, high molecular weight n-alkanes can be used as internal standards[258] since they give ions at intervals of 14 amu. Fully deuterated n-alkanes have been used as reference compounds to avoid obscuring alkyl ions that appear in the mass spectrum of the sample compound.[213] Alkanes of a sufficiently high molecular weight are less volatile than PFK; they are therefore less convenient to use since they cannot be admitted via a gas inlet system at room temperature.

2. Sample Introduction

The direct insertion probe that is used so commonly in EI mass spectrometers can be used satisfactorily in CI or FI sources. In a CI source, the probe, which is usually made of a metal, is inevitably floated at the accelerating potential of the mass spectrometer. If this is a high voltage, as it is in most magnetic sector machines, the probe becomes a hazard to the operator. Dealing with this is a rather minor problem and various simple protection devices are available. Any circuits involving the probe, such as probe heaters and thermocouples among others, will also be floated at this high voltage; all of these problems tend to increase the cost of the peripheral equipment. In spite of all this, direct insertion probes in CI sources work well: in fact, there is evidence[251] that they are more useful in a CI source than in an EI source.

The interfacing of a gas chromatograph with a mass spectrometer greatly increases the power of both machines; in EI mass spectrometry this interface has been the object of considerable study. In contrast to this, very little work has been reported on the interfacing of a gas chromatograph with an FI mass spectrometer. There would seem to be few problems in such an undertaking and, in fact, a report[137] on a coupled gas chromatograph-FI mass spectrometer revealed that the procedure was fairly straightforward.

With CI mass spectrometers, there is no requirement for a molecular separator. The carrier gas can be admitted to the CI source, where it is used as the reagent gas,[219,245-248] but any high voltages must be insulated from the gas chromatograph with some care. There is the further problem that

the total-ion-current monitor, which can be used as a gas chromatographic detector in EI (but apparently not in FI[137]) mass spectrometry, cannot be used in CI mass spectrometry because the bulk of the total ion current here is contributed by reagent ions. The effluent from the chromatograph must therefore be split and a flame ionization detector used, if possible.

Both of these difficulties are overcome with ease if the mass analyzer used in the combination is a quadrupole,[219-221] dodecapole,[222] or time-of-flight system.[246] Here, no high accelerating voltage is necessary and the rapid scanning possible with such machines, together with the technique of measuring total ion current by integration of the collector current, provides a gas chromatogram in what is effectively real time. The data acquisition problems, moreover, are considerably simplified by the mass spectrometers having linear mass scales. This combination is a very powerful one and is subject only to the present limitations of such linear mass analyzers, viz., low mass range and resolving power.

C. Ionization of Different Types of Compounds

If a molecule can be volatilized unchanged, then the EI, FI, or CI mass spectrum can be measured with equal ease. The EI mass spectrum will almost always contain more fragment ions than either the FI or the CI mass spectrum; in general, the molecular weight of an unknown must, as a first step, be assumed to be that indicated by the highest mass ion (ignoring ions containing minor isotopes) in the FI and CI mass spectra.

While the molecular weight of a compound can be derived most reliably from its FI or CI mass spectrum, care must be taken in this exercise. In FI mass spectra, most notably of polar compounds such as sugars,[156,157] the intensity of the ion at m/e (M + 1) often exceeds that of the $M^{+\cdot}$ ion, which may in fact be absent. In CI mass spectra, the MH^+ ion is often accompanied by ions of higher mass such as those at m/e (M + 29) and (M + 41) in CI(CH_4) mass spectra. Such ions can usually be correctly identified and present no serious problems. Some molecules give intense ions at m/e (M + 1) and also m/e (2M + 1); in some of these cases it may be difficult to choose between M and 2M for the molecular weight. In both CI and FI mass spectra, then, some care must be taken with molecular weight assignments; in spite of this they are far more powerful techniques than EI mass spectrometry in this particular area.

There are now many cases in the literature where satisfactory $M^{+\cdot}$ or MH^+ ions are observed in the FI or CI mass spectra, while only fragment ions are found in the EI mass spectrum. Some generalizations are possible in this connection. The identity of functional groups emerges most clearly from the CI mass spectrum, while information as to the carbon skeleton of the molecule is more likely to be found in the EI mass spectrum. The similarities between FI mass spectra and low voltage EI mass spectra have been noted,[116] and fragmentation reactions that are important in EI may also be seen in FI. There is, however, much less common ground between EI and CI. Bonds such as -C(OH)-C(OH)- that tend to cleave readily in EI are frequently quite stable towards reagent ions such as $C_4H_9^+$ that tend to promote quite different routes of fragmentation. In this sense CI is truly complementary to EI.

FI can complement EI in a different sense because they both are concerned with the formation and stability of the odd-electron ion $M^{+\cdot}$, though on different time scales. Thus the field ionization kinetic studies of Burlingame and Derrick and their co-workers[108,109,116,117] have succeeded in resolving some of the very fast unimolecular decompositions of ions. Work in CI mass spectrometry, for its own part, has potentially important implications in the gas phase and condensed-phase chemistry of ions.

In cases where the compound under study cannot be volatilized without some thermal breakdown, the only useful technique of the four under consideration is FD. Mass spectrometry has been applied for years to the analysis of large, polar, biologically important molecules such as proteins, nucleic acids, and their degradation products, but with limited success; this new method promises to open up these areas of biochemistry to mass spectrometry.

Both CI and FI can be used successfully with a wider variety of molecules than EI, but CI in particular has some weaknesses. For compounds of moderate polarity, such as amino acids[152] and alkaloids,[273] it is an ideal technique; it is perhaps less satisfactory for nonpolar molecules such as alkanes and alkenes. Its most serious difficulty is with relatively nonpolar, acid-labile molecules; with compounds of this sort, such as triglycerides and the simpler sterols and their esters, the

ionization method of choice is either EI or FI. The greatest strength of CI is the range of available reagent gases, which can be exploited to increase or decrease the stability of the protonated molecular ion that is formed.

In general, compounds are more likely to give stable molecular ions in FI than stable MH^+ ions in CI. However, the absence of $M^{+\cdot}$ ions in the FI mass spectra of some large molecules such as cerberoside (16)[162] and the low intensities of these ions in the spectra of thermally labile molecules [e.g., PETN (3, R = ONO_2)][146] has been noted. Another of the problems of FI mass spectra is the relative absence of useful fragment ions such as peptide sequencing ions.[144]

D. Advantages and Disadvantages of Field Ionization, Field Desorption, and Chemical Ionization

Like EI, FI is a fairly standardized and relatively inflexible technique. The only parameter of significance that can be easily changed in FI mass spectrometry is the temperature of the FI anode; while temperature is important in FD,[97] it has rather little effect upon FI mass spectra.[100,101]

Compared to FI, CI is extremely flexible in that a wide range of reagent gases may be drawn from, permitting one to alter at will the proton affinity of the reagent gas. Thus the selectivity of protonation and also the extent of fragmentation in the protonated molecular ions formed can also be altered. This latter feature may be varied widely by changing the source temperature.

Both CI and FI can be made to produce very simple mass spectra from most types of compounds; this has made possible the mass spectrometric analysis of mixtures of compounds. The EI mass spectra that are obtained from such mixtures are prohibitively complex, even for computer handling; the traditional approach to this problem has been to separate the mixture into its components, and record the EI mass spectra one at a time. Recently, the technique of mass fragmentography[249] has received increasing attention as a method of monitoring a specific compound in a mixture by focusing on an ion in the mass spectrum that is peculiar to this compound. The MH^+ ion in the CI mass spectrum, like the $M^{+\cdot}$ ion in the FI mass spectrum, is often the only intense ion present; it can be used as a fragment ion is used in mass fragmentography to quantitate the individual components of a mixture. A molecular ion may, in some cases, be more unique than a fragment ion, while precisely the reverse may be true in other cases. The analysis of mixtures of drugs using CI mass spectrometry has been reported,[271,292] and FI mass spectrometry has been used similarly for the analysis of the hydrocarbons in crude oil.[130,141]

The advantages of FD are very clear, but its disadvantages are less easy to define since there are relatively few FD data available to date. From the papers that have been published, it would appear that the greatest single problem associated with FD mass spectrometry is with the temperature of the FD anode. In order to produce an FD mass spectrum, the conditioned anode is coated with the compound. Inserted into the ion source, the anode is then maintained at a high voltage and heated electrically, whereupon material diffuses over the surface of the anode until it reaches the tip of a microneedle where FD takes place. The difficulty with electrical heating of the anode is that the microneedles, which are appendages on the wire, are usually cooler than the wire; because of this, FD may be spasmodic and, more importantly, in competition with pyrolysis. Winkler and Beckey[98] have recognized this problem and have explored the possibility of indirect heating of the anode. This improves the situation, but the question of local temperature gradients remains one of the more poorly understood aspects of FD mass spectrometry.

E. Future Prospects in Ionization Techniques

Research in CI and, to a lesser extent, in FI and FD mass spectrometry is at a turning point in that the initial phase of exploratory work is essentially complete; the general possibilities of each of the methods are known. It is, therefore, of some interest to speculate about the areas that may become important in the future.

CI mass spectrometry is slowly coming into use as an analytical technique, complementary to EI mass spectrometry. However, some of the exciting possibilities of CI have been a little neglected; it is hoped that more work will be done on, for example, proton affinities. The studies of gas-phase equilibria carried out by Field[315,316,319] and Kebarle[317,318] may well lead to a superior method for the determination of proton affinities. As is suggested by the work of Dzidic[225] and Munson[226] and others, the judicious use of several reagent gases of accurately known proton affinities could yield a wealth of structural information that

is currently inaccessible. The question of whether or not functional groups per se have identifiable proton affinities has yet to be answered. Entirely too little work has been done with very mild reagent ions with a view to distinguishing between structural isomers and between stereoisomers. In view of the very low exothermicities possible in protonation reactions, this may prove to be an interesting field. Finally, the possibilities of high-pressure mass spectrometers with a very high level of sensitivity are very exciting, as the initial results with the plasma chromatography[11] indicate.

Some of the interest surrounding FI has been stolen by FD; nevertheless, there are many unanswered questions in FI mass spectrometry that may receive some attention in the near future. The technique may prove to be especially valuable in some specific problems such as the sequencing of oligosaccharides. The possibilities of combining CI or FI with collisional activation[33] seem to be of particular promise; much of the experience that may be gained here could be applied directly to FD mass spectrometry. The recent work of Burlingame and Derrick[108,109,116,117] on field ionization kinetics represents the first extensive work in a surprisingly neglected area; it is safe to say that much more work of this sort is under way.

In the area of large intractable molecules, however, the future clearly belongs to FD. An enormous area of analytical capability has been opened up by Beckey's few papers on the subject. It is certain that as more FD mass spectrometers are built by different groups, the technical problems will be simplified and the method will become very important indeed.

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