Mass Spectrometry of Large, Fragile, and Involatile Molecules

Kenneth L. Busch and R. Graham Cooks

After years of limited success, it is now both possible and relatively straightforward to obtain mass spectra of large, involatile, and thermally fragile molecules. This situation has extensive implications for the biological scientist. In this article we characterize the current art and place in perspective the steps that resulted in the present capabilities.

Summary. Desorption ionization makes it possible to obtain mass spectra of molecules whose vaporization by heating may lead to thermal degradation. Several methods are in use, but in general desorption is achieved by particle or photon bombardment of the sample and the mass spectra obtained by different methods are fundamentally similar. Desorption ionization techniques have been used to obtain mass spectra of biomolecules, including peptides, antibiotics, and oligosaccharides, for which normal mass spectral methods have been of limited power. Several examples are given of recent applications of these new techniques, and prospects for their further evolution are discussed.

Since its first use in organic analysis, mass spectrometry has relied largely on electron ionization (EI) to create ions from vaporized neutral molecules. An electron with an energy of 70 electron volts interacts with the neutral molecule in the gas phase and, in an electronic transition, strips an electron to leave a positive radical ion, $M^{+}\cdot$. When observed, this radical molecular ion reflects the molecular weight of the analyte, and this ion and the fragment ions resulting from its dissociation make up the mass spectrum, which can be interpreted to reveal the structure of the molecule. Electron ionization often results in an internally excited parent ion which fragments so extensively that it is not observed; information on molecular weight is then not easily retrieved from the spectrum. In 1965 Munson and Field (*1, 2*) discovered that ions themselves, rather than electrons, could act as ionizing reagents, and they termed the technique chemical ionization (CI). For example, in a source held at the relatively high pressure of 1 torr, methane can yield CH_5^{+}, which ionizes gas-phase sample molecules by exothermic proton transfer to form $(M+H)^{+}$. Many samples whose molecular weight is not indicated in the EI spectrum give CI spectra with abundant ions in the molecular weight region.

Both EI and CI require that a distinct ionizing agent act on gas-phase sample molecules; thus, vaporization of the analyte is a prerequisite. For nonvolatile or thermally fragile samples, heating the sample to vaporize it often leads to thermal degradation. Various alternative strategies have been developed to make possible the analysis of such compounds. The key to these approaches is the desorption of ions directly from a condensed phase (solid or liquid). Desorption ionization (DI) is a general term (*3*) which includes secondary ion mass spectrometry (SIMS), fast atom bombardment (FAB), plasma desorption (PD), field desorption (FD), electrohydrodynamic ionization (EHMS), and thermal desorption (see Table 1).

Desorption ionization is markedly different from EI and CI in dispensing with bulk sample vaporization and even with an ionization process per se. In essence, energy, in a variety of forms, is deposited in the sample, causing transfer of molecules and preexisting ions from the condensed into the gas phase. The simplicity of the underlying phenomenon and the ability of delicate biomolecules to withstand the impact of energetic particle beams are two striking features of DI. The various DI methods differ in rate of energy deposition, volume of sample energized, and use of electric fields to assist in desorption.

A short summary of developments in DI will be given here. In 1969 Beckey (*4*) published a mass spectrum of glucose, a compound of low volatility. The method he used, field desorption (*5*), proved to be the precursor of a family of ionization methods, the power of which has been enhanced by the availability of mass spectrometers with extended mass ranges. Beckey's work was founded on a thorough understanding of the physics of field emission and ionization. His contribution was the translation of this knowledge into a device having practical value in the molecular sciences.

Plasma desorption developed as an outgrowth of work in which the decay of californium was studied by time-of-flight measurement of its fission fragments. In one of the more direct transpositions of physics to biology, Macfarlane and coworkers in 1974 (*6*) used californium fission fragments to ionize thermally fragile compounds such as arginine and cystine and used the time-of-flight measurement for mass analysis. The original apparatus barely changed in becoming the plasma desorption mass spectrometer. In recent work, a portion of a thin film of insulin coated on a solid support was energized by a high-energy ^{127}I ion to produce a mass spectrum containing ions characteristic of the molecular weight of insulin, as well as fragment ions that gave useful information on the structure (Fig. 1) (*7*).

In spite of impressive successes during the 1970's with FD and PD, neither technique achieved the level of routinely successful operation and widespread availability that allowed CI, for example, to join EI as a major ionization method. In response, other techniques were developed based on desorption of a sample from an inert support. Considerable success was achieved by heating the sample with a laser beam (*8*) or placing it directly in the beam of an EI source (*9*) or in the plasma of a CI source (*10*). These latter two methods are simple and represent an advance over traditional ionization methods for biomolecules, but did not attain the ability to analyze the larger, less volatile, more fragile molecules tractable with FD and PD.

A second response to the limitations of FD and PD was the development of desorption techniques of similar power, but far greater convenience, based on secondary ion mass spectrometry. In this case, both the phenomenon—the

Kenneth L. Busch is an assistant research scientist and R. Graham Cooks is a professor of chemistry at Purdue University, West Lafayette, Indiana 47907.

0036-8075/82/1015-0247$01.00/0

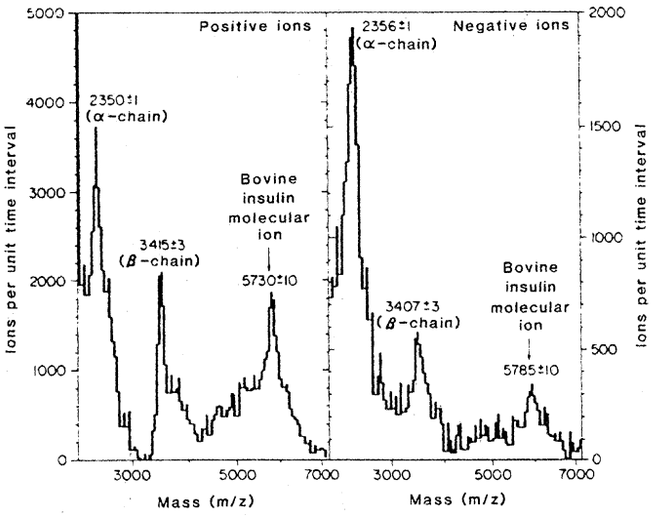


Fig. 1. Plasma desorption positive and negative ion mass spectra of bovine insulin. [Reprinted from Hakansson *et al.* (*7*) with permission from the American Chemical Society]

sputtering of ions upon particle impact—and its utilization in mass spectrometry have a long history (*11*), and SIMS is widely used today as a sensitive method of elemental analysis, capable of microscopic resolution and depth profiling (*12*). The key to translating this into a procedure for molecular analysis was found in a concept introduced by Benninghoven (*13*). If the flux of primary ions is kept sufficiently low, there is a negligible probability that any area of the surface will twice suffer ion impact during an experiment. This "static" SIMS condition was developed to avoid sputtering through monolayers during an experiment. It was soon found by Benninghoven (*14*) and others (*15*) that, under these conditions, bulk organic compounds at surfaces could be examined without thermal damage. Spectra similar to those recorded by FD or PD were obtained, but the experiment was far simpler. Samples persist for minutes to hours under the low flux of bombarding ions, allowing spectral features of interest to be carefully examined. Many of these first molecular SIMS studies utilized quadrupole mass analyzers of limited mass range, and the stated potential for desorption of higher mass molecules was not appreciated. The need for sensitive analyses and structurally informative spectra of biomolecules (*16*) was such that when FAB became commercially available in 1981, the response from potential users was overwhelming. FAB is distinguished from SIMS in two ways. First, the sample is energized by a neutral rather than an ion beam. Fundamentally, this is a minor difference, and indeed neutral beams had been used before for inorganic and organic analysis (*17*). A practical advantage of the neutral beam is that sector instruments with sources at high voltages can be easily fitted with such DI sources. The second and more significant difference involves sample preparation. By desorbing ions from a solution of glycerol, this method sidesteps the static SIMS requirement by providing a method, diffusion within a solution, of continuously supplying analyte to the interface.

The analytical capabilities of these new DI methods have opened up exciting new areas of biomolecular analysis. Figure 2 shows the SIMS spectrum of vitamin B_{12}; just a few years ago it would have seemed impossible to produce such a spectrum. FAB has been vigorously applied to structural problems in biological and pharmaceutical studies. Figure 3 shows the FAB spectrum for the antibiotic vancomycin, and the spectrum of insulin has also been reported (*18*). The literature is replete with examples of this nature, testifying to the rapid acceptance of these new methods.

An aspect of DI which holds promise for all molecular analysis is the prospect of further improvements in the already notable sensitivity of mass spectrometry. The reason is fundamental. Losses to the vacuum system as the sample is vaporized are avoided; in DI the analyte need never be in an uncharged state in the gas phase. Given sufficient time, a significant fraction of the sample initially present in the ionic form can be desorbed. Consider that although detector efficiencies are almost 100 percent, 1 microgram of methyl stearate yields in EI and CI a total charge of only about 10^{-8} coulomb, giving an overall efficiency of about 1 part in 10^6. The losses are thought to be equally distributed between the ionization process and the extraction–mass analysis step. High-quality instruments have a transmission

Table 1. Techniques of desorption ionization. The dates correspond to the first sustained use for biological/organic analysis by these techniques.

Field desorption (FD). 1969. Samples are placed on microdendrites, usually carbon grown on a fine metal wire. Ions are desorbed by the combined action of heat and the very high fields present in the source. Commercial sources are widely available, but the technique has a reputation for erratic performance, and ion currents are transient and not intense. Nevertheless, laboratories specializing in the technique have an impressive record of achievement.

Plasma desorption (PD). 1974. Samples are supported on a thin foil and energized by the passage of high-energy fission fragments (from ^{252}Cf) or ions from a particle accelerator. Mass analysis is normally performed by time-of-flight measurements. No commercial systems available.

Secondary ion mass spectrometry (SIMS). 1977. Samples, usually in solid form but often mixed with a solid matrix, are energized by ions of kilo-electron-volt energy. Low fluxes of ions are used for recently developed (middle 1970's) molecular SIMS and high fluxes for inorganic analysis and depth profiling. Commercial systems are designed for elemental analysis and are not optimized for organic analysis. Add-on units are commercially available.

Electrohydrodynamic ionization (EHMS). 1978. Samples are dissolved in glycerol containing an electrolyte. Desorption takes place directly from solution under the influence of high fields without the application of heat. Spectra are marked by the almost complete absence of fragment ions. Success has been attained in determining the molecular weight distributions of lower polymers. Not commercially available.

Laser desorption (LD). 1978. Samples are prepared in various ways since both reflection and transmission experiments are performed. Applications in inorganic analysis predated the first organic studies in the late 1960's. The tendency toward thermal degradation is greater than in other desorption ionization methods. Commercial systems available with time-of-flight mass analysis.

Thermal desorption. 1979. Samples are introduced on a direct probe into the source. Heating of the probe tip desorbs ions and neutrals; no ionization filament is used. Historically, the technique has been used for inorganic analysis; recent work has included analyses of organic salts.

Fast atom bombardment (FAB). 1981. Samples, usually in solution (often glycerol), are energized by atoms of kilo-electron-volt energy. Fluxes are higher than in SIMS. Add-on sources of several types are available. Widely used for biological samples, especially in the pharmaceutical industry.

of approximately 10^{-3}, and this cannot be easily improved. The potential of DI lies in reducing losses during the ionization process; estimates of the sampling efficiency for certain precharged organic species have been as high as 25 percent.

Characteristics of Desorption Ionization Mass Spectra

In this section we seek to illustrate, with examples involving small molecules, some of the characteristics of the ionization methods under discussion. Choices are available with respect to the type of ion generated to represent the intact molecule. For example, pyridine oximes (*19*) can be characterized as the cationized molecule, that is, the adduct with a metal ion. Alternatively, the sample can be pretreated, for example, with acid or base, to favor the $(M+H)^+$ or $(M-H)^-$ ions. More extensive derivatization, for example, preparation of a pyridinium salt, is another approach. Here chemical selectivity is exercised in the derivatization reaction, and spectral selectivity is exercised in the formation of a distinctive ion desorbed intact with high efficiency. The results of these choices are illustrated in Fig. 4. Not only can cross-checks be made on molecular weight by using these several types of ionized molecules, but low detection limits and long examination times can be attained as well, particularly in the analysis of solid samples. In SIMS it is usual to be able to obtain spectra for many hours from a 1-μg sample; for precharged organic salts, 1 to 10 nanograms produces a full spectrum.

Ions resulting from fragmentation of the intact molecule occur in most DI measurements, although markedly reduced fragmentation is observed in both EHMS and FD. The other DI methods are not "soft" ionization methods in the sense of minimizing fragmentation. Rather, they tend to provide both molecular weight data and structural information, the latter reflected in the fragmentation pattern. While detailed gas-phase fragmentation chemistry is of interest to few nonspecialists, users of DI mass spectrometry should recognize that DI spectra are interpretable in analogous fashion to other mass spectra, particularly CI spectra, which also derive from precursor ions with closed electron shells. Figure 5 shows fragmentation patterns for a SIMS spectrum of the quaternary salt candicine chloride.

Control over the internal energy of the ions is a valuable feature of any ionization method (*20*). It allows fragmentation patterns to be adjusted to emphasize reactions of interest. An excellent method of achieving internal energy control is through the use of tandem mass spectrometry (MS-MS) (*21*). In this procedure, the desorbed parent ion is isolated from all others and then excited in a controlled fashion; resulting ionic products are mass analyzed to give an MS-MS spectrum. The energy imparted is controlled through selection of either the collision energy or the angle through which the collision products are scattered. This feature is one of a number of reasons why the combination of MS-MS with DI is eliciting intense interest. Other methods for achieving control of ion internal energy in DI are not well established. Laser wavelength (*22*) and kinetic energy of the bombarding particles do not exhibit the direct control over DI mass spectra that is shown by energy of the ionizing electrons in EI or proton affinity of the reagent ion in CI. The flux of the energizing radiation is correlated with the extent of fragmentation, but the effects are not large. Desorption from solution seems to decrease the degree of internal excitation (Fig. 5).

The matrix from which DI spectra are recorded has significant spectral effects. In particular, the formation of adduct ions in which cations are attached to analyte molecules involves reactions between the matrix and analyte. Matrices may isolate substrate molecules and minimize intermolecular reactions. Diffusion in liquid matrices maintains a high concentration of analyte at the surface, despite depletion by sputtering, allowing higher fluxes of bombarding particles to be employed. This effect forms the basis for FAB and should have general merit in DI. A physical mixture of sample in a solid matrix shows analogous behavior to a solution; the quality (signal-to-noise ratio) of SIMS spectra improved as the analyte was diluted as much as 100-fold in solid ammonium chloride (*23*). The low heat of vaporization of this salt may account for its effectiveness. Use of sim-

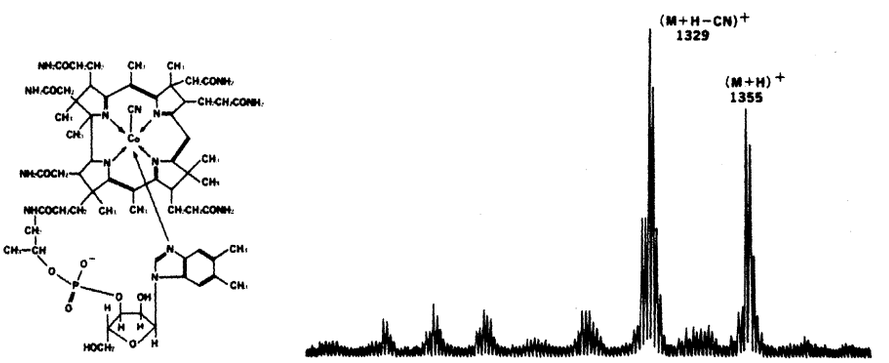


Fig. 2. Secondary ion mass spectrum of vitamin B_{12}. [Courtesy of Hitachi Instruments, Tokyo]

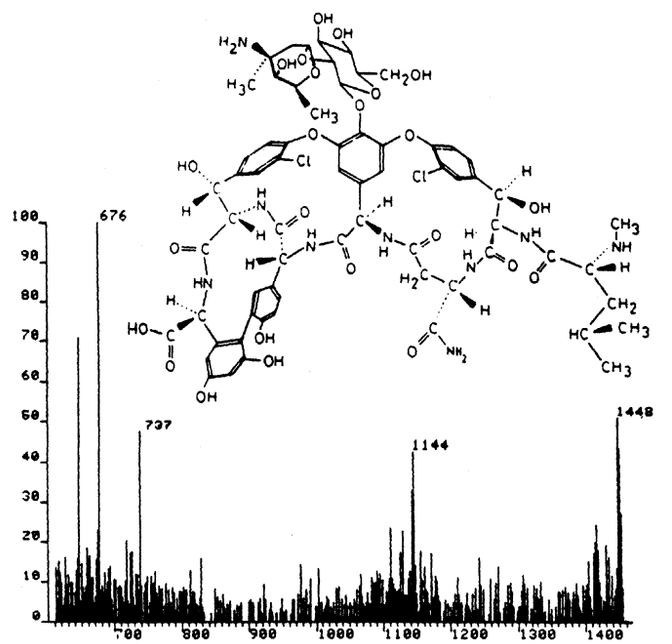


Fig. 3. Fast atom bombardment spectrum of vancomycin. [Courtesy of Kratos Instruments, Manchester, United Kingdom]

ple solid matrices avoids one major limitation of solution matrices, namely, the high level of chemical noise and poor signal-to-noise ratios typical of these spectra. This in turn suggests that matrix isolation procedures such as those of Michl and co-workers (*24*) may prove useful for attaining the lowest possible detection limits.

A characteristic of DI spectra not often noted when large molecules are examined is the occurrence of cluster ions. These ions, familiar from spectra of metals and salts obtained by sputtering, are also evident, for example, in the SIMS spectrum of frozen benzene, which extends beyond mass 1000 (*25*), in the PD spectrum of chlorophyll (*26*), and in LD spectra of smaller organics (*27*). Individual molecular units may retain their identity in the cluster, but in some circumstances they do not, as in the chemically interesting but not analytically useful spectra of nitrogen oxides obtained at cryogenic temperatures (*28*).

The use of DI, rather than EI or CI, does not engender any compromises in mass spectrometer performance. Thus, exact mass measurements have the expected value as a means of assigning molecular formulas. They are made in the usual way with a conventional double-focusing mass spectrometer, or they may be made by ion cyclotron resonance mass spectrometry. Comparisons of the reproducibility of FAB spectra, made with a suite of biological samples, a recipe for sample preparation, and a variety of neutral beam sources and instrumental configurations, showed results similar to interlaboratory comparisons of EI spectra, with coefficients of variation of about 20 percent for selected ions (*29*).

Ion Production in Desorption Ionization

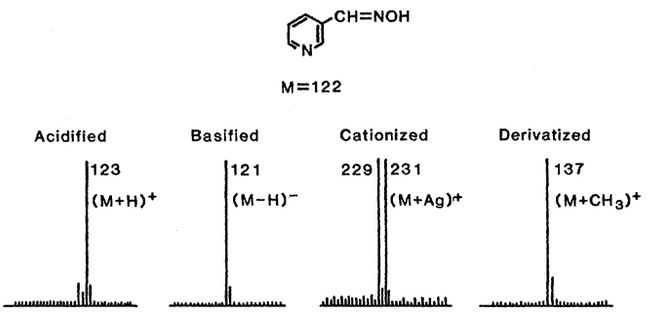
The most striking feature of DI is that similar spectra are produced by ion impact, fission particle impact, laser irradiation, and other methods of energization of the sample (*30, 31*). For example, the PD and SIMS spectra of nucleosides are virtually superimposable (*32, 33*). These similarities are explicable on the basis of a few dominant mechanisms for ion formation and a single mode of ion dissociation.

The views of a number of investigators are converging on a description of the overall DI process as an isomerization of energy (however supplied) into a common form in which it can effect desorption. The energy supplied to the sample appears as vibrational energy, couples particularly with intermolecular modes, and desorbs molecules and clusters of molecules from the condensed into the gas phase. A species can be ionized or neutralized as it traverses the near-surface region (the selvedge) and collisions can result in stabilization or internal excitation. Excess internal energy is used to jettison solvent molecules or other adduct molecules that solvate the ionic site. Once the ion leaves the selvedge, its internal energy determines the degree of fragmentation, and this is dependent on both the ionization method and the conditions employed. Nevertheless, the available set of fragmentation reactions is a function of ion structure and is, to a first approximation, independent of the ionizing procedure. (There may be exceptions in which the imprint of the ionization method is evident in the mass spectrum, as in contributions to field desorption from field ionization evident in the formation of molecular cation radicals.)

A phenomenological description of the ionization process in SIMS recognizes the operation of three mechanisms (*34*): (i) direct desorption of precharged species, (ii) cationization and anionization in which a neutral analyte M is observed as the adduct $(C+M)^+$ or $(A+M)^-$ with a cation C^+ or an anion A^-, and (iii) electron ionization processes leading to radical ions $M^{+}\cdot$ and $M^{-}\cdot$. Cluster ions occur under the various headings. These three mechanisms, listed in order of decreasing yield and utility in analysis, cover many dozens of individual observations in SIMS, and suffice to describe ions observed in DI spectra in general. For example, the role of solvent in decreasing lattice energies of ionic solids and thus allowing desorption of intact anions or cations is accommodated. These simple concepts have been tested in a variety of ways. Chemical manipulation of the sample, including derivatization reactions designed to generate ionic forms of the substrate (*35*), results in the expected enhancement of the intact anion or cation. For example, acid treatment of zwitterions results in a prominent cation at $(M+H)^+$, and base treatment results in an anion at $(M-H)^-$; the neutral zwitterion itself is desorbed in poor yield for ions of either polarity. The preferential desorption of preformed ions is a significant concept underlying DI methods. Second, simply mixing and coirradiating metals or metal salts with organic molecules results in cationization. Phenanthroline yields adducts with about half of the elements in the periodic table (*36*). Finally, the ionization energy of the compound determines if ion-radical formation is likely; such ions appear in the spectra of compounds with low ionization potentials, such as diphenylamine. The full sensitivity of DI is available only for the direct desorption mechanism in which the relatively inefficient reactions of electron ionization and ion-molecule adduct formation are circumvented. This has reversed the requirements for derivatizing samples in mass spectrometry. Traditionally, ionic or polar compounds have been derivatized to make them less polar and more volatile so that they could be vaporized and ionized. The new reverse derivatization methods (*35*) seek to create ionic derivatives for DI mass spectrometry.

In the description of DI above we recognized two zones of activity: the selvedge and the vacuum region. In the selvedge, bonds are made and broken and ion-molecule reactions lead to adducts such as cationized species. In the vacuum region, collisions are precluded and only fragmentation reactions occur. Little is known about the detailed ion-molecule chemistry of the selvedge, but the unimolecular chemistry in the vacuum region follows a well-established precedent. The best guide to predicting fragmentations (*22, 37*) in DI is a knowledge of unimolecular dissociations in CI. (Electron impact mass spectrometry is a poorer guide because of the odd-electron nature of the parent ions.) Thus the attractiveness of DI lies in its ability to provide both intact molecules and their fragments. At first it was not apparent that fragment ions had such an origin, rather than being themselves primary products of the energy deposition process. However, the evidence is now compelling that this is the usual situation. Ions can be isolated and internally activated by collision (the MS-MS experiment); the masses and relative abundances of the resulting fragment ions match those in the DI spectrum. Direct evidence that the bulk of the fragmentation occurs in the gas phase comes from experiments in which fragments generated from high-energy parent ions are selectively removed from the spectrum by use of an appropriate electric field (*38*). However, some reactions do not parallel gas phase processes, and thus might occur on the surface prior to desorption. These include the formation of small $(C-2)^+$ ions, where C is an intact cation, in the SIMS spectra of organic and organometallic salts. Low-mass fragment ions may also form on the surface, perhaps as indicators of thermal degradation. The use of high-power lasers for desorption may lead to more surface fragmentation than is caused by ion or atom bombardment.

The events that occur in the condensed phase have also been described. For incident particles with kilo-electron-



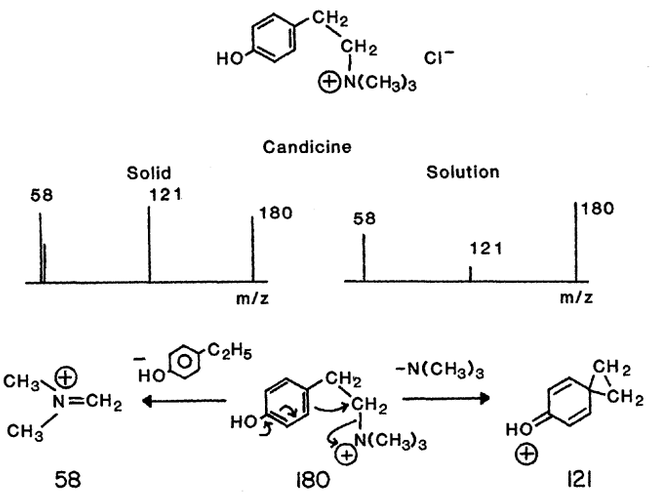


Fig. 4 (left). Four types of ions which serve to characterize an intact molecule in DI are the protonated molecule, the deprotonated molecule, the cationized molecule, and the intact cation of a simple ionic derivative resulting from quaternization. Fig. 5 (right). Fragmentation observed in DI from the desorbed intact cation of candicine chloride. The spectra also show the effect of decreased internal energy in the cation desorbed directly from solution.

volt energy, Sigmund and Claussen (*39*) have developed in some detail a collision theory which invokes the formation of energy "spikes." This model explains most aspects of secondary ion sputtering. Garrison and Winograd (*40*) have used a classical dynamics model which follows the nuclear motions of all the particles in the disrupted area of the surface with time. This calculation yields the velocities and directions of particles in the selvedge, with the premise that cluster ion formation can be predicted from a consideration of the motion of ions leaving the surface. Murray and Rabalais (*41*) have considered chemical effects of the selvedge in terms of charge exchange processes such as resonance neutralization and Auger processes. All of these models have value when applied to simple systems, but are not yet sophisticated enough to deal with some intriguing experimental observations in SIMS, particularly the desorption of intact high molecular weight molecules. The outstanding mechanistic question is whether desorption occurs by a quasi-thermal process or as a result of a collision cascade. It seems likely that the delay time between energy input and ion desorption will help answer this question. Several measurements (*21, 42, 43*) show long delays (many microseconds) for laser desorption to occur, and similar experiments for SIMS and FAB should reveal much about the emission phenomenon.

Current Applications of Desorption Ionization Mass Spectrometry

Williams *et al.* (*44*) have published a detailed DI study in which molecular weight and sequence information were determined for peptides with up to 21 residues. By using FAB, spectra were obtained without the derivatization procedures necessary in EI and CI. The samples could be examined for a much longer time than in a comparable FD analysis, allowing several distinct experiments to be performed. In agreement with the concept of preferential desorption of preformed ions, this study confirmed that the net charge of the protein (established at neutral *p*H through electrophoresis) is conserved in the DI spectrum. (The matrix *p*H serves to transfer ion current from the positive to the negative ion spectrum under the control of the analyst.) Peptides with mass-to-charge ratios (*m/z*) up to 2000 were sequenced with sample sizes of about 20 nanomoles. The most significant aspect of this work is that a systematic interpretation of the fragmentation chemistry was used and applied to the elucidation of unknown peptide sequences. With such a detailed spectral interpretation, isobaric amino acid residues could be distinguished.

Time-of-flight mass spectrometry in conjunction with PD has produced spectra of biomolecules in the molecular weight range of 4000 to 10,000 (*45*). Use of these spectra has been limited, however, to the confirmation or determination of molecular weight. Limitations arise because of a low level of secondary ion production, long data acquisition times, and relatively low resolution. Nevertheless, impressive studies at high mass have been published—for example, the study of insulin which we mentioned earlier (see Fig. 1) and the observation of the sodium-cationized molecular ion of a protected decanucleotide at *m/z* 6298. Molecular weights in the range 1500 to 4000 have been determined for protected oligonucleotides, and in addition, a detailed interpretation of the fragmentation processes allowed the oligonucleotides to be sequenced (*32*).

For antibiotics and oligosaccharides normal mass spectral methods of analysis have been of limited power. By using a SIMS source and a sector instrument, Kambara and co-workers (*46*) have completed systematic investigations of oligosaccharides and aminoglycosidic antibiotics. Reproducible spectra were obtained and interpreted, and structural isomers could be distinguished. Cationized molecules were observed with high abundances, and the fragmentation chemistry was analogous to that in CI processes. Spectra taken without the use of solutions, as in FAB, were notably free of interfering solvent peaks, and the signal-to-noise ratio was markedly improved. So-called in-beam methods have also been used to characterize these compounds without the complications due to a solvent (*47*).

The new DI methods have not been used exclusively in the analysis of organic molecules or biomolecules. Both LD and SIMS have been used to analyze various types of nonvolatile organometallic and coordination compounds (*48, 49*). Even when these compounds can be analyzed by EI or CI, their vaporization leads to metal deposits in the source. Sampling in DI is a much cleaner process. Using an LD instrument with time-of-flight analysis, Muller *et al.* (*48*) observed molecular ions for transition metal complexes with sulfur-containing ligands when EI failed to produce such ions. The fragmentation observed in the spectrum was controlled by varying the laser power. Higher fluxes of energy led to increased fragmentation, sometimes to the metal and ligand ions themselves. Lower power resulted in a completely interpretable spectrum, including molecular weight information. This control was used to advantage in screening complex asphaltene mixtures for metals and their associated ligands. Pierce *et al.* (*50*) used both SIMS and LD to investigate some aspects of cluster ion formation in DI analysis of metal acetylacetonates,

and used LD with MS-MS to investigate some of the cluster ions observed. Cluster ions formed between metal ions and ligands are a source of both novel chemistry and basic thermochemical information. In the higher mass ranges now accessible, cluster ions of the simple alkali halides of the form $C_{x+1}A_x^+$ have been observed at masses in excess of 25,000. Regular atomic arrays such as 3×3×1, 3×3×3, and 3×3×5 have been recognized (*51*) in sputtered CsI clusters of the type $[Cs(CsI)_n]^+$.

The first examples of direct analysis of complex mixtures in DI mass spectrometry are now appearing (*52*). It is often necessary to detect and quantitate a targeted compound in the presence of a large excess of endogenous material. The tendency for precharged species to be desorbed with high efficiency makes the detection of such species possible even in complex extracts of biological tissue. However, the recommended procedure is to examine simple mixtures of chemically similar compounds, as in the detection of opiates in urine by Benninghoven and co-workers (*53*), to employ chromatography in conjunction with DI, or to use MS-MS techniques. For example, LD has been combined with MS-MS in the identification of the quaternary alkaloid candicine in simple aqueous extracts of cactus tissue (*54*). Here, the selective desorption of the precharged species from the complex matrix was combined with the high selectivity of MS-MS to allow an unambiguous identification. This experiment is suited to screening of a large number of samples, and stands in contrast to more time-consuming methods of extraction and isolation.

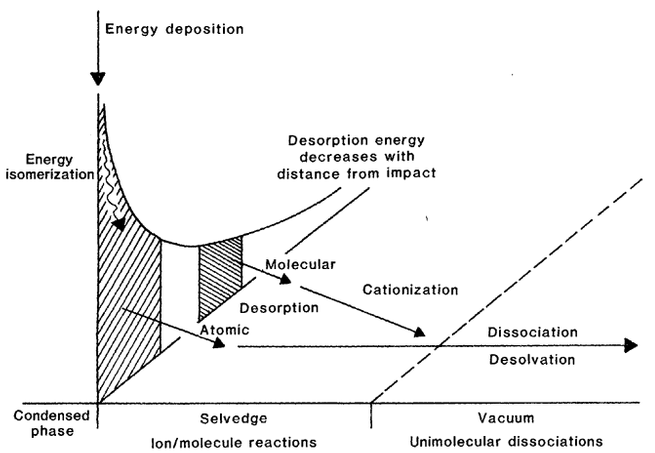


Fig. 6. Simple model for desorption ionization. [Reprinted with permission from Busch *et al.* (*55*)]

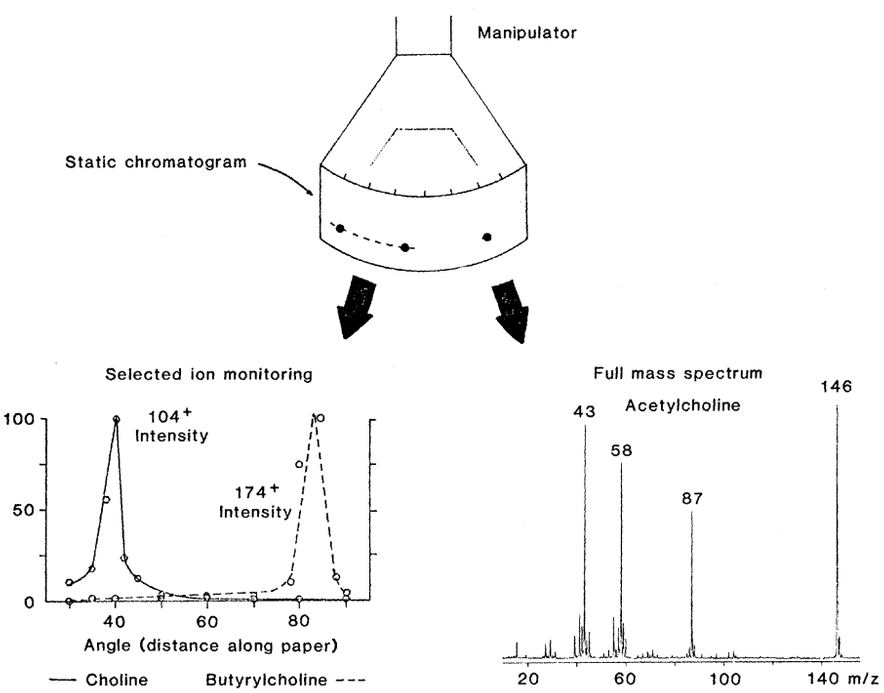


Fig. 7. Interface of DI mass spectrometry with paper and thin-layer chromatography and electrophoresis. Data are for choline and butyrylcholine examined on paper. [Reprinted from Unger *et al.* (*69*) with permission from the American Chemical Society]

Developments in Desorption Ionization

Desorption ionization has so recently emerged as a technique for molecular analysis that the range of applications is not yet fully evident. Much of the available information on DI is summarized in Fig. 6 (*55*). A key role is played by chemical rather than purely physical processes, as recognized for FD several years ago (*56*). Dynamical models of sputtering and a consideration of electron transfer processes inform our understanding of the desorption step. But it is the chemical reactions in the selvedge and the unimolecular reactions in the vacuum region which have dominant effects. The limited importance of the physical processes is indicated by the similarities between photon and particle desorption spectra of large molecules and by the long delays (tens and hundreds of microseconds) measured (*22, 42, 43*) between energy input in a laser pulse and ion desorption.

There are compelling reasons for applying MS-MS in analytical mass spectrometry (*57*). These include capabilities for direct mixture analysis with high speed and molecular specificity, and for the identification, not only of targeted compounds, but of groups of compounds sharing common structural features. MS-MS is particularly effective in removing chemical noise, that is, the background signal due to contaminant species. It also allows ion internal energy, and hence degree of dissociation, to be controlled (which is valuable when the DI method produces a molecular ion with minimal fragmentation, as in FD). MS-MS has been coupled with FD (*58*), LD (*59*), SIMS (*60*), and FAB (*61*). The rapid sequencing of peptides by MS-MS experiments on whole hydrolyzate mixtures (*61*) illustrates the type of situation in which the capabilities of DI can be expected to extend the molecular weight range of the problems amenable to solution. Commercial mass spectrometers with mass ranges of about 5000 have recently become available and will facilitate such applications as well as key experiments on nucleosides (*62*) and polysaccharides (*63*). In the future sector instruments as well as those based on time of flight and ion resonance should continue to improve in mass range specifications.

In another area of activity, chemical rather than instrumental concerns are paramount. For DI mass spectrometry there is considerable scope for the development of new derivatization procedures which are simple one-step processes that yield ionic products. Reactions can be carried out in situ, for example, in glycerol solutions or directly from the gas phase in the mass spectrometer. Chemical treatment prior to mass spectrometric examination of mixtures allows different types of components to be enhanced and characterized, and it provides additional data on individual compounds by preforming different ions with distinctive fragmentation chemistries. Simple redox and acid-base reactions provide clues (*35*) to the power of this approach (Fig. 2).

Molecular analysis of surfaces is an established area (*64*) of application for DI, and interest is likely to continue at a high level. The successful characterization of inorganic compounds dispersed on substrates such as alumina and silica (*65*) would appear to presage the molecular analysis of catalyst surfaces by DI. Transition metal complexes such as $Ni[P(C_6H_5)_3]_2Cl_2$ and $(\eta^5\text{-}C_5H_5)Ni[P(C_6H_5)_3]Cl$ have been desorbed from both silica and alumina surfaces. The particle and photon beams employed in DI can induce surface reactions. For example, thiophene adsorbed on a polycrystalline silver surface can be hydrogenated under argon ion bombardment (*66*). This reaction, discovered in a SIMS spectrometer, was confirmed in experiments in which thiophene vapor was passed over powdered silver at elevated temperature.

Several aspects of DI mass spectrometry seem to offer new advantages for the combination of mass spectrometry with chromatography. In liquid chromatography, the sample is provided to the mass spectrometer dispersed in distance on a solid support; the distance dimension represents the time dimension in chromatography. On-line experiments with continuous belt introduction systems have been reported (*67, 68*). In thin-layer and related forms of chromatography, such as paper chromatography and electrophoresis, distance dispersal is also inherent in the separation, and the chromatogram can be directly examined by photon or particle forms of DI (Fig. 7) (*69*). The advantage of coupling this type of chromatography to DI is the ability to work off-line, partitioning spectral acquisition time according to the demands of the analysis, and not the dictates of chromatography. A felicitous combination is that of electrophoresis (*70*), which separates ions, and DI, which is efficient at ion desorption. The structure and molecular weight information provided by mass spectrometry should be a valuable adjunct to the rapid separation and empirical characterization achieved by electrophoresis. Extensions to two-dimensional electrophoresis are awaited. Laser desorption may be superior in these experiments because it allows a greater sampling depth.

There is a remarkable similarity in mechanism (*71*) between DI and the ionization process which occurs in thermospray methods of interfacing a liquid chromatograph to a mass spectrometer (*72*). In the latter case, small droplets are desolvated by thermal evaporation until a single molecule of analyte remains. Adventitious alkali ions can remain associated with the analyte molecule to give cationized species analogous to those observed in DI. Alternatively, for precharged ionic compounds, solvent evaporation yields the ions directly. Sputtering in DI also involves the ejection of large clusters of ions, which undergo desolvation as a result of their excess internal energies. The common mechanistic features of sputtering, laser ablation, and thermal nebulization or evaporation should further facilitate applications of DI and lead to a more detailed understanding of its mechanism.

An exciting area of future application may be in molecular microscopy. Microprobe methods, which provide elemental distributions with a resolution of 1 micrometer or less, are well established. This form of SIMS has been important for years, and a commercial laser desorption instrument (*73–75*) was designed for such measurements in biological tissue. For molecular analysis on this scale, two conflicting demands must be met: (i) the need to maintain a low bombarding energy flux to minimize thermal damage to the substrate and (ii) the need for a high flux to provide a sufficient signal. A great contribution to biology could be made by the successful development of a device based on the combination of desorption ionization with microscopy.

We have attempted to provide a glimpse of the creativity, expressed through instrument development, that has led to the new methods for mass spectrometric analysis. Progress has been steady from the first rapid heating experiments by Friedman and co-workers (*76*), in which biological compounds were supported on an inert polymer surface, to the current proliferation of techniques for desorption and ionization directly from solution (*77*) and present interest in the bombardment of surfaces by massive particles (inorganic and organic) (*78, 79*). The endeavor has been fueled by both the needs of nonspectroscopists for a sensitive, specific, and flexible method of compound identification, and the interest of the investigators themselves in understanding and extending the capabilities of their methods.

References and Notes

1. M. S. B. Munson and F. H. Field, *J. Am. Chem. Soc.* **88**, 2621 (1966).
2. B. Munson, *Anal. Chem.* **49**, 772A (1977).
3. This terminology is widely, but not universally, accepted. Compare W. V. Ligon, *ibid.* **54**, 588 (1982).
4. H. D. Beckey, *Int. J. Mass Spectrom. Ion Phys.* **2**, 500 (1969).
5. ———, *Principles of Field Ionization and Field Desorption Mass Spectrometry* (Pergamon, Oxford, 1977).
6. D. F. Torgerson, R. P. Skowronski, R. D. Macfarlane, *Biochem. Biophys. Res. Commun.* **60**, 616 (1974).
7. P. Hakansson, I. Kamensky, B. Sundqvist, J. Fohlman, P. Peterson, C. J. McNeal, R. D. Macfarlane, *J. Am. Chem. Soc.* **104**, 2948 (1982).
8. M. A. Posthumus, P. G. Kistemaker, H. L. C. Meuzelaar, M. C. Ten Noever de Brauw, *Anal. Chem.* **50**, 985 (1978).
9. M. A. Baldwin and F. W. McLafferty, *Org. Mass Spectrom.* **7**, 1353 (1973).
10. G. Hansen and B. Munson, *Anal. Chem.* **50**, 1130 (1978).
11. H. Liebl, *ibid.* **46**, 22A (1974).
12. J. A. McHugh, in *Methods of Surface Analysis*, A. Czanderna, Ed. (Elsevier, New York, 1975), pp. 223–278.
13. A. Benninghoven, *Surf. Sci.* **35**, 427 (1973).
14. ——— and W. Sichtermann, *Org. Mass Spectrom.* **12**, 595 (1977).
15. H. Grade, N. Winograd, R. G. Cooks, *J. Am. Chem. Soc.* **99**, 7725 (1977).
16. R. A. Kerr, *Science* **216**, 163 (1982).
17. F. M. Devienne and J.-C. Roustan, *Org. Mass Spectrom.* **17**, 171 (1982).
18. A. Dell and H. R. Morris, *Biochem. Biophys. Res. Commun.* **106**, 1456 (1982).
19. A. Vincze, K. L. Busch, R. G. Cooks, *Anal. Chim. Acta* **136**, 143 (1982).
20. S. A. McLuckey and R. G. Cooks, in *Tandem Mass Spectrometry*, F. W. McLafferty, Ed. (Wiley, New York, 1982).
21. S. A. McLuckey, G. L. Glish, R. G. Cooks, *Int. J. Mass Spectrom. Ion Phys.* **39**, 219 (1981).
22. G. J. Q. van der Peyl, J. Haverkamp, P. G. Kistemaker, *ibid.* **42**, 125 (1982).
23. S. E. Unger, R. J. Day, R. G. Cooks, *ibid.* **39**, 231 (1981).
24. H. T. Jonkman, J. Michl, R. N. King, J. D. Andrade, *Anal. Chem.* **50**, 2078 (1978).
25. G. M. Lancaster, F. Honda, Y. Fukuda, J. W. Rabalais, *J. Am. Chem. Soc.* **101**, 1951 (1979).
26. J. E. Hunt, R. D. Macfarlane, J. J. Katz, R. C. Dougherty, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1745 (1980).
27. B. Schueler, P. Feigl, F. R. Krueger, F. Hillenkamp, *Org. Mass Spectrom.* **16**, 502 (1981).
28. R. G. Orth, H. T. Jonkman, J. Michl, *J. Am. Chem. Soc.* **103**, 1564 (1981).
29. R. C. Murphy and K. L. Clay, paper presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics, Honolulu, 1982.
30. C. J. McNeal, *Anal. Chem.* **54**, 43A (1982).
31. G. D. Daves, Jr., *Acc. Chem. Res.* **12**, 359 (1979).
32. C. J. McNeal, S. A. Narang, R. D. Macfarlane, H. M. Hsuing, R. Brousseau, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 735 (1980).
33. W. Ens, K. G. Standing, J. B. Westmore, K. K. Ogilvie, M. J. Nemer, *Anal. Chem.* **54**, 960 (1982).
34. R. J. Day, S. E. Unger, R. G. Cooks, *ibid.* **52**, 557A (1980).
35. K. L. Busch, S. E. Unger, A. Vincze, R. G. Cooks, T. Keough, *J. Am. Chem. Soc.* **104**, 1507 (1982).
36. R. J. Day, S. E. Unger, R. G. Cooks, *Anal. Chem.* **52**, 353 (1980).
37. H. Grade and R. G. Cooks, *J. Am. Chem. Soc.* **100**, 5615 (1978).
38. B. T. Chait and F. H. Field, *Int. J. Mass Spectrom. Ion Phys.* **41**, 17 (1981).
39. P. Sigmund and C. Claussen, *J. Appl. Phys.* **52**, 990 (1981).
40. B. J. Garrison, N. Winograd, D. E. Harrison, Jr., *J. Vac. Sci. Technol.* **16**, 789 (1979).

41. P. T. Murray and J. W. Rabalais, *J. Am. Chem. Soc.* **103**, 1007 (1981).
42. D. Zakett, A. E. Schoen, R. G. Cooks, P. H. Hemberger, paper presented at the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, 1981.
43. R. J. Cotter and R. B. vanBreemen, paper presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics, Honolulu, 1982.
44. D. H. Williams, C. V. Bradley, S. Santikarn, G. Bojeson, *Biochem. J.* **201**, 105 (1982).
45. R. D. Macfarlane, in *Biochemical Applications of Mass Spectrometry*, G. R. Waller and O. C. Dermer, Eds. (Wiley, New York, 1980).
46. K.-I. Harada, M. Suzuki, N. Takada, A. Tatematsu, H. Kambara, *J. Antibiot.* **35**, 102 (1982).
47. V. N. Reinhold and S. A. Carr, *Anal. Chem.* **54**, 499 (1982).
48. J. F. Muller, C. Bethe, J. M. Magar, *Fresenius Z. Anal. Chem.* **308**, 312 (1981).
49. J. Pierce, K. L. Busch, R. A. Walton, R. G. Cooks, *J. Am. Chem. Soc.* **103**, 2583 (1981).
50. J. L. Pierce, K. L. Busch, R. G. Cooks, R. A. Walton, *Inorg. Chem.* **21**, 2597 (1982).
51. T. M. Barlak, J. R. Wyatt, R. J. Colton, J. J. DeCorpo, J. E. Campana, *J. Am. Chem. Soc.* **104**, 1212 (1982).
52. F. W. Crow, K. B. Tomer, M. L. Gross, paper presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics, Honolulu, 1982.
53. W. Sichtermann, M. Junack, A. Eicke, A. Benninghoven, *Fresenius Z. Anal. Chem.* **301**, 115 (1980).
54. D. V. Davis, thesis, Purdue University (1982).
55. K. L. Busch, S. E. Unger, R. G. Cooks, in *Proceedings of the 1980 Münster Conference on Ion Formation from Organic Solids*, A Benninghoven, Ed. (Springer-Verlag, New York, 1982).
56. J. F. Holland, B. Soltmann, C. C. Sweeley, *Biomed. Mass Spectrom.* **3**, 340 (1976).
57. F. W. McLafferty, *Science* **214**, 280 (1981).
58. F. W. Röllgen, U. Giessmann, F. Borchers, K. Levsen, *Org. Mass Spectrom.* **13**, 459 (1978).
59. D. Zakett, A. E. Schoen, R. G. Cooks, P. H. Hemberger, *J. Am. Chem. Soc.* **103**, 1295 (1981).
60. G. L. Glish and P. J. Todd, paper presented at the 29th Annual Conference on Mass Spectrometry and Allied Topics, Minneapolis, 1981.
61. D. F. Hunt, W. M. Bone, J. Shabanowitz, J. Rhodes, J. M. Ballard, *Anal. Chem.* **53**, 1704 (1981).
62. B. Schueler and F. R. Krueger, *Org. Mass Spectrom.* **15**, 295 (1980).
63. H. Kambara and S. Hishida, *ibid.* **16**, 167 (1981).
64. R. J. Colton, *J. Vac. Sci. Technol.* **18**, 737 (1981).
65. J. L. Pierce, thesis, Purdue University (1982).
66. S. E. Unger, R. G. Cooks, B. J. Steinmetz, W. N. Delgass, *Surf. Sci.* **116**, L211 (1982).
67. A. Benninghoven, A. Eicke, M. Junack, W. Sichtermann, J. Krizek, H. Peters, *Org. Mass Spectrom.* **15**, 459 (1980).
68. R. D. Smith, J. E. Burger, A. L. Johnson, *Anal. Chem.* **53**, 1603 (1981).
69. S. E. Unger, A. Vincze, R. G. Cooks, R. Chrisman, L. D. Rothman, *ibid.*, p. 976.
70. K. L. Busch and R. G. Cooks, paper presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics, Honoloulu, 1982.
71. P. J. Arpino and G. Guichon, *J. Chromatogr.* **251**, 153 (1982).
72. C. R. Blakely, J. J. Carmody, M. L. Vestal, *J. Am. Chem. Soc.* **102**, 5931 (1980).
73. A. Mathey, *Fresenius Z. Anal. Chem.* **308**, 249 (1981).
74. H. Vogt, H. J. Heinen, S. Meier, R. Wechsung, *ibid.*, p. 195.
75. D. M. Hercules, R. J. Day, K. Balasanmugam, T. A. Dang, C. P. Li, *Anal. Chem.* **54**, 280A (1982).
76. R. J. Beuhler, E. Flanagan, L. J. Greene, L. Friedman, *J. Am. Chem. Soc.* **96**, 3990 (1974).
77. M. Tsuchiya and T. Taira, *Int. J. Mass Spectrom. Ion Phys.* **34**, 351 (1980).
78. R. Stoll, U. Schade, F. W. Röllgen, U. Giessmann, D. F. Barofsky, *ibid.* **43**, 227 (1982).
79. S. S. Wong, R. Stoll, F. W. Röllgen, *Z. Naturforsch. Teil A*, in press.
80. Supported by grants from the National Science Foundation (CHE 78-08728 and CHE 81-14410) and the MRL Program DMR 77-23798 and DMR 80-20249. J. W. Amy, W. N. Delgass, D. F. Hunt, S. E. Unger, F. Hillenkamp, F. W. Röllgen, and R. D. Macfarlane provided valuable suggestions.

Fast Atom Bombardment Mass Spectrometry

Kenneth L. Rinehart, Jr.

Summary. Fast atom bombardment mass spectrometry has become a powerful structural tool since the first reports of its use in 1981. Samples are ionized in the condensed state, usually in a glycerol matrix, by bombarding the matrix with xenon or argon atoms with energies of 5000 to 10,000 electron volts. This yields both positive and negative secondary ions, which are sputtered from the surface. The technique has been used to detect inorganic ion clusters to mass 25,800 and biologically active peptides to mass 5700, and it gives molecular ions of such highly polar or labile organic compounds as glycosphingolipids and polyene antibiotics. It can be especially valuable in determining the sequences of amino acids in polypeptides.

Recent developments in mass spectrometry have made it possible to ionize molecules of interest to biologists and biologically oriented chemists and to provide answers to vexing biological problems (*1, 2*). It is now possible to obtain mass spectra of compounds with molecular weights above 12,500 (*3*) and to obtain detailed structural information on compounds with molecular weights as high as 2800 (*4, 5*). In addition to being of high molecular weight, many of these compounds are highly polar and of generally low volatility.

The new developments in mass spectrometry which have achieved this success partly involved building larger magnets to deal with the larger molecules, but mainly involved designing methods for ionizing molecules in the solid state and desorbing them into the vapor state, where they can be detected. A number of these methods are discussed elsewhere in this issue by Busch and Cooks (*6*).

Among the recently developed ionization methods, fast atom bombardment (FAB) is the newest (*7–9*) and in several respects the most successful. Not only can it provide precise masses for molecular ions of biologically active peptides with high molecular weights, but it can provide nearly complete amino acid sequences of many of these peptides and structural information deduced from their fragmentation loci on other classes of compounds as well. FAB mass spectrometry and its applications to peptides and other biologically important compounds will be the subject of this article.

Fast Atom Bombardment Mass Spectrometry

The overall diagram of the equipment required for FAB mass spectrometry is shown in Fig. 1. This method is closely related to another condensed state ionization method, secondary ion mass spectrometry (*9a*), but different from SIMS in that the accelerated inert gas ions (preferably xenon) (*5, 10, 11*) employed in SIMS undergo charge neutralization by electron capture or charge exchange, giving rise to accelerated inert gas atoms. The residual ions may be removed by deflector plates (Fig. 1) and the accelerated atoms allowed to bombard the sample, which is usually dispersed in a glycerol solution or matrix, giving rise to both "molecular" ions, which include M+H and M+Na ions, and fragment ions. As with SIMS, a reasonable mechanism may involve "sputtering" or "splashing" the sample

Kenneth L. Rinehart, Jr., is a professor of chemistry at the University of Illinois at Urbana-Champaign, Urbana 61801.

0036-8075/82/1015-0254$01.00/0