Chemical Aspects of Fast Atom Bombardment

CATHERINE FENSELAU* and ROBERT J. COTTER*

Department of Pharmacology and Molecular Sciences, The Johns Hopkins University, Baltimore, Maryland 21205

Received October 14, 1986 (Revised Manuscript Received February 13, 1987)

Contents

Catherine Fenselau and Robert J. Cotter are co-directors of the Middle Atlantic Mass Spectrometry Laboratory, an NSF Shared Instrumentation Facility. Professor Fenselau is well-known for her publications on the use of mass spectrometry to investigate biochemical problems. Among the outstanding accomplishments of her research are the identification of the human metabolite of cyclophosphamide responsible for killing tumor cells, the identification and synthesis of Laetrile, and the characterization of acyl-linked glucuronides as a class of metabolites that can alkylate biopolymers. More recently, her research interests have included the development of mass spectrometric techniques for analysis of heavy compounds and for applications in biotechnology. Dr. Fenselau is editor-in-chief of *Biomedical and Environmental Mass Spectrometry* and past president of the American Society for Mass Spectrometry and was the 1985 Garvan Medalist of the American Chemical Society. Dr. Cotter's interests have been primarily in the development and application of new ionization techniques to the analysis of complex biomolecules. These include thermal desorption, laser desorption, fast atom bombardment, and plasma desorption mass spectrometry. He has also been involved in the development of time-of-flight mass analysis using laser desorption and liquid SIMS and has published a number of papers using a "time delay" technique for studying the mechanisms of ion formation and metastable fragmentation of heavy ions.

I. Introduction

The name "fast atom bombardment" and its popular acronym "FAB" were introduced by two research groups in Manchester[1,2] to describe a new technique for secondary ion mass spectrometry, which made it possible to easily desorb intact molecular ions from large and complex organic molecules and biomolecules. Five years later, with more than 1000 papers reporting FAB measurements, it is clear that their significant innovation was the use of the liquid matrix[3,4] rather than neutral beams, which had in fact been reported earlier.[5,6] This review addresses the chemical aspects of the formation and desorption of complex organic ions from such fluid matrices under the impact of particles possessing kilovolt translational energies. Key and exemplary references are cited; however, referencing is not inclusive, and readers interested in other aspects may consult a number of earlier reviews.[3,7–11] Except in instances where it is useful to do so, we have also not included the large body of results reported for desorption of ions in the absence of liquid matrices. These techniques, generally referred to as SIMS (secondary ion mass spectrometry), have been reviewed previously[12] and are discussed in another section of this issue.[13]

The community has spent a great deal of energy debating how best to designate and/or distinguish desorption when atoms or ions are employed as primary particles, when high- or low-energy beams are used, and when desorption takes place from liquids or dry surfaces.[14–16] To distinguish methods according to primary particle energy, the term "electronic sputtering" has been suggested by Sundqvist[16] for desorption induced by MeV particles, as such sputtering yields are associated with electronic stopping power. Analogously, therefore, one might term desorption by keV particles "nuclear sputtering", reflecting the conversion of pri-

0009-2665/87/0787-0501$06.50/0

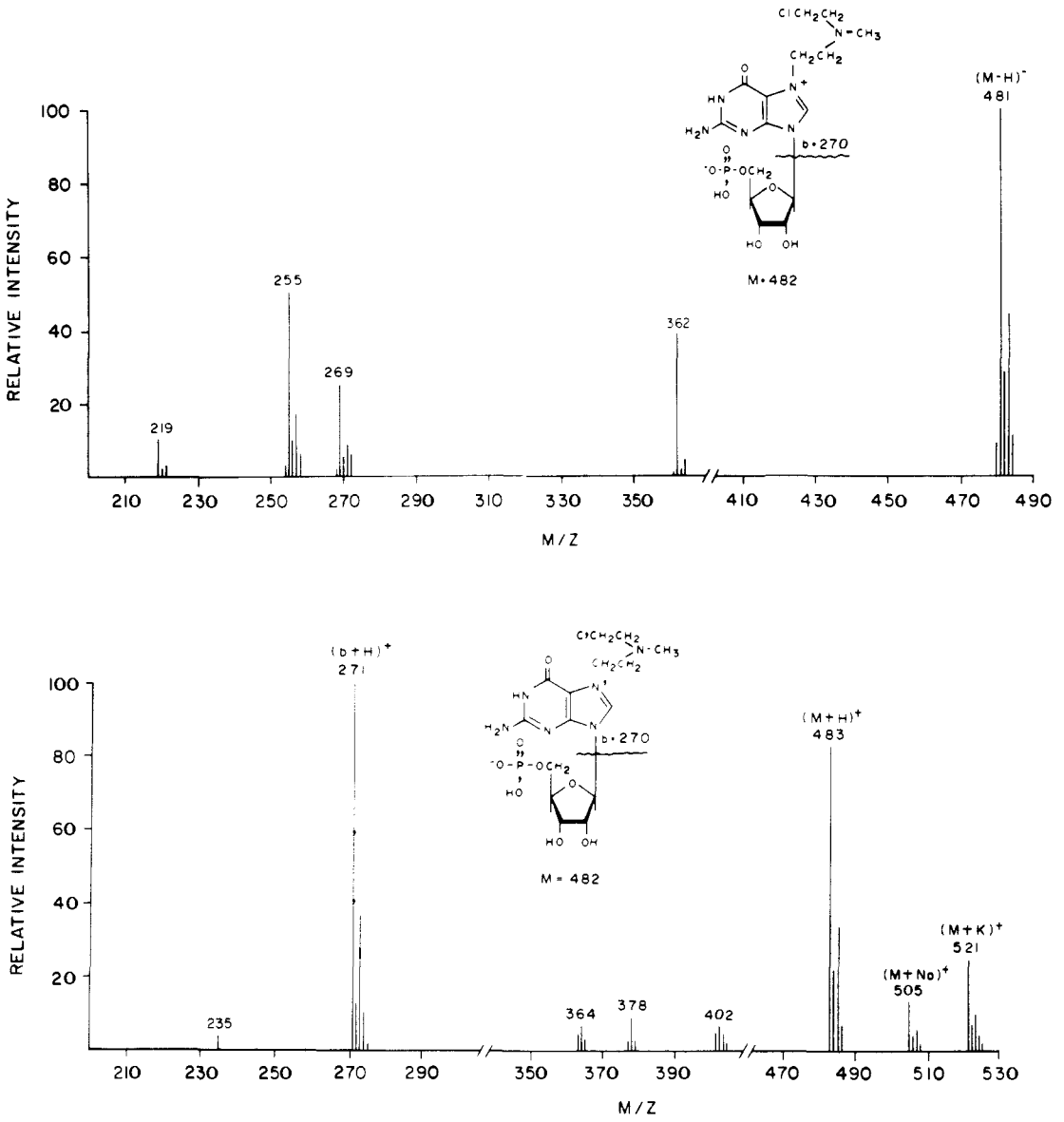

Figure 1. Partial positive- and negative-ion FAB mass spectra of the major product formed by alkylation of guanosine monophosphate by nitrogen mustard.[146]

mary particle energy into lattice vibrations in the target.[17] However, writing in an historical context, we will use the term FAB for nuclear sputtering by keV particles whenever a liquid matrix is involved, regardless of whether the primary particle is an ion or neutral atom. We note as well that such methods have also been referred to as "liquid SIMS".[14]

Spectra obtained in this configuration are characterized by high sample ion currents, comparable to those produced by electron impact, 10^{-11}–10^{-10} C/μg for the MH^+ species,[18] or secondary ion currents of 10^{-10} A.[8] Ion currents can also be prolonged, even through several hours, a feature that has made the technique compatible and highly successful with sector instruments scanning over large mass ranges.[10] The sustained strong ion current permits high resolution and MS/MS measurements.[19,20] Both positive and negative ions can be produced by fast atom bombardment. The choice between obtaining positive or negative ion spectra (or both) depends primarily upon the compound to be analyzed, rather than any inherent differences in ionization efficiency, and examples of both kinds of spectra are common in the literature.

Most FAB spectra resemble chemical ionization spectra in comprising primarily even-electron cations or anions. These often include a suite of molecular ion species formed by protonation/deprotonation, adduction with alkali metal ions, and replacement of active protons by alkali metal ions. Preformed or salt-derived organic cations and anions are also readily desorbed. Fragment ions are nearly always rationalizable by elimination of a neutral molecule from an even-electron molecular cation or anion. Hydrogen transfer is often involved, and this fragmentation also resembles hydrolysis. These features are illustrated in the positive ion and negative ion FAB spectra of a zwitterionic adduct formed between guanosine monophosphate and nitrogen mustard, shown in Figure 1. Note that in some cases the same bond is broken to produce even-electron anions and cations 2 mass units apart. These spectra illustrate the applicability of the technique to ionic, involatile, thermolabile molecules.

On the whole, molecular ion species are quite abundant in FAB spectra, and coherent fragmentation occurs to only a modest extent. Figure 2 presents the FAB spectrum of a cyclobutadiene complex of molyb-

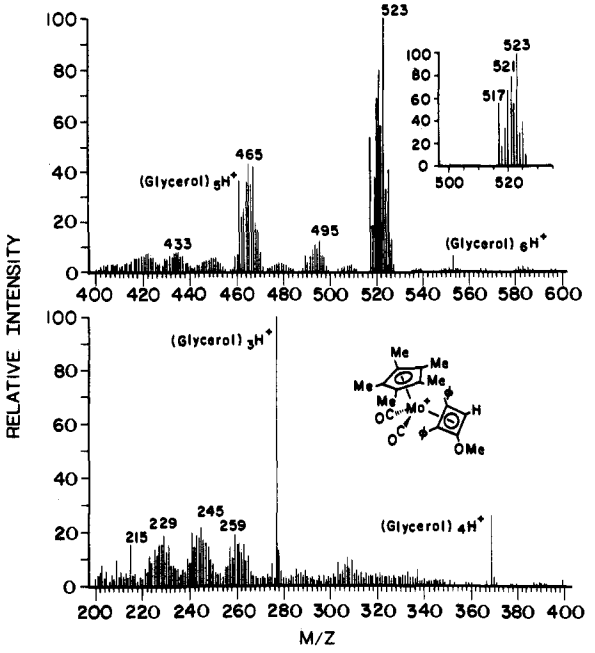

Figure 2. Partial positive-ion FAB mass spectrum of a cationic cyclobutadiene complex of molybdenum obtained on a Kratos MS-50 instrument. The inset shows the distribution of molecular ions predicted from the empirical formula.[21] The sample was provided by J. W. Reisch, Dartmouth College.[183]

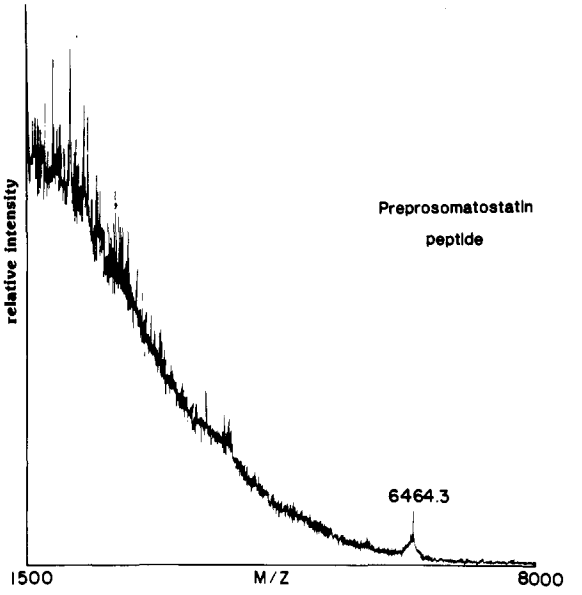

Figure 3. Partial positive-ion FAB mass spectrum[184] of an isolated preprosomatostatin peptide. The sample was provided by Dr. Phillip Andrews, Purdue University. The spectrum is plotted at a resolution of about 1000. (Reprinted with permission from ref 37. Copyring 1983 American Chemical Society.)

denum with these features. The inset portrays the molecular ion envelope calculated from the empirical formula.[21] This spectrum also illustrates the background of chemical noise, incoherent fragmentation, or peak-at-every-mass, and prominent peaks resulting from the matrix itself, which are common features of FAB spectra. The use of the liquid matrix with particle beam desorption made possible the extension of mass spectrometry to many classes of fragile organometallic compounds and complexes, such as that shown in Figure 2.[11,22-28]

This technique has also permitted many laboratories to use mass spectrometry to characterize heavier samples such as epidermal growth factors,[29,30] recombinant eglin,[31] and proinsulins[32,33] and has spurred the development of analyzers and detectors with higher mass capabilities. The capability of the technique to desorb heavy ions is illustrated in Figure 3 in a wide mass range spectrum of an unknown peptide hormone. Several low-resolution scans were signal averaged to produce the spectrum.[34] Arguments have been made for the use of lower resolution over this mass range on the grounds of increased signal-to-noise ratio and sensitivity and also on the grounds that for many questions average masses provide the most meaningful answers to the chemist or biochemist.[35-37] On the other hand, for those situations where it is advantageous to determine isotopic distributions at unit resolution, the high secondary ion currents of the FAB technique have been found to provide accurate assessments of relative abundances of isotopes. For example, theoretical and measured distributions of the complex molecular ion regions of glucagon[38] and porcine insulin[39] have been found to compare well. Strong secondary ion currents permit the use of isotope-labeled analogues as internal standards for absolute quantitation.[18,40-44]

The question of analytical reproducability has been addressed in an interlaboratory comparison led by Murphy.[45] The coefficients of variation of relative intensities of peaks in the FAB spectra were found to be comparable to those for relative intensities of peaks in an electron impact spectrum measured by the same instrumentalists on a variety of instrumental configurations.

In an early review of FAB,[8] three mechanistic models were presented for ionization and/or desorption. These were desorption of ions preformed in solution by a localized nonequilibrium vibrational process analogous to that considered operational in SIMS; evaporation of preformed ions from splash droplets analogous to proposals by Iribarne[46] and Vestal[47] for aerosols; and gas-phase ion molecule reactions analogous to the thermalized processes in chemical ionization. Subsequently, each of these mechanisms has received some experimental interrogation and support. It seems likely that multiple mechanisms exist in FAB, as they do in field desorption, and that their relative contributions vary with different kinds of samples, liquid matrices, and ionization chambers.

II. Energy Transfer and Secondary Ion Emission

Although this review focuses on chemical aspects of methods that employ liquid matrices, a comparison of the currently accepted mechanisms for energy transfer and emission of secondary particles from solid surfaces with some recent results for liquid matrices is useful, in order that chemical properties of the matrix may be discussed in that context. In particular, the "collision cascade" model, introduced by Thompson[48] and Sigmund,[49] is most often employed to describe the direct sputtering of atomic ions from metal surfaces. Secondary ion yields are directly proportional to the primary energy deposited at the surface and inversely proportional to the surface binding energy, which may be rather high (on the order of a few electronvolts) for a metal. Kinetic energy distributions are broader than

those for ions produced by thermionic emission, with maxima between 1 and 5 eV (depending again upon the surface binding energy), and the high energy "tail" falls off in a fairly predictable way.

Recent papers by Kistemaker and colleagues[50,51] and Kelner and Markey[52] have reported measurements of the kinetic energies of several small ions desorbed from a glycerol matrix and analyzed these in terms of various models for transfer of kinetic energy from the primary, impacting ion. The first group observed energy distributions of 0.3–0.7 eV (fwhm), a high-energy tail, and broadening of the distribution after evaporation of the glycerol.[50] The narrow energy distribution can be explained in part by the lowering of the surface binding energy (relative to metal surfaces) through the use of an organic matrix, which in turn increases the contribution of ions desorbed by a "collective" thermal process relative to those that are "individually sputtered" and are reflected in the high-energy tail. These workers propose a continuum of events, from a linear collision cascade, through a dense collision cascade or thermal spike, leading to a hydrodynamic process,[50] which results in ejection of large quantities of material from the sample/matrix and further reduces the binding energy. This "extended collision cascade" interpretation had been previously described by Michl[53] for the sputtering of frozen gases. In addition, this flexible model is consistent with the whole body of experimental observations, including the desorption from fluid matrices of polyatomic molecules exceeding 10 000 daltons and clusters containing more than 50 atoms. Desorption of such molecules requires interruption of many noncovalent bonds simultaneously and release of interactions with the surface, neighboring molecules, etc., without significant vibrational excitation in the molecule itself. Other scientists have suggested shock-wave[54] and percussive sputtering[53,55] to characterize rapid transmission of energy or momentum through the fluid matrix.

Another component and consequence of this emerging hydrodynamic model is the continuous spraying or removal of part of the underlying matrix along with the sample molecules or ions. This intuitively reasonable phenomenon is now being supported experimentally by studies with the kinds of thermally labile polyatomic molecules whose facile analysis distinguishes FAB from dry SIMS.[15] Measurements of the volume of glycerol sputtered per incident 6-keV Xe particle suggest that as many as 1700 molecules of glycerol are removed by each impact.[56] In another laboratory[57] 850 molecules of poly(ethylene glycol) (PEG-400) were estimated as the sputtering yield per 6-keV indium ion, based on gravimetric measurements and corrected for evaporation. A second, widespread observation that supports the occurrence of bulk sputtering is the temporal constancy of the level of incoherent fragmentation or chemical noise throughout a FAB experiment.[58,59] The radiolysis damage produced by the high-flux primary beam does not accumulate, but is continuously removed, at least until the matrix is nearly evaporated. Ablation[60] or peeling[55] of the surface in bulk has implications for surfactant samples and focuses questions on ion migration, diffusion, and mixing in the fluid matrix. This hydrodynamic splashing sputters sample ions already formed in the matrix; however, collisions and dissociations in the microdroplets provide opportunities for ion–molecule chemistry and for vibrational cooling prior to evaporation and ion ejection. This high-pressure region between the condensed phase and the vacuum has also been termed the selvedge.[61]

III. Ion Formation

A. Chemistry in Solution

The sputtering process itself does not form analytically useful ions. It seems to be generally accepted that the best way to form ions for FAB analysis is through solution chemistry in the liquid matrix, and most reports on improved spectra involve changes to the matrix. In a recent pertinent study, Watson and coworkers have correlated detection of ions in the mass spectrum with the protonation of a porphyrin in acidic solution quantitated by visible spectroscopy.[62] No ions were detected in the mass spectrometer until protonation could also be detected spectroscopically, after which the correlation was quantitative. In a similar vein, Schronk et al.[63] have noted that the relative abundances of MH^+, MH_2^{2+}, and MH_3^{3+} ions from bovine insulin directly reflect the pH of the solution. Such multiple protonation is rarely observed in chemical ionization spectra, so these experiments provide direct support for the early hypothesis that ions preformed in the solution are desorbed through the emission processes described above. Exploitation of this model usually provides the largest ion currents. Many reports confirm increases in currents of protonated molecular ions when the acidity of the matrix is increased, and anion formation by loss of a proton is enhanced in basic solutions. Organic salts, quaternary ammonium ions, for example, form a special class of preformed ions and are readily desorbed in FAB analyses. Ionization by adduction with ammonium, sodium, potassium, lithium, silver,[64] and other cations can also be promoted by addition of appropriate salts to the matrix solution. Opinions differ as to whether the cation attachment occurs in solution[65,66] or in the selvedge,[67] and it has been suggested that cation attachment depends upon the ability of the sample molecules to compete with the liquid matrix for alkali ions.[65,66]

Compounds with low redox potentials, such as quinones, can be ionized to varying extents by one-electron processes,[68–71] and these can be facilitated by addition of charge-transfer reagents to the solution.[72] The source of one-electron reducing equivalents appears to be the primary beam itself, and this has been augmented electrochemically.[73]

B. Chemistry in the Selvedge

Studies have been reported which show that ion intensity parallels proton affinity, the gas-phase property fundamental to ion–molecule reactions or chemical ionization,[74–76] and some workers argue that these trends support the ionization of volatile molecules by ion–molecule reactions in bubbles, splash droplets, or selvedge. In most of these studies solution-phase basicity parallels gas-phase basicity. In all of these studies the samples have been volatile. The extent to which this mechanism contributes to ionization vis-à-vis solution reactions probably varies greatly according to the sample and the matrix.

The suggestion made by a number of workers that molecular conglomerates and large ion clusters are sputtered, comprising sample, solvent, and additives, is an attractive one because it provides an explanation for observed analogies to ion–molecule or chemical ionization reactions, and a mechanism for cooling the sputtered ions by solvent shedding[61] to species with the relatively low internal energies which have been measured[50–52] and deduced from rates of gas-phase fragmentation.[77,78] Experimentally, larger inorganic and organic cluster ions are desorbed with greater absolute abundances and longer lifetimes from glycerol matrices than from solid samples under high fluxes. Desolvation reactions have been recorded in the metastable time frame (i.e., after ion formation and acceleration in sector instruments) in a few instances,[65,79–81] e.g.

$$[\text{trehalose} + Na^+ + \text{glycerol}] \rightarrow [\text{trehalose} + Na^+]$$

$$[\text{trehalose} + H^+ + \text{glycerol}] \rightarrow [\text{trehalose} + H^+]$$

$$[\text{glucose} + Na^+ + (\text{glycerol})_n] \rightarrow [\text{glucose} + Na^+ + (\text{glycerol})_{n-1}]$$

where $n = 1$–5. Cluster ions also offer another opportunity for proton distribution according to relative affinities. Bojeson[82] has shown that relative abundances of a_1H^+ and a_2H^+ from the metastable decomposition of cluster ions $a_1a_2H^+$ formed from binary mixtures reflect the relative proton affinities of the amino acids a_1 and a_2.

C. Ionization of Gaseous Samples

More recently several laboratories have demonstrated that ion currents can be detected from volatile samples introduced in the gas phase directly into the primary beam.[83,84] Odd-electron ion radicals are formed. Bojesen argues that this ionization takes place by charge-transfer processes.[84] Huang points out that both solution- and gas-phase ionization can be detected in spectra of some samples, the former contributing even-electron ions and the latter ion radicals.[83] (With some samples ion radicals can also be formed in solution by the redox processes discussed elsewhere.)

IV. Bringing the Sample to the Surface

In many cases ion currents can be increased when various chemical processes are employed to continuously enrich the concentration of sample at the surface. Studies with a high-flux beam pulsed at varied intervals show that the sputtering proceeds more rapidly than equilibrium can be reestablished.[85] However, plateaus are often seen in the temporal profiles of secondary ion currents (e.g., Figure 4). This suggests that some kind of steady state is achieved between removal of sample and solvent from the surface of the matrix by sputtering and evaporation and the dynamic forces of convection, diffusion, and ion migration[86,87] which create sample gradients in the matrix.

A. Surface Activity

A number of scientists have called attention to the highly efficient desorption of surface-active samples,[54,60,88,89] and discontinuities in several spectral phenomenon have been found to correlate well with the

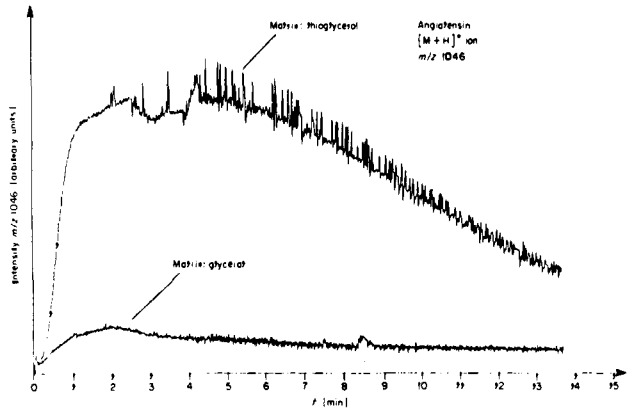

Figure 4. Profiles of the abundance as a function of time of the protonated molecular ion of angiotensin in thioglycerol and glycerol. (Reprinted with permission from ref 18. Copyright 1984 John Wiley & Sons Ltd.)

discontinuity in surface tension which indicates that the surface is covered. These include the drop to zero of the ratio of glycerol cluster ions to sample ions observed by Barber et al.[88] and the abrupt stabilization of relative abundances of sample cluster ions when sample concentration in the matrix is raised sufficiently to provide complete surface coverage.[90] Barber[88] and others[54,60,89] have pointed out that competition for the surface, i.e., differing surface activities, provides one compelling explanation for the selective desorption of components of mixtures, each of which provides a spectrum independently. This suppression phenomenon was first reported with mixtures of peptides[91] and has been of particular concern in the FAB mapping of tryptic and other digests of proteins.

Desorption efficiency for amino acids and peptides has been correlated with sample hydrophobicity.[89,92,93] The best correlations of peptide hydrophobicities and FAB sensitivities were obtained by using the hydrophobicity scale devised by measuring peptides at water/air interfaces,[93] and it seems likely that increased hydrophobicity in very polar polyfunctional compounds such as peptides and carbohydrates improves their surfactant properties in glycerol and thioglycerol. It should be remembered that a hydrophobic peptide is still much more polar than truly hydrophobic samples such as cholesterol and polystyrene which appear not to produce molecular ion species under fast atom bombardment. Conversion to hydrophobic derivatives will decrease the secondary ion yields of many samples.

Empirical efforts can also be made to achieve the optimal balance between solubility and surface activity by evaluating many solvents for a given sample. This requires larger amounts of sample.

Experimental measurements are readily made on surface tension, and thus surface-active samples have been most readily studied. However, other methods of concentrating the sample in the upper layer of the matrix[94–96] should also lead to improvements in sample desorption efficiency and in sample-to-matrix signal ratio. Recent studies[86] of electrophoretic behavior under the conditions of the FAB source, i.e., in a field approximating that imposed by fields and ion currents in the source, confirm the movement of peptides (migration) through the matrix toward the surface. This was facilitated by charging some samples by derivatization or by changing the pH of the matrix.

B. Mixture Analysis

Several approaches to mixture analysis have evolved from the theory that differential surface activities lead to differential desorption. More homogeneous desorption of components in some mixtures has been achieved by chemically converting the entire mixture to more hydrophobic derivatives.[93] Repetitive scanning has been used to detect components that may be fractionated by their different surface activities. Additions of surfactants of the same sign as the sample ions under analysis[89] and of surfactants of the opposite charge sign[97] have also been reported to reduce differences in desorption efficiencies of components of mixtures and to suppress desorption of matrix solvent ions. Surfactant additives of the opposite sign may be "transparent", not contribute ions to the spectrum, and the suggestion is made that these long-chain surfactants attract more lipophilic samples to the surface by creating a lipophilic layer there. It may be possible to quantify relative molarities from spectra of mixtures of compounds with unequal desorption efficiencies if standard curves are constructed through the concentration range of interest and spectra are scanned at consistent time intervals or ion currents are integrated through the entire sample lifetime.[98-100]

On the other hand, selectivity by the FAB process in the desorption of components of mixtures can be used to advantage. Selected molecules have been successfully analyzed directly from algae,[100] lyophilized bacteria,[101] TLC plates,[102,103] and charcoal[104] submersed in the liquid matrix and from crude extracts of such things as lyophilized tomatoes[105] and amniotic fluid.[43]

V. Choosing the Matrix

Although all aspects of the primary beam and ion optics of the mass spectrometer are critical to obtaining good FAB spectra, many of these parameters are not variable or optimized on commercial instruments. The experimental variable that is readily varied, and which can spell the difference between success and failure, is the liquid matrix from which the sample is desorbed.

The multiple contributions of the matrix have been discussed.[10,70,87,96,106] The liquid matrix provides the opportunity for formation of ions by solution chemistry. The solution lowers the energy required to desorb ions by solvating and separating the ions; the fluid matrix provides a hydrodynamic mechanism for secondary ion emission which includes desorption of the products of radiolysis damage. The matrix, which is the essence of the fast atom bombardment technique, should be designed with the objectives of optimizing absolute secondary ion currents and spectral persistence. Its selection can also influence fragmentation and signal-to-noise ratios.

A. Volatility

Two important physical characteristics of the matrix are viscosity and low vapor pressure. Low volatility is important, since the desorption of ions is terminated when the matrix is evaporated. Ion currents can be prolonged by cooling (but not freezing) volatile matrices,[107] and continuous flow FAB probes have also been designed to prolong ion currents.[108] Solvents with lower vapor pressure have been used to prolong sample lifetimes.[109] However, one involatile and viscous high molecular weight poly(ethylene glycol) was found not to be self-cleaning, i.e., not to be sputtered in bulk.[58]

Some of the liquid matrices found to be suitable include glycerol, monothioglycerol,[38] tetraglyme,[90] diethanolamine, and a 1:1 mixture of dithioerythritol and dithiothreitol.[28] Protonation (i.e., formation of MH^+ ions) is generally improved by the use of thioglycerol rather than glycerol. Since thioglycerol is more volatile, glycerol can be mixed with thioglycerol to lower the evaporation rate of the matrix and extend analysis time.

B. Fluidity

Strong ion currents are temporally prolonged under the high-flux primary beams used in FAB only if the matrix is fluid.[56,110] Several investigators report obtaining better spectra by heating viscous matrices or samples.[58,111-113] One interesting heated matrix is the saturated glucose solution (syrup) suggested by Watson and co-workers for carbohydrate analysis.[113] Spectra contain less matrix-contributed background; however, ion lifetimes appear not to be as long as those provided by a glycerol system well matched to the sample.

C. Solvency

There is general agreement that secondary ion currents are stronger and more prolonged if the sample is actually dissolved, in either the matrix liquid or in a cosolvent, as opposed to being presented as a mull or suspension. This is readily understood if reactions in the solution are being used to form the sample ions. Cosolvents such as water, methanol, chloroform, and dimethyl sulfoxide have been used, miscible with the less volatile matrix solvents. Some workers recommend that the matrix liquid be layered over the cosolvent sample solution,[114] and other suggest the opposite.[94] It is uncertain how much of the miscible cosolvent remains in the matrix under vacuum in the mass spectrometer.

As a first approximation, more concentrated samples produce better spectra. However, the relationship of the ions in the spectrum to the concentration of the sample in the matrix is not always continuous, either quantitatively or qualitatively.[88,90] Both theoretical[10] and empirical considerations suggest that fragmentation occurs more extensively with more concentrated samples. As a corollary, conclusions of fundamental studies using fairly high concentrations (e.g., ref 69, 85, and 115) may not extend to the dilute solutions often employed at the limits of sensitivity in analytical applications. Cook and Todd recommend that FAB matrices be characterized by their dielectric constants as a measure of their solvency for different classes of samples, and they have commenced to measure and tabulate these physical constants for the most popular matrix solvents.[106]

D. Acidity and Basicity

The acidity or basicity of the solvent relative to that of the sample is critical to production of ions with good abundances and should be considered in conjunction with the charge sign of the ions to be analyzed. As noted above, thioglycerol improves the production of MH^+ ions relative to glycerol for samples with low proton affinities. For samples that are anionic, i.e.,

sulfates, phosphates (nucleotides), and carbohydrates containing anionic sugars (sialic acid), detection of $(M - H)^-$ ions in the negative ion mass spectrum is enhanced by using a suitable proton acceptor matrix, such as diethanolamine.

E. Surfactant Properties

The experimental evidence suggesting the important role of the surface activity of the sample in the matrix is discussed in section IV. Surface activity for a given sample may be improved by changing the matrix,[87,106] by altering the sample,[60,93] or by judicious use of added surfactants.[96]

F. Additives

Additional chemicals are often added to the matrix solution toward the objectives of more fully ionizing the sample and/or drawing the sample to the surface of the solution. The most common additives are, of course, those which change the pH, such as HCl, NH_4Cl, and *p*-toluenesulfonic acid. The effect of pH (through the range 0.3–3.0) on the production of singly and multiply protonated insulin molecules has been reported.[63] Addition of NH_4Cl and NH_4SCN promotes the formation of $(M + NH_4)^+$ ions in carbohydrates and glycosides in analogy with chemical ionization. Additions may also be made to the matrix cocktail in order to promote chemical reactions before or during the analysis.

G. Background Contributions

Consideration must also be given to the masses of background ions that the matrix solvent and additives can contribute to the spectrum. That is, a matrix is preferred which provides a clear window in the mass range of analytical interest. Glycerol produces a series of peaks corresponding to protonated clusters with masses of $92n + 1$ and a similar series $(92n + 23)$ in the presence of sodium salt impurities. Similar series are encountered for thioglycerol ($108n + 1$ and $108n + 23$) and additional cluster ion peaks result from NH_4Cl impurities. Mixtures of glycerol/thioglycerol, of course, increase the multiplicity of matrix ion peaks. In general, the relative abundance of background peaks from the matrix depends upon the nature and concentration of the sample as well. This is discussed more extensively in section VII.

At the present time the literature contains many empirical reports of the efficacy of one solvent or another for one kind of sample or another,[116-118] with no correlation with physical or chemical properties beyond that well accepted for pH and pK_a. In the interest of encouraging rationalization of these phenomena, Cook and Todd have recently prepared a summary of relevant physical constants for a number of liquids used as matrices for FAB.[106] These physical constants include viscosity, dielectric constant, heats of vaporization, and pK_a or proton affinity.

VI. Reactions in the Matrix

A. Ion Formation

Almost as soon as the liquid matrix came into use, the correlation was made between the production of protonated molecular ions and the pK_a or pH of the matrix solution relative to the pK_a or isoelectric point (pI) of the sample. The enhanced detection of protonated molecular ions in the FAB spectrum when protonation reactions are carried out in the solution has been widely confirmed. This has been accomplished by working with more acidic matrix solvents, for example, adding acids to glycerol[119] or using thioglycerol,[38] dithiothreitol, and dithioerythritol.[28] A quantitative study is summarized in Figure 5[63] of the absolute and relative abundances of monovalent, divalent, and trivalent molecular ion species of bovine insulin desorbed from dimethyl sulfoxide with a range of acidic pH values. In a number of studies protonated molecular ion intensities have been correlated with proton affinities of samples[74,80,120,121] with the conclusion that for a given matrix more basic compounds are detected with greater sensitivities. Proton affinities have now been measured for many of the most popular matrix solvents.[76,106] However, the use of this gas-phase property instead of solution pK_a values is not meant to preclude considerations of ionization by solution chemistry. Basicities are parallel in the gas and solution phases for most compounds, and the role of solution ionization seems clearly established for involatile compounds.[62,63,86]

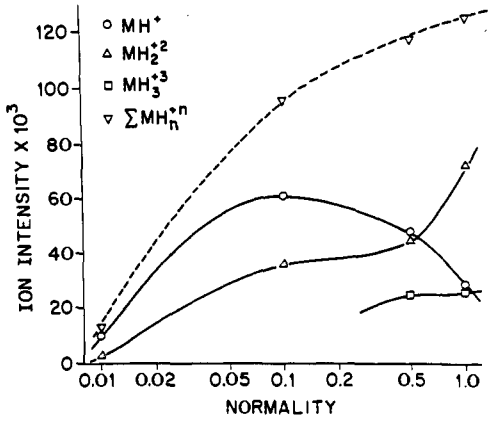

Figure 5. Abundances of mono-, di-, and trivalent molecular ion species of bovine insulin as a function of the pH of the matrix. Hydrochloric acid was added to a Me_2SO/thioglycerol matrix to adjust the pH. (Reprinted with permission from ref 63. Copyright 1986 John Wiley & Sons, Ltd.)

The corollary was also quickly established experimentally that $(M - H)^-$ ions could be detected with greater sensitivity in anion spectra if these were preformed by proton abstraction in basic solution. Diethanolamine and triethanolamine are most popularly used; however, chelation of protons by addition of an appropriate crown ether has been suggested as an alternate approach.[122] Formation of anions by adduction of Cl^-[123,124] and SCN^-[125] have been reported.

The addition of controlled amounts of ammonium or alkali metal salts to the matrix generates $(M + NH_4)^+$, MNa^+, etc. ions and has been recommended as one way to produce molecular ion species from samples that are not basic enough to protonate well. Studies of the relative abundances of MH^+ and $(M + NH_4)^+$ or MNa^+ ions throughout the entire emission period[56,79,126] show that these ratios can change with time and that the apparent efficacy of cationization with added salt may depend on when the spectrum is measured. Salt addition has been particularly utilized for analysis of carbohydrates,[125,127,128] where ammonium adduction was initially encountered by contaminant ammonium salts

in thioglycerol. Researchers have various opinions about the relative contributions of gas-phase vs. solution adduction.[65,66,79] In some instances MNa^+ ions have been shown to produce a different set of fragment ions from those produced by MH^+ ions.[107,120]

The uncontrolled presence of alkali metal salts, e.g., in samples isolated from biological matrices, can lead to a multiplicity of molecular ion species, MH^+, MNa^+, MK^+, etc., which have the advantage of providing confirming measurements on the molecular ion but the considerable disadvantage of reducing the sensitivity of the analysis by dividing the ion current among several signals. Although FAB is considered to be more tolerant of contaminating salt than field desorption, at the extreme, too much salt can obscure or suppress organic sample ion current. Consequently, most biological samples and many chemical samples must be freed from contaminating salts for FAB analysis. Reversed-phase liquid chromatography,[114,129] acidic ion-exchange columns,[106,130] and cryptofix columns[131] have been recommended for this. Alternatively, derivatization to a chloroform-soluble product is recommended,[132] from which salts can be removed by water washes. Volatile buffers are recommended for all sample isolation and purification procedures that require buffers.

B. Directed Reactions in the Matrix

A number of important and intriguing demonstrations have already been reported of mass spectrometric studies of transient intermediates and of dynamic processes in the FAB matrix. Saito and Kato have followed the formation of a short-lived glutathione conjugate formed by chemical reaction with an arylnitroso carcinogen in the glycerol matrix.[133] Kalinoski et al. have observed unstable intermediates formed in situ by palladium-catalyzed reactions between glycals and organomercurials.[134] Horman[135] has characterized open-chain forms of thiamin produced in the matrix in the presence of strong bases. Photoproducts of chlorpromazine have been characterized[136] generated in situ. The analytical potential for studies of dynamic chemical processes in solution by high-sensitivity mass spectrometry techniques is very great indeed.

Caprioli has demonstrated that FAB can be used to follow hydrolytic enzyme reactions carried out in the glycerol matrix.[137,138] However, he has expressed reservations[139] about the effect of glycerol on enzyme activities.

Derivatization can also be carried out in situ. Reagents have been added to the matrix to form acetates[114,140] and boronates[141] for acidolytic cleavage of peptides[142] and to reduce disulfides.[143] Methods have been proposed to count active hydrogens by exchanging them with deuterium in a deuterium-labeled matrix[30,144,145] or by exchanging them with alkali metal cations, e.g., in a matrix containing sodium chloride.[146] The former approach is demonstrated to be applicable to heavier compounds than could previously be analyzed, containing as many as 28 exchangeable protons.[147]

C. Sampling Solution Equilibria

The possibility of sampling peptide binding complexes and other equilibria in solution was examined early on[91] and caution was urged, since many different factors can influence relative abundances of detected ions. The possibility has been evaluated with more optimism for a carbohydrate–lipid complex,[148] for complexes between imidazole and electron donors such as trimethyl phosphate,[149] and for equilibria in cation binding by macrocyclic ligands.[150] However, equilibria between aluminum chloride and butylpyridinium chloride,[151] alkali metal cations and phthalic acid,[152] alkali metal cations and phospholipids,[153] and inorganic anions and cationic surfactants[154] are reported not to be quantitatively reflected in the FAB spectra. Caprioli suggests that pK_a values can be quantitatively correlated with ion abundances by including a correction factor.[155] The ambiguities of this approach may be resolved as the active chemical role of the matrix solvent is recognized and studied.

D. Reactions with the Matrix

Chemical reactions can also occur in the matrix between the sample and the solvent or cosolvent. Generally these reactions may be grouped as nucleophilic displacements, e.g., of halide groups[156] and coordination ligands,[124,157] esterification,[158,159] and reductions, e.g., of peptide disulfide bonds,[160,161] azo groups in dyes,[68] NADP,[70] and other compounds with low-energy unoccupied molecular orbitals.[162] The relative contributions of a reducing matrix, such as thioglycerol, of electrons in the primary beam[83,84] or of secondary electrons in these reductions have not been delineated.

A less predictable artifact is the occurrence of M + 12 ions shown to arise by reaction of amine-containing sample molecules with glycerol or thioglycerol.[18,163] The mass of these artifact ions shifted to M + 14 when pentadeuterioglycerol was used as the matrix. A mechanism involving formation of a Schiff base has been proposed by Lehmann et al.[18]

Sindona and co-workers[164] attribute some of the anions recorded from cationic carnitine to reactions in the matrix "energized" by the primary beam and commend the study of reactions in the energized matrix as a new field of chemistry.

VII. Chemical Background and Noise

Matrix ions are sputtered along with sample ions under most circumstances. The mass range of ions contributed to a cation spectrum by glycerol, for example, may be extended considerably beyond its molecular weight by the formation of cluster ions, $(glycerol)_n + H^+$, beyond 900 atomic mass units, although intensity falls off rapidly as the cluster number increases (Figure 2). Fragment ions are also formed from the cluster ions. A sample with surfactant properties usually suppresses matrix ions of this kind, depending on its concentration. Consequently, it is not always easy to obtain an appropriate matrix spectrum for background subtraction.[98,129] The presence of alkali metal salts, e.g., sodium chloride, in the matrix further confound the pattern of ions contributed by the matrix, since these may also include clusters with metal ions. Matrix molecules may also form cluster ions with sample molecules, e.g., sample + H^+ + matrix, usually accompanying protonated or natriated molecules at the expected mass increment.

Sometimes overlap of matrix and sample ions can be avoided by selecting a matrix with the right window. For example, the heated glucose syrup is said to provide a clear window below 300 atomic mass units.[111] Often

the contributions of matrix ions to the spectrum can be reduced by increasing the concentration of sample or by increasing the surface activity of the sample.

One characteristic feature of FAB spectra is the chemical noise, also called the peak-at-every-mass (Figure 2) or the incoherent fragmentation. This becomes particularly prominent in spectra of heavy compounds such as that in Figure 3, where it extends somewhat beyond the mass of the molecular ion and increases steeply at the low-mass end. Although this noise does not accumulate through time in a well-chosen matrix (see above), it is thought to derive from radiolysis damage by the impacting primary beam. Several groups have given thought to subtracting or allowing for this background in quantitative studies.[98,129] Others report improving signal-to-noise ratios and dynamic range by careful sample cleanup[165] or by the use of MS/MS techniques. Signal may also be distinguished from overlapping noise by high-resolution measurements. Such measurements have demonstrated 10 or more isobaric contributions to a single peak in the incoherent noise.[166]

VIII. Directing Fragmentation

Chemical parameters influence many aspects of the spectrum, the charge sign and charge state of the molecular ions, the nature and multiplicity of adduct ions, the presence and identity of matrix ions, and the overall abundance of sample ions. Suggestions are beginning to appear toward the objectives of promoting and directing fragmentation. One line of thought is to reduce the sizes of the clusters desorbed, so that fewer solvent molecules can be shed to dissipate internal energy. Approaches include increasing sample concentration, working with only one or two monolayers of glycerol,[109] and reducing the energy of the primary beam so that smaller clusters are ejected.[77] Observations have been made which suggest that different matrix solvents favor detection of different peptide sequence ions.[142]

A rational approach to directing fragmentation has been proposed by Spiteller,[167] who argues that fragmentation occurs in the vicinity of the charge in gaseous ions originating in a FAB source, just as in chemical ionization, electron impact, and other techniques. He finds that if he acetylates the N-terminus of a small peptide, fragmentation (i.e., production of sequence ions) is more evenly distributed along the amide backbone, while in the peptide with a free amino terminus the charge and the fragmentation are localized there. Presumably this same effect is the basis for the "high diagnostic value" of fragment ions from permethylated carbohydrates reported relative to underivatized carbohydrates.[112] Of course, derivatives designed to distribute charge more evenly throughout the molecule may reduce sensitivity compared to derivatives that introduce and localize the charge. Schram and Slowikowski[168] report that FAB spectra of trimethylsilylated adenosine and adenosine monophosphate contain many more fragment ions, including ion radicals, than spectra of the underivatized samples. One interpretation is that derivatization renders these samples volatile and subject to gas-phase ionization as discussed in section III.C.

Although it is not yet known if these lines of reasoning can be extended to promote and direct fragmentation in peptides with masses above 5000 daltons or to samples other than peptides and carbohydrates, it is nonetheless encouraging to find work beginning in this area.

IX. Optimizing Sensitivity

A. Instrumental Setup

Although the focus of this review is primarily on chemical aspects, it is not possible to discuss optimization of sensitivity without some consideration of the primary beam and sample stage. Various studies have shown that secondary ion yields do not depend on whether or not the primary particles are charged or neutral[14] and that they are largely insensitive to variations of the high-flux beam between 1 and 10 kV of translational energy. On the other hand, the secondary ion yields are influenced by the mass of the primary particles, by full coverage of the sample stage by the primary beam, by the distance between the gun and the stage, and by the angle of impact and thus the dispersion of secondary ions with respect to source exit slits and the ion trajectory of the mass analyzer.[169-171] A focused primary beam has been shown[170] to permit better control of angular trajectories and to foul the source less. Inadequate vacuum or increased source pressure reduces absolute secondary ion yields.[172] Gold-plated sample stages have been found to provide higher analytical sensitivity.[160,173] The stage must be fully covered by the matrix to avoid sputtering metal, and smaller sample stages permit higher matrix concentrations to be achieved with smaller amounts of sample.

B. Better Sensitivity through Chemistry

The several considerations in both the design of the matrix and the preparation of the sample, including derivatization, will be summarized here. The matrix should be selected by considering sample solubility, surface activity, and mechanisms for ionization. Controlling the pH of the matrix to promote ionization is simple and effective, as is indicated in Figure 4. Alternatively, sensitivity can be significantly enhanced by derivatization designed to precharge the sample,[174,175] to increase its surfactant properties,[96,132] or both.[56,95] Some time before matrix parameters controlling ion formation in FAB mass spectrometry began to be defined, the relative desorption efficiencies of different kinds of ions were noted[176] in a qualitative generalization which still holds:

$$R_4N^+ > MH^+, \quad MNa^+ \gg M^+$$

For optimal sensitivity spectra should be scanned for ions of the appropriate sign, e.g., anion spectra for phosphorylated samples[96,177-180] and cation spectra for quaternary ammonium-containing samples.[146,181,182]

C. Temporal Variations

The chemical properties of the matrix solution change throughout the sputtering experiment, and as a consequence the abundances of all ions in the spectrum have a temporal profile. On the long time scale of the ideal experiment, the abundance of a particular molecular ion species declines smoothly as the sample is depleted from solution. Fine structure is also observed in these profiles,[126] particularly at the beginning of the analysis

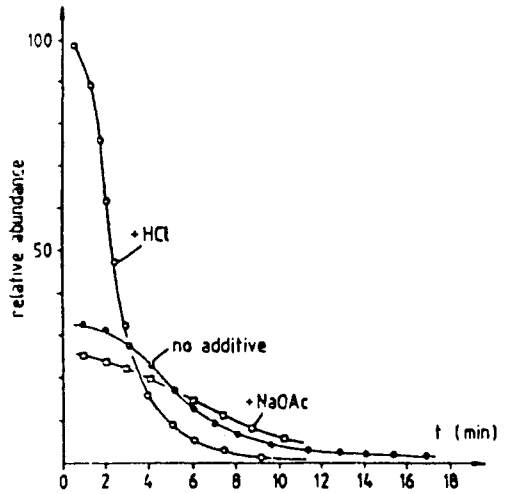

Figure 6. Dependence of the abundances of the protonated molecular ion of Pz-Pro-Leu-Gly-Pro-Arg on sputtering time with and without additives. The matrix is glycerol. (Reprinted with permission from ref 56. Copyright 1986 Springer-Verlag.)

when steady state is being established between sputtering and evaporation at the surface and the several phenomena which continue to enrich the surface and depend on the overall concentration.[18,54,58,59,80,105,107,126] Deterioration of spectra at the end of the emission period is correlated with the loss of the liquid matrix and the accumulation of radiolysis damage.[109]

Initial settling times can be seen in Figure 4, in which the abundances of protonated angiotensin molecular ions are presented as a function of time and of matrix. This figure also makes the point that the sensitivity of peptides can be enhanced by using a more acidic matrix. Sensitivity is more correctly evaluated by integrating the ion current throughout the entire emission period. A case in point is shown in Figure 6, which presents the effects of several additives on the abundance of a protonated peptide species. Abundance could appear to be increased or decreased, depending on when the spectrum is recorded. Similarly, the relative abundances of matrix ions, e.g., protonated glycerol, and sample ions vary through time.[54,126] Familiarity with the time profile of a particular sample at a defined concentration in a given matrix will allow the invetigator to optimize the reproducability and sensitivity of the measurements. These considerations have been found to be important in quantitative analysis of mixtures.[43,44,98,105] In Figure 7, relative molecular ion abundances of two glycerophosphocholines in an equimolar mixture are displayed as a function of time. Relative ion abundance is constant when triethanolamine is the matrix solvent; however, in glycerol it varies with time.[44]

X. Conclusions

Both quantitative and qualitative features of the FAB spectrum are influenced and even controlled by various aspects of solution chemistry in the matrix. Thus far, efforts to these ends have mostly been directed empirically. Increased availability and discussion of the various physical constants of matrix fluids should encourage more systematic variation and increase our understanding at a predictive level.

The possibility of exploring the dynamic chemistry of the liquid state with a technique that analyzes gas-

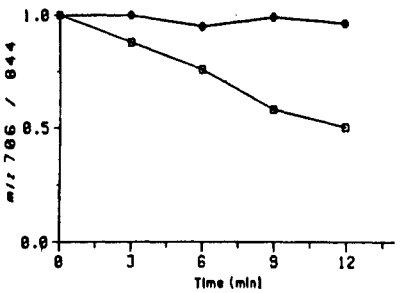

Figure 7. Molecular ion ratios of 14:0a/16:0 glycerophosphocholine (m/z 706) and 18:0a/22:0 glycerophosphocholine (m/z 844) analyzed as a function of time from an equimolar mixture in (□) glycerol and (◆) triethanolamine. (Reprinted with permission from ref 44. Copyright 1986 John Wiley & Sons, Ltd.)

eous ions opens a new future for mass spectrometry.

XI. Acknowledgments

We thank the National Science Foundation for Grants PCM 82-09954 and DMB 85-15390 and the National Institutes of Health for Grants GM 21248 and GM 33967.

XII. References

(1) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325–326.
(2) Surman, D. J.; Vickerman, J. C. *J. Chem. Soc., Chem. Commun.* **1981**, 324.
(3) Barber, M.; Bordoli, R. S.; Elliott, G. J.; Sedgwick, R. D. *Anal. Chem.* **1982**, *59*, 645A–657A.
(4) Surman, D. J.; Vickerman, J. C. *J. Chem. Res., Synop.* **1981**, *6*, 170–172.
(5) Lehrle, R. S.; Robb, J. C.; Thomas, D. W. *J. Sci. Instrum.* **1962**, *39*, 458–463.
(6) Devienne, F. M.; Roustan, J.-C. *Org. Mass Spectrom.* **1982**, *17*, 173–181.
(7) Rinehart, K. L. *Science (Washington, D.C.)* **1984**, *218*, 254–260.
(8) Fenselau, C. In *Ion Formation from Organic Solids*; Benninghoven, A., Ed.; Springer-Verlag: Berlin, 1983; pp 90–100.
(9) Cochran, R. L. *Appl. Spectrosc. Rev.* **1986**, *22*, 137–187.
(10) DePauw, E. *Mass Spectrom. Rev.* **1986**, *5*, 191–212.
(11) Miller, J. M. *Adv. Inorg. Chem. Radiochem.* **1984**, *28*, 1–27.
(12) Day, R. J.; Unger, S. E.; Cooks, R. G. *Anal. Chem.* **1980**, *52*, 557A–572A.
(13) Cooks, R. G. *Chem. Rev.*, this issue.
(14) Burlingame, A. L.; Baillie, T. A.; Derrick, P. J. *Anal. Chem.* **1986**, *58*, 165R–211R.
(15) Rollgen, F. W.; Giessmann, U. *Int. J. Mass Spectrom. Ion Phys.* **1984**, *56*, 229–231.
(16) Sundqvist, B.; Hedin, A.; Hakansson, P.; Salehpour, M.; Saeve, G.; Johnson, R. E. *Nucl. Instrum. Methods Phys. Res., Sect. B* **1986**, *14*, 429–435.
(17) McNeal, C. J. *Anal. Chem.* **1982**, *54*, 43A–50A.
(18) Lehmann, W. D.; Kessler, M.; Konig, W. A. *Biomed. Mass Spectrom.* **1984**, *11*, 217–220.
(19) Carr, S. A.; Reinhold, V. N.; Green, B. N.; Hass, J. R. *Biomed. Mass Spectrom.* **1985**, *12*, 288–295.
(20) Barber, M.; Bordoli, R.; Elliott, G.; Tyler, A.; Bill, J.; Green, B. N. *Biomed. Mass Spectrom.* **1984**, *11*, 182–186.
(21) Yergey, J. A. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *52*, 337–339.
(22) Boland-Lussier, B. E.; Hughes, R. P. *Organometallics* **1982**, *1*, 635–639.
(23) Donaldson, W. A.; Hughes, R. P. *J. Am. Chem. Soc.* **1982**, *104*, 4846–4859.
(24) Cowens, J. W.; Stevie, F. A.; Alderfer, J. L.; Hansen, G. E.; Pendyala, L.; Creaven, P. J. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *48*, 177–179.
(25) Cohen, A. I.; Glavin, K. A.; Kronauge, J. F. *Biomed. Mass Spectrom.* **1983**, *10*, 287–291.
(26) Miller, J. M. *J. Organomet. Chem.* **1983**, *249*, 299–302.
(27) Lee, J. J.; Chen, T. S.; Chang, C. J.; Fenselau, C.; Floss, H. G. *J. Antibiot.* **1985**, *10*, 1444–1446.
(28) Finke, J.; Oroege, M. W.; Cook, J. C.; Suslick, K. S. *J. Am. Chem. Soc.* **1984**, *106*, 5750–5753. Suslick, K. S.; Cook, J. C.; Rapko, B.; Droege, M. W.; Finke, R. G. *Inorg. Chem.* **1986**, *25*, 241–243.

(29) DiAugustine, R. P.; Walker, M. P.; Klapper, D. G.; Grove, R. I.; Willis, W. D.; Harvan, D. J.; Hernandez, O. *J. Biol. Chem.* **1985**, *260*, 2807–2811.
(30) Hyver, K. J.; Campana, J. E.; Cotter, R. J.; Fenselau, C. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 1287–1293.
(31) Richter, W. J.; Raschdorf, F.; Maerki, W. In *Mass Spectrometry in the Health and Life Sciences*; Burlingame, A. L., Castignoli, N., Eds.; Elsevier: Amsterdam, 1985; pp 193–208.
(32) Barber, M.; Bordoli, R. S.; Elliott, G. J.; Horoch, N. J.; Green, B. N. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 753–757.
(33) Cottrell, J. S.; Frank, B. H. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 1032–1038.
(34) Cotter, R. J.; Larsen, B. S.; Heller, D. N.; Campana, J. E.; Fenselau, C. *Anal. Chem.* **1985**, *57*, 1479–1480.
(35) Lattimer, R. P.; Hansen, G. E. *Macromolecules* **1981**, *14*, 776.
(36) Yergey, J.; Cotter, R. J.; Heller, D.; Fenselau, C. *Anal. Chem.* **1984**, *56*, 2262–2263.
(37) Yergey, J.; Heller, D.; Hansen, G.; Cotter, R. J.; Fenselau, C. *Anal. Chem.* **1983**, *55*, 353–358.
(38) Fenselau, C. *Anal. Chem.* **1982**, *54*, 104A–111A.
(39) Fenselau, C.; Yergey, J.; Heller, D. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 5–20.
(40) Fenselau, C.; Feng, P. T.; Johnson, L. P. *Drug Metab. Dispos.* **1983**, *10*, 316–319.
(41) Gaskell, S. J.; Brownsey, B. G.; Brooks, P. W.; Green, B. N. *Biomed. Mass Spectrom.* **1983**, *10*, 215–219.
(42) Clay, K. L.; Steve, D. O.; Murphy, R. C. *Biomed. Environ. Mass Spectrom.* **1984**, *11*, 47–49.
(43) Ho, B. C.; Fenselau, C.; Hansen, G.; Larsen, J.; Daniel, A. *Clin. Chem. (Winston-Salem, N.C.)* **1983**, *29*, 1349–1354.
(44) Chilton, F. H.; Murphy, R. C. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 71–76.
(45) Clay, K. L.; Wahlin, L.; Murphy, R. C. *Biomed. Mass Spectrom.* **1983**, *10*, 489–491.
(46) Iribarne, J. V.; Thomson, B. A. *J. Chem. Phys.* **1976**, *65*, 2287.
(47) Blakley, C. R.; Vestal, M. L. *Anal. Chem.* **1983**, *55*, 750–754.
(48) Thompson, M. W. *Philos. Mag.* **1968**, *18*, 377.
(49) Sigmund, P. *Phys. Rev.* **1969**, *184*, 383.
(50) Van der Peyl, G. J. Q.; Van der Zande, W. J.; Hoogerbrugge, R.; Kistemaker, P. G. *Int. J. Mass Spectrom. Ion Processes* **1985**, *67*, 147–159.
(51) Hoogerbrugge, R.; Van der Zande, W. J.; Kistemaker, P. G. *Int. J. Mass Spectrom. Ion Processes*, in press.
(52) Kelner, L.; Markey, S. P. *Int. J. Mass Spectrom. Ion Processes* **1984**, *59*, 157–167.
(53) Michl, J. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 255–272. See also: David, D. E.; Magnera, T. F.; Tian, R.; Michl, J. *Radiat. Eff.* **1986**, *99*, 247. David, D. E.; Magnera, T. F.; Tian, R.; Suslick, D.; Michl, J. *Nucl. Instrum. Methods Phys. Rev., Sect. B* **1986**, *B14*, 378. Urbassek, H. M.; Michl, J. *Nucl. Instrum. Methods Phys. Rev., Sect. B*, in press.
(54) Wong, S. S.; Rollgen, F. W. *Nucl. Instrum. Methods Phys. Res., Sect. B* **1986**, *14*, 436–447.
(55) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Nature (London)* **1981**, *293*, 270–275.
(56) Wong, S. S.; Wirth, K. P.; Rollgen, F. W. In *Ion Formation from Organic Solids IFOS III*; Benninghoven, A., Ed.; Springer-Verlag: Berlin, 1986; pp 91–95.
(57) Barofsky, D. F.; Barofsky, E. In *Ion Formation from Organic Solids IFOS III*; Benninghoven, A., Ed.; Springer-Verlag: Berlin, 1986; pp 86–90.
(58) Ligon, W. V. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *52*, 183–187.
(59) Wong, S. S.; Rollgen, F. W.; Manz, I.; Przybylski, M. *Biomed. Mass Spectrom.* **1985**, *12*, 43–45.
(60) Ligon, W. V.; Dorn, S. B. *Int. J. Mass Spectrom. Ion Processes* **1984**, *57*, 75–90.
(61) Cooks, R. G.; Busch, K. L. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 111–124.
(62) Musselman, B. D.; Watson, J. T.; Chang, C. K. *Org. Mass Spectrom.* **1986**, *21*, 215–219.
(63) Schronk, L. R.; Cotter, R. J. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 395–400.
(64) Musselman, B. D.; Allison, J.; Watson, J. T. *Anal. Chem.* **1985**, *57*, 2425–2427.
(65) Keough, T. *Anal. Chem.* **1985**, *57*, 2027–2034.
(66) Bursey, M. M.; Marbury, G. D.; Hass, J. R. *Biomed. Mass Spectrom.* **1984**, *11*, 522–526.
(67) Sunner, J. A.; Kulatunga, R.; Kebarle, P. *Anal. Chem.* **1986**, *58*, 2009–2014.
(68) Gale, P. J.; Bentz, B. L.; Chait, B. T.; Field, F. H.; Cotter, R. J. *Anal. Chem.* **1986**, *58*, 1070–1076.
(69) Pelzer, G.; De Pauw, E.; Dao Viet, D.; Marien, J. *J. Phys. Chem.* **1984**, *83*, 5065–5068.
(70) Fenselau, C. *J. Nat. Prod.* **1984**, *47*, 215–225.
(71) Clanton, E.; Wakefield, A. *J. Chem. Soc., Chem. Commun.* **1984**, 969–970.
(72) De Pauw, E. *Anal. Chem.* **1983**, *55*, 2195–2196.
(73) Bartmess, J.; Phillips, L. B. *Adv. Mass Spectrom.* **1985**, *10*, 1581.
(74) Fenselau, C.; Liberato, D.; Yergey, J.; Cotter, R. J.; Yergey, A. L. *Anal. Chem.* **1984**, *56*, 2759–2763.
(75) Sunner, J. A.; Kulatunga, R.; Kebarle, P. *Anal. Chem.* **1986**, *58*, 1312–1316.
(76) Sunner, J.; Kulatunga, R.; Kebarle, P. *Proceedings of the 34th Annual Conference on Mass Spectrometry and Allied Topics*, Cincinnati, OH; American Society for Mass Spectrometry: East Lansing, MI, 1986; pp 639–640, 649–650.
(77) De Pauw, E.; Pelzer, G.; Marien, J. In *Advances in Mass Spectrometry 1985*; Todd, J. F. J., Ed.; Wiley: Chichester, UK, 1986; pp 1563–1564.
(78) Wong, S. S.; Rollgen, F. W. *Int. J. Mass Spectrom. Ion Processes* **1986**, *70*, 135–144.
(79) Puzo, G.; Prome, J. C. *Org. Mass Spectrom.* **1985**, *20*, 288–291.
(80) Puzo, G.; Prome, J. C. *Org. Mass Spectrom.* **1984**, *19*, 448–451.
(81) Tondeur, Y.; Clifford, A. J.; De Luca, L. M. *Org. Mass Spectrom.* **1985**, *20*, 157–160.
(82) Bojesen, G. *J. Chem. Soc., Chem. Commun.* **1986**, 244–245.
(83) Huang, Q.-W.; Wu, G.-L.; Tang, H.-T. *Int. J. Mass Spectrom. Ion Processes* **1986**, *70*, 145–152.
(84) Bojesen, G.; Moller, J. *Int. J. Mass Spectrom. Ion Processes* **1986**, *68*, 239–248.
(85) Olthoff, J. K.; Cotter, R. J. *Nucl. Instrum. Methods Phys. Rev., Sect. B*, in press.
(86) Wirth, K. P.; Wong, S. S.; Rollgen, F. W. *Proceedings of the 34th Annual Conference on Mass Spectrometry and Allied Topics*, Cincinnati, OH; American Society for Mass Spectrometry: East Lansing, MI, 1986; pp 647–648.
(87) Todd, P. J. *Proceedings of the 34th Annual Conference on Mass Spectrometry and Allied Topics*, Cincinnati, OH; American Society for Mass Spectrometry: East Lansing, MI, 1986; pp 651–652.
(88) Barber, M.; Bordoli, R. S.; Elliott, G. J.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Faraday Trans. 1* **1983**, *79*, 1249–1255.
(89) De Pauw, E.; Pelzer, G.; Dao Viet, D.; Marien, J. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 27–32.
(90) Heller, D. N.; Fenselau, C.; Yergey, J.; Cotter, R. J.; Larkin, D. *Anal. Chem.* **1984**, *56*, 2274–2277.
(91) Williams, D. H.; Bradley, C.; Bojesen, G.; Santikarn, S.; Taylor, L. C. E. *J. Am. Chem. Soc.* **1981**, *103*, 5700–5704.
(92) Pelzer, G.; De Pauw, E.; Marien, J. *Analysis* **1985**, *13*, 368–372.
(93) Naylor, S.; Findeis, A. F.; Gibson, B. W.; Williams, D. H. *J. Am. Chem. Soc.* **1986**, *108*, 6359–6363.
(94) Zhang, M. Y.; Liang, X. Y.; Chen, Y. T.; Liang, X. G. *Anal. Chem.* **1984**, *56*, 2288–2290.
(95) Ligon, W. V.; Dorn, S. B. *Anal. Chem.* **1986**, *58*, 1889–1892.
(96) Ligon, W. V.; Dorn, S. B. *Fresenius' Z. Anal. Chem.* **1986**, *325*, 626–627.
(97) Ligon, W. V.; Dorn, S. B. *Int. J. Mass Spectrom. Ion Processes* **1984**, *61*, 113–122.
(98) Townsend, R. R.; Heller, D. N.; Fenselau, C.; Lee, Y. C. *Biochemistry* **1984**, *23*, 6389–6392.
(99) Beckner, C. F.; Caprioli, R. M. *Biomed. Mass Spectrom.* **1984**, *11*, 60–62.
(100) Ross, M. M.; Neihoff, R. A.; Campana, J. E. *Anal. Chim. Acta* **1986**, *181*, 149–157.
(101) Heller, D. N.; Fenselau, C.; Cotter, R. J.; Demirev, P.; Olthoff, J. K.; Honovich, J. Uy. M.; Tanaka, T.; Kishimoto, Y. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 194–199.
(102) Iwatani, K.; Nakagawa, Y. *Mass Spectrom. (Tokyo)* **1986**, *34*, 189–195.
(103) DiDonato, G. C.; Busch, K. L. *Anal. Chem.* **1986**, *58*, 3231–3232.
(104) Ross, M. M.; Kidwell, D. A.; Campana, J. E. *Anal. Chem.* **1984**, *56*, 2142–2145.
(105) Price, K. R.; Fenwick, G. R.; Self, R. *Food Addit. Contam.* **1986**, *3*, 241–246.
(106) Cook, K.; Todd, P. J. *Biomed. Environ. Mass Spectrom.*, in press.
(107) Falick, A. M.; Walls, F. C.; Laine, R. A. *Anal. Biochem.* **1986**, *159*, 132–137.
(108) Caprioli, R. M.; Fan, T.; Cottrell, J. S. *Anal. Chem.* **1986**, *58*, 2949–2954. Mahoney, J. F.; Perel, J. *Proceedings of the 32nd Annual Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX; American Society for Mass Spectrometry: East Lansing, MI, 1984; pp 94–95.
(109) Todd, P. J.; Groenewold, G. S. *Anal. Chem.* **1986**, *58*, 895–899.
(110) Katz, R. N.; Chaudhary, T.; Field, F. H. *J. Am. Chem. Soc.* **1986**, *108*, 3897–3903.
(111) Ackermann, B. L.; Watson, J. T.; Holland, J. F. *Anal. Chem.* **1985**, *57*, 2656–2663.
(112) Dell, A.; Morris, H. R.; Egge, H.; von Nicolai, H.; Strecker, G. *Carbohydr. Res.* **1983**, *115*, 41–52.
(113) Ackermann, B. L.; Watson, J. T.; Newton, J. F.; Hork, J. B.; Braselton, W. E. *Biomed. Mass Spectrom.* **1984**, *11*, 502–511.

(114) Martin, S. A.; Costello, C. E.; Biemann, K. *Anal. Chem.* **1982**, *54*, 2362–2368.
(115) Glish, G. L.; Todd, P. J.; Busch, K. L.; Cooks, R. G. *Int. J. Mass Spectrom. Ion Phys.* **1984**, *56*, 177–192.
(116) Gower, J. L. *Biomed. Mass Spectrom.* **1985**, *12*, 191–196.
(117) Gower, J. L. In *Advances In Mass Spectrometry 1985*; Todd, J. F. J., Ed.; Wiley: Chichester, UK, 1986; pp 1537–1538.
(118) *Proceedings of the 33rd Annual Conference on Mass Spectrometry and Allied Topics*, San Diego, CA; American Society for Mass Spectrometry: East Lansing, MI, 1985; pp 998–1000. *Proceedings of the 34th Annual Conference on Mass Spectrometry and Allied Topics*, Cincinnati, OH; American Society for Mass Spectrometry: East Lansing, MI, 1986.
(119) Malorini, A.; Marino, G.; Milone, A. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 477–482.
(120) Harada, K.; Kimura, I.; Masuda, K.; Suzuki, M. *Org. Mass Spectrom.* **1985**, *20*, 582–588.
(121) Isobe, R.; Komori, T.; Abe, F.; Yamauchi, T. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 585–594.
(122) Fujii, I.; Isobe, R.; Kanematsu, K. *J. Chem. Soc., Chem. Commun.* **1985**, 405–406.
(123) Fraisse, D.; Tabet, J. C.; Becchi, M.; Raymond, J. *Biomed. Mass Spectrom.* **1986**, *13*, 1–14.
(124) Siegel, M. M.; Bitha, P.; Child, R. G.; Hlauka, J. J.; Lin, Y.; Chang, T. T. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 25–32.
(125) Dell, A.; Oates, J. A.; Morris, H. R.; Egge, H. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *46*, 415–418.
(126) Barofsky, D. F.; Giessmann, U.; Barofsky, E. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 319–322.
(127) Dell, A.; Ballou, C. E. *Biomed. Mass Spectrom.* **1983**, *10*, 50–56.
(128) Fenselau, C.; Yelle, L.; Stogniew, M.; Liberato, D.; Lehman, J.; Feng, P.; Colvin, M. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *46*, 411–414.
(129) Ackermann, B. L.; Watson, J. T.; Newton, J. F.; Hook, J. B.; Braselton, W. E., Jr. *Biomed. Mass Spectrom.* **1984**, *11*, 502–511.
(130) Przybylski, M. *Fresenius' Z. Anal. Chem.* **1983**, *315*, 402–421.
(131) Harada, K. I.; Masuda, K.; Okumura, K.; Suzuki, M. *Org. Mass Spectrom.* **1985**, *20*, 533–534.
(132) Tsai, P.; Dell, A.; Ballou, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *82*, 4119–4123.
(133) Saito, K.; Kato, R. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 1–5.
(134) Kalinoski, H. T.; Hacksell, U.; Barofsky, D. F.; Barofsky, E.; Daves, G. D., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 6476–6482.
(135) Horman, I. *Helv. Chim. Acta* **1984**, *67*, 1478–1483.
(136) Dung, D. V.; Marien, J.; De Pauw, E.; Decuyper, J. *Org. Mass Spectrom.* **1984**, *19*, 276–279.
(137) Smith, L. A.; Caprioli, R. M. *Biomed. Mass Spectrom.* **1983**, *10*, 98–106.
(138) Caprioli, R. M.; Smith, L. *Anal. Chem.* **1986**, *58*, 1080–1083.
(139) Caprioli, R. M.; Fan, T. *Anal. Biochem.* **1986**, *154*, 596–603.
(140) Morris, H. R.; Panico, M. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 623–631.
(141) Rose, M. E.; Logstaff, C.; Dean, P. D. *Biomed. Mass Spectrom.* **1983**, *10*, 512–527.
(142) Przybylski, M.; Dietrich, I.; Manz, I.; Bruckner, H. *Biomed. Mass Spectrom.* **1984**, *11*, 569–582.
(143) Yazdanparast, R.; Andrews, P.; Smith, D. L.; Dixon, J. E. *Anal. Biochem.* **1986**, *153*, 348–353.
(144) Sethi, S.; Smith, D. L.; McCloskey, J. A. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 126–131.
(145) Lam-Tranh, H.; Gandin, D.; Virelizier, H.; Hagemann, R.; Fermandjian, S.; Jankowski, K. *Spectrosc. Lett.* **1986**, 563–594.
(146) Fenselau, C.; Vu, V. T.; Cotter, R. J.; Hansen, G.; Heller, D.; Chen, T.; Colvin, O. M. *Spectrosc.: Int. J.* **1982**, *1*, 132–151.
(147) Verma, S.; Pomerantz, S. C.; Sethi, S. K.; McCloskey, J. A. *Anal. Chem.* **1986**, *58*, 2898–2902.
(148) Ballou, C. E.; Dell, A. *Carbohydr. Res.* **1985**, *140*, 139–143.
(149) Brown, S. J.; Miller, J. M.; Theberge, R.; Clark, J. H. *Can. J. Chem.* **1986**, *64*, 1277–1279.
(150) Johnstone, R. A.; Rose, M. E. *J. Chem. Soc., Chem. Commun.* **1983**, 1268–1269.
(151) Ackerman, B. L.; Tsarbopoulos, H.; Allison, J. *Anal. Chem.* **1985**, *57*, 1768–1770.
(152) Bursey, M. M.; Marbury, G. D.; Hass, J. R. *Biomed. Mass Spectrom.* **1984**, *11*, 522–528.
(153) Demirev, P. *Proceedings of the 34th Annual Conference on Mass Spectrometry and Allied Topics*, Cincinnati, OH; American Society for Mass Spectrometry: East Lansing, MI, 1986; pp 363–364.
(154) Ligon, W. V.; Dorn, S. B. *Int. J. Mass Spectrom. Ion Processes* **1986**, *68*, 337–340.
(155) Caprioli, R. M. *Anal. Chem.* **1983**, *55*, 2387–2391.
(156) Sethi, S. K.; Nelson, C. C.; McCloskey, J. A. *Anal. Chem.* **1984**, *56*, 1977–1979.
(157) Divisia-Blohorn, B.; Kyriakakou, G.; Ulrich, J. *Org. Mass Spectrom.* **1985**, *20*, 463–466.
(158) Rose, M. E.; Prescott, M. C.; Wilby, A. H.; Galpin, I. J. *Biomed. Mass Spectrom.* **1984**, *11*, 10–23.
(159) Chan, K. W. S.; Cook, K. D. *Anal. Chem.* **1983**, *55*, 1422–1424.
(160) Buko, A. M.; Fraser, B. A. *Biomed. Mass Spectrom.* **1985**, *10*, 577–585.
(161) Demirev, P.; Alai, M.; van Breemen, R.; Cotter, R. J.; Fenselau, C., In *Secondary Ion Mass Spectrometry SIMSV*; Benninghoven, A., Colton, R. J., Simons, D. S., Werner, H. W., Eds.; Springer-Verlag: Berlin, 1986; pp 527–530.
(162) Cerney, R. L.; Gross, M. L. *Anal. Chem.* **1985**, *57*, 1160–1163.
(163) Pang, H.; Costello, C. E.; Biemann, K. *Proceedings of the 32nd Annual Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX; American Society for Mass Spectrometry: East Lansing, MI, 1984; pp 751–752.
(164) Lignori, A.; Sindona, G.; Uccella, N. *J. Am. Chem. Soc.* **1986**, *107*, 7488–7491.
(165) Wagner, R. M.; Fraser, B. A. *Biomed. Mass Spectrom.* **1987**, *14*, 69–72.
(166) Caprioli, R. M.; Desiderio, D. *Biomed. Mass Spectrom.* **1982**, *9*, 557–566.
(167) Renner, D.; Spiteller, G. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 405–410.
(168) Schram, K. M.; Slowikowski, D. L. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 263–264.
(169) Aberth, W.; Burlingame, A. L. *Anal. Chem.* **1984**, *56*, 2915–2918.
(170) Aberth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* **1982**, *54*, 2029–2034.
(171) Falik, A. M.; Wang, G. H.; Walls, F. C. *Anal. Chem.* **1986**, *58*, 1308–1311.
(172) Freas, R. B.; Ross, M. M.; Campana, J. E. *J. Am. Chem. Soc.* **1985**, *107*, 6195–6201. Private communication.
(173) Buko, A. M.; Phillips, L. R.; Fraser, B. A. *Biomed. Mass Spectrom.* **1983**, *10*, 408–416.
(174) Busch, K. L.; Unger, S. E.; Vincsze, A.; Cooks, R. G.; Keough, T. *J. Am. Chem. Soc.* **1982**, *104*, 1507–1511.
(175) Kidwell, D. A.; Ross, M. M.; Colton, R. J. In *Advances in Mass Spectrometry 1985*; Todd, J. F. J., Ed.; Wiley: Chichester, UK, 1986; pp 1607–1608.
(176) Fenselau, C.; Cotter, R. J. In *IUPAC Frontiers of Chemistry*; Laidler, K. J., Ed.; Pergamon: Oxford, 1982; pp 207–216.
(177) Fenselau, C.; Heller, D. N.; Miller, M. S.; White, H. B., III *Anal. Biochem.* **1985**, *150*, 309–314.
(178) Grotjahn, L.; Frank, R.; Bloecher, H. *Nucleic Acid Res.* **1982**, *10*, 4671.
(179) Sherman, W. R.; Ackermann, K. E.; Bateman, R. M.; Green, B. N.; Lewis, I. *Biomed. Mass Spectrom.* **1985**, *12*, 409–411.
(180) Munster, H.; Stein, J.; Budzikiewicz, H. *Biomed. Environ. Mass Spectrom.* **1986**, *13*, 423–428.
(181) Vu, V. T.; Fenselau, C.; Colvin, O. M. *J. Am. Chem. Soc.* **1981**, *103*, 7362–7363.
(182) Cotter, R. J.; Hansen, G. E.; Jones, T. R. *Anal. Chim. Acta* **1982**, *136*, 135–138.
(183) Reisch, J. W. Ph.D. Thesis, Dartmouth College, 1985.
(184) Fenselau, C.; Hyver, K. In *Ion Formation from Organic Solids IFOS III*; ed. Benninghoven, A., Ed.; Springer-Verlag: Berlin, 1986; pp 109–112.