

Mass Spectrometry of Involatile and Thermally Unstable Molecules

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Mass spectrometry, after several decades of vigorous development, impressive innovation, and creative application, includes a large family of related techniques. Collectively, they constitute perhaps the most powerful and widely applicable tool for study of the structure of matter. It was early recognized that three properties are often encountered in substances for which mass spectrometric analysis is desirable—high mass, low volatility, and thermal instability. These properties present special analytical challenges and, despite many technical advances, still frequently preclude satisfactory analyses. Recent advances in mass spectrometry of involatile¹ and/or thermally unstable compounds² constitute the subject of this Account.³

Our interest in mass spectrometry of polar, thermally unstable biomolecules dates from attempts to use mass spectrometry in structural studies of thyrotropin-releasing hormone (TRH), later shown, using other methods, to be the tripeptide amide pyroglutamyl-histidylprolinamide.^{4,5} These initial efforts were not successful, and we obtained an interpretable mass spectrum (electron ionization) of TRH only after synthetic samples became available.⁶ Desiderio⁷ obtained a chemical ionization mass spectrum of TRH at about the same time. These spectra appear to be the first reported in which a molecular ion⁶ (or protonated molecular ion, MH⁺)⁷ of an underivatized tripeptide was observed.

TRH and other hypothalamic substances⁸⁻¹⁰ are obtainable only in extremely small quantities following laborious, large-scale tissue fractionation. The problem of their structure elucidation is representative of many practical analytical challenges which provide stimuli for development of new mass spectrometric methods suitable for direct analysis of underivatized polar compounds.

The event which marked the onset of the current widespread interest and research in mass spectrometry of nonvolatile and thermally unstable compounds was the announcement in 1969 by Beckey¹¹ that a new technique, termed field desorption mass spectrometry (FDMS), permitted ionization of substances adsorbed on a surface without prior volatilization. Beckey selected the monosaccharide glucose to exemplify the capability of the new technique since sugars are widely known to be of both low volatility and low thermal stability.¹² Conceptually, Beckey's development of

FDMS has had a profound effect on mass spectrometry.

Prior to the introduction of FDMS, the presumed necessity to provide gaseous samples at vapor pressures of 10⁻⁶–10⁻⁷ torr¹³ had appeared to preclude mass spectrometric analysis of compounds for which appreciable vapor pressures are not attainable without sample decomposition. During the 1950s, techniques were developed¹⁴ for the introduction of a solid sample directly into a mass spectrometer ion chamber via a probe which vaporizes the sample within a few centimeters of the ionization region. These techniques extended mass spectrometric analysis to large numbers of compounds which possess relatively low volatility but relatively good thermal stability. Prior to Beckey's announcement of FDMS¹¹ and the onset of the research discussed in this Account, compounds too involatile or too unstable for mass spectrometric analysis by conventional solid samples introduction techniques were analyzed (a) following derivatization for volatility enhancement, (b) following inadvertent or deliberate pyrolysis,^{14,15} or (c) not at all. During the decade since Beckey's initial report, a number of other new techniques for mass spectrometric analysis of involatile,

(1) "Involatile" and "nonvolatile" in this Account are used interchangeably with and to mean "very low volatility", i.e., volatility too low to permit analysis by conventional direct-probe electron ionization mass spectrometric techniques.

(2) The emphasis in this Account is on mass spectrometry of involatile and/or thermally unstable organic compounds. However, much of the discussion is equally pertinent to mass spectrometry of inorganic compounds or salts.

(3) Research in mass spectrometry of molecules of high mass (10⁴–10⁶ amu), although not discussed in this Account, is also progressing. See: Dole, M.; Cox, H. C., Jr.; Gieniec, J. *Adv. Chem. Ser.* 1973, No. 125, 73–84.

(4) Folkers, K.; Enzmann, F.; Boler, J.; Bowers, C. Y.; Shally, A. V. *Biochem. Biophys. Res. Commun.* 1969, 37, 123.

(5) Burgus, R.; Dunn, T. F.; Ward, D. N.; Vale, W.; Amoss, M.; Guillemin, R. C. R. *Hebd. Seances Acad. Sci.* 1969, 268, 2116.

(6) Chang, J. K.; Sievertsson, H.; Bogentoft, C.; Currie, D.; Folkers, K.; Daves, G. D. Jr. *J. Med. Chem.* 1971, 14, 481.

(7) Desiderio, D. M.; Burgus, R.; Dunn, T. F.; Vale, W.; Guillemin, R.; Ward, D. N. *Org. Mass. Spectrom.* 1971, 5, 221.

(8) Folkers, K.; Knudsen, R.; Lam, Y. K.; Humphries, J.; Wan, Y. P.; Bowers, C. Y.; Frick, W.; Daves, G. D., Jr.; Barofsky, D. F.; Barofsky, E. *Proc. 2nd Eur. Coll. Hypothalamic Hormones* 1978, 47–58.

(9) Lam, Y. K.; Knudsen, R.; Folkers, K.; Frick, W.; Daves, G. D., Jr.; Barofsky, D. F.; Bowers, C. Y. *Biochem. Biophys. Res. Commun.* 1978, 81, 680.

(10) Knudsen, R.; Lam, Y. K.; Folkers, K.; Frick, W.; Daves, G. D., Jr.; Barofsky, D. F.; Bowers, C. Y. *Biochem. Biophys. Res. Commun.* 1978, 80, 735.

(11) Beckey, H. D. *Int. J. Mass Spectrom. Ion Phys.* 1969, 2, 500.

(12) Glucose itself is sufficiently volatile and thermally stable for field ionization mass spectrometry. See Giessmann U.; Röllgen, F. W. *Org. Mass Spectrom.* 1976, 11, 1094.

(13) An ion cyclotron resonance mass spectrometer that can detect vapor pressures of 10⁻¹⁰ torr has been reported. McIver, R. R.; Ledford, E. B., Jr.; Miller, J. S. *Anal. Chem.* 1974, 47, 692.

(14) Beynon, J. H. "Mass Spectrometry and its Applications to Organic Chemistry"; Elsevier: Amsterdam, 1969; Chapter 5.8.

(15) Posthumus, M. A.; Nibbering, N. M. M.; Boerboom, A. J. H.; Schulten H. R. *Biomed. Mass. Spectrom.* 1974, 1, 352.

G. Doyle Daves, Jr., was born in Clayton, NM, and obtained his undergraduate education at New Mexico Highlands University and Arizona State University. Following 2 years at Midwest Research Institute, he attended Massachusetts Institute of Technology and received a Ph.D. degree in organic chemistry under John C. Sheehan in 1964. After postdoctoral research with Karl Folkers at Stanford Research Institute, he became a member of the founding faculty at the Oregon Graduate Center in 1967. His current research involves isolation, structure elucidation, and synthesis of biologically potent molecules and emphasizes applications of mass and nuclear magnetic resonance spectrometries.

Table I
Mass Spectrometric Procedures for Ionization and Analysis of Nonvolatile and/or Thermally Unstable Compounds

technique	introduction
field desorption mass spectrometry (FDMS)	1969 (Beckey ^a)
²⁵² Cf plasma (fission fragment induced) desorption mass spectrometry (PDMS)	1974 (Macfarlane and Torgerson ^{b,c})
electrohydrodynamic ionization mass spectrometry (EHDMS)	1974 (Evans and co-workers ^{d-f})
secondary ion (ion bombardment) mass spectrometry (SIMS) ^g	1974-1976 (several investigators ^{h-i})
laser desorption mass spectrometry (LDMS)	1978 (FOM Institute, Amsterdam ^{m,n}) (1968, Vastola and Pirone ^o)
chemical ionization (CI) in beam	1973 (Baldwin and McLafferty ^p)
desorption	1977 (Hunt and co-workers ^q)
electron ionization (EI) in beam	1975 (Dell et al.; Ohashi et al. ^s) (1963, Reed and co-workers ^t)
rapid heating	1972 (Friedman and co-workers ^u)
desorption	1977 (Soltmann, Sweeley, and Holland ^v)
flash desorption	1977 (Anderson, Daves, and co-workers ^w)

^a Reference 11. ^b Torgerson, D. F.; Skowronski, R. P.; Macfarlane, R. D. *Biochem. Biophys. Res. Commun.* 1974, 616.
^c Macfarlane, R. D.; Torgerson, D. F. *Science* 1976, 191, 920. ^d Simons, D. S.; Colby, B. N.; Evans, C. A., Jr. *Int. J. Mass Spectrom. Ion Phys.* 1974, 15, 291. ^e Stimpson, B. P.; Evans, C. A., Jr. *Biomed. Mass Spectrom.* 1978, 5, 52. ^f Stimpson, B. P.; Simons, D. S.; Evans, C. A., Jr. *J. Phys. Chem.* 1978, 82, 660. ^g Secondary ion mass spectrometry (SIMS) is a long-established technique for analysis of atomic composition of surfaces (Benninghoven, A. *Surf. Sci.* 1975, 33, 596). In this Account consideration is restricted to analysis of an adsorbed substance (or substances) on a surface. ^h Duhamel, A. P. *Pittsburgh Conf. Anal. Chem. Appl. Spectrosc.* 1974, No. 352. ⁱ Benninghoven, A.; Jaspers, D.; Sichtermann, W. *Appl. Phys.* 1976, 11, 35. ^j Benninghoven, A.; Sichtermann, W. *Anal. Chem.* 1978, 50, 1180. ^k Karasek, F. W. *Res./Dev.* 1976, 25, 42. ^l Reference 50. ^m Posthumus, M. A.; Kistemaker, P. G.; Meuzelaar, H. L. C.; Ten Noever de Brauw, M. C. *Anal. Chem.* 1978, 50, 985. ⁿ Boerboom, A. J. H.; Kistemaker, P. G.; Posthumus, M. A.; Meuzelaar, H. L. C. *Dyn. Mass Spectrom.* 1978, 5, 114. ^o Vastola, F. J.; Pirone, A. J. *Adv. Mass Spectrom.* 1968, 4, 107. ^p Reference 33. ^q Reference 23. ^r Reference 29. ^s Reference 30. ^t Reference 28. ^u Reference 19-21. ^v Reference 22. ^w Reference 24, 25.

thermally unstable compounds have been reported (Table I).

Strategies for Obtaining Mass Spectra of Nonvolatile and Thermally Unstable Compounds

The techniques listed in Table I have utilized two general strategies: (1) optimization of sample vaporization and (2) direct ionization of molecules from a surface. They have extended mass spectrometry to analysis of compounds much less volatile and much less thermally stable than was possible previously. These two strategic concepts will be discussed separately for convenience, although, for most of the techniques listed (Table I), there is ambiguity concerning the precise events which lead to ion production; it is probable that in some instances both sample volatilization and direct ionization of an adsorbed molecule from a surface occur.

Optimization of Conditions for Sample Vaporization. Vaporization of a thermally stable molecule depends only on the provision of adequate energy to the molecule. Consequently, high-temperature vaporization techniques using a Knudsen cell followed by electron ionization¹⁶ and spark source mass spectrometric techniques¹⁷ have been used to obtain spectra of stable inorganics.¹⁸ Transfer of energy to a relatively involatile, thermally labile compound by heating produces a kinetic competition between vaporization (i.e., dissociation of intermolecular bonds) and sample decomposition (dissociation of intramolecular bonds) in which, under many conditions, sample decomposition is favored. Friedman and co-workers¹⁹⁻²¹ addressed this

problem and suggested two approaches to the enhancement of volatility: (a) reduction of the energy necessary to achieve vaporization by sample deposition onto an inert surface and (b) selection of conditions for transfer of energy to the sample molecule such that vaporization is favored kinetically over decomposition, i.e., rapid sample heating. A third approach—minimization of the distance from site of volatilization to point of ionization—has led to the techniques denoted "in beam" (Table I).

Dispersal of molecules onto a relatively inert surface reduces surface-molecule bonding energy. Beuhler et al.¹⁹ calculated an activation energy for desorption of the tripeptide amide TRH⁴⁻⁷ from Teflon of 30 kcal/mol compared with 60 kcal/mol for desorption of TRH from glass. They have noted analytically useful improvements in volatilities of peptides and other polar molecules through use of a Teflon-coated probe.¹⁹⁻²¹ More recently, it has been shown that activated carbonaceous microneedle tungsten wire emitters, developed for FDMS, provide good surfaces from which to volatilize relatively involatile and thermally unstable compounds for electron²² and chemical²³ ionization. Presumably the principal advantage of microneedle emitters in this application is their large, relatively inert surface which permits effective sample dispersal and minimizes molecule-molecule interactions. An important limitation of this approach to volatility enhancement is the fact that polar compounds on inert surfaces tend to aggregate.²⁰ Unless effective sample dispersion is achieved, the bulk of the bonding energy to be overcome in vaporization of a polar compound from an inert surface will derive from molecule-molecule interactions.

(16) Boerboom, A. J. H. In "Mass Spectrometry"; Reed, R. L., Ed.; Academic Press: New York, 1965, p 251.

(17) Rechsteiner, C. E.; Buck, R. P.; Pedersen, L. *J. Chem. Phys.* 1976, 65, 1659.

(18) The use of spark source techniques for production of mass spectra of organic compounds has also been reported. See, for example: Beynon, J. H. In "Mass Spectrometry"; Reed, R. L., Ed.; Academic Press: New York, 1965, pp 373-376.

(19) Beuhler, R. J.; Flanigan, E. O.; Greene, L. J.; Friedman, L. *Biochem. Biophys. Res. Commun.* 1972, 46, 1082.

(20) Beuhler, R. J.; Flanigan, E.; Greene, L. J.; Friedman, L. *J. Am. Chem. Soc.* 1974, 96, 3990.

(21) Beuhler, R. J.; Flanigan, E.; Greene, L. J.; Friedman, L. *Biochemistry* 1974, 13, 5060.

(22) Soltmann, B.; Sweeley, C. C.; Holland, J. F. *Anal. Chem.* 1977, 49, 1164.

(23) Hunt, D. F.; Shabanowitz, J.; Botz, F. K.; Brent, D. A. *Anal. Chem.* 1977, 49, 1160.

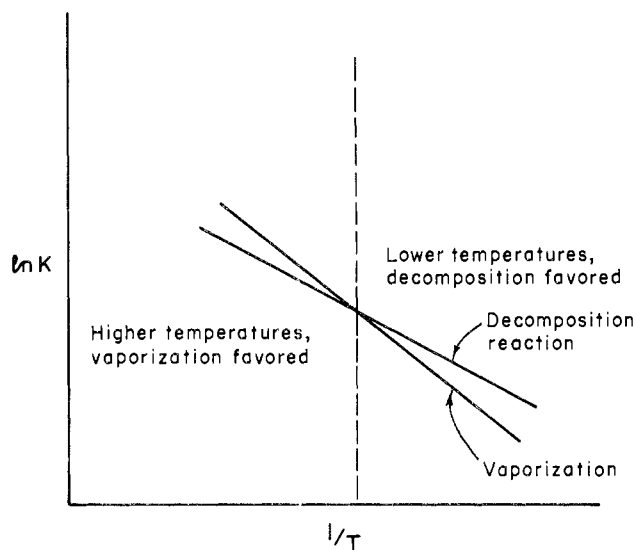


Figure 1. Relationship between the temperature dependencies of vaporization and molecular decomposition processes of an involatile, thermally labile compound. Recognition of this relationship led Friedman and co-workers¹⁹⁻²¹ to advocate rapid sample heating in mass spectrometric analysis of thermally labile compounds.

The second approach to enhancement of sample vaporization introduced by Friedman and co-workers¹⁹⁻²¹ is rapid sample heating. This has been shown to be advantageous based on a kinetic analysis of the competitive processes of sample vaporization and sample decomposition (Figure 1). Even though the activation energy for a decomposition reaction is lower

than the activation energy for vaporization of a thermally labile compound, Arrhenius plots of the rates of the two processes vs. $1/T$ must intersect at some value of $1/T$.²⁰ The implication of this fact for mass spectrometry of thermally labile compounds is that molecular species will be relatively more abundant in the gas phase at temperatures above the point of intersection of the Arrhenius plots than at lower temperatures where decomposition is favored (Figure 1). This was well illustrated by Friedman and co-workers in a series of experiments employing TRH.²⁰ Under chemical ionization conditions, at a sample temperature of $\sim 160^\circ\text{C}$, the mass spectrum of TRH exhibited a protonated molecular ion (m/e 363) which is only one-third as intense as an ion at m/e 235 indicative of a thermal decomposition process. In contrast, when the TRH sample was heated rapidly ($10^\circ/\text{s}$) to $\sim 215^\circ\text{C}$, the intensities of the two ions had an inverse relationship; i.e., the intensity of the protonated molecular ion was now three times that of the fragment ion intensity.²⁰

In our laboratory, we have combined even more rapid sample heating ($25^\circ\text{C} \rightarrow >1000^\circ\text{C}$ in ~ 0.2 s) (flash desorption) with photographic detection of the ions produced during the brief desorption period in an electron ionization mass spectrometer.^{24,25} Heating a sample in this way minimizes the residence time in the temperature region where decomposition is favored, and the high temperature achieved compensates for lack of knowledge concerning the precise point of intersection of the Arrhenius plots for the vaporization and decomposition processes (Figure 1).

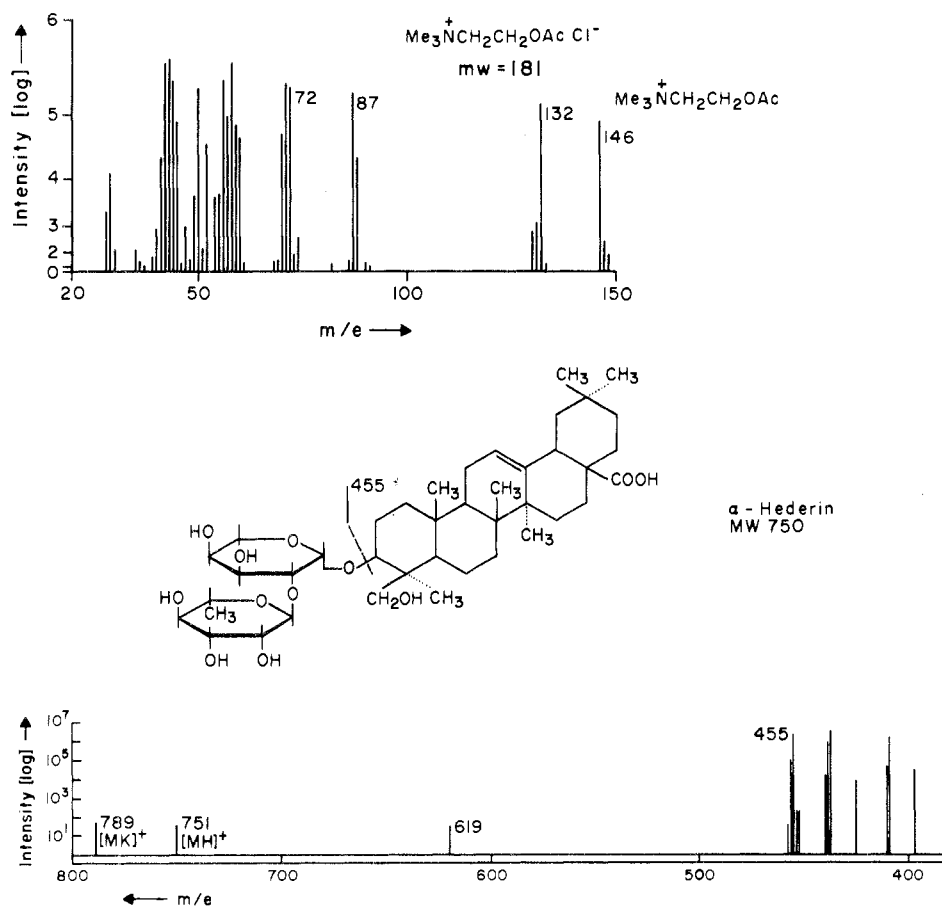


Figure 2. Mass spectra of acetylcholine (top) and α -hederin (bottom) obtained by the electron ionization-flash desorption technique.²⁵

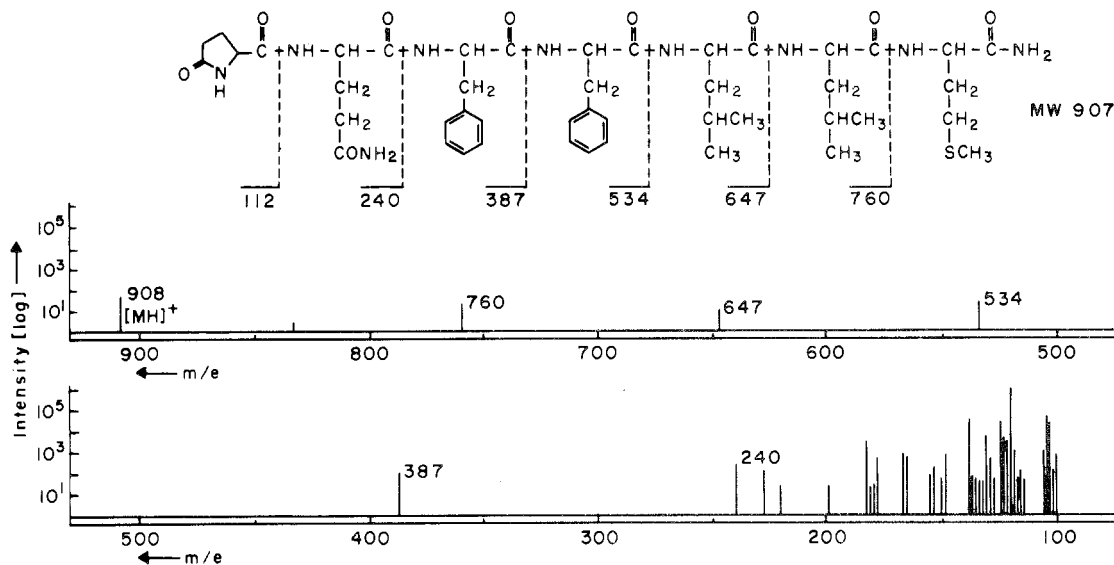


Figure 3. Mass spectrum of the heptapeptide amide *p*-Glu-Gln-Phe-Phe-D-Leu-Leu-MetNH₂²⁴ obtained by electron ionization-flash desorption.^{24,25} Fragment ions which define the amino acid sequence are noted.

The utility of the flash desorption technique^{24,25} is apparent from the spectra contained in Figures 2 and 3. These figures show ions indicative of molecular weight of a quaternary ammonium ion²⁶ (acetylcholine, Figure 2, top), of a relatively involatile, thermally unstable plant saponin (α -hederin,²⁷ Figure 2, bottom), and of a heptapeptide amide²⁴ (Figure 3). Because electron ionization was used rather than a "soft" ionization technique like FDMS, the spectra are rich in structurally significant fragment ions. This is particularly evident in the spectrum of the heptapeptide amide (Figure 3) in which cleavage ions necessary to define the amino acid sequence are seen.

In 1963, as a logical extension of direct sample insertion techniques then under development, Reed and co-workers²⁸ discovered that placement of a sample of an involatile, thermally unstable compound in very close proximity to the electron beam of an electron ionization mass spectrometer allowed spectra exhibiting protonated molecular ions to be recorded. More conventional techniques invariably failed to yield ions characteristic of molecular weight. Recently, Dell et al.²⁹ rediscovered this phenomenon, termed "in beam" mass spectrometry. Ohashi and co-workers have employed it effectively in a number of challenging analytical applications.³⁰⁻³² A closely related chemical ionization technique was reported by Baldwin and McLafferty³³ and has been used successfully by others.^{34,35} Particularly impressive is a recent report by

Ganguly et al.³⁶ in which Cl⁻ attachment negative ion mass spectra of underivatized di-, tri-, and tetrasaccharides were recorded.

Direct Ionization of Molecules from a Surface. The techniques discussed in the previous section are characterized by relatively minor modifications of methods used in conventional electron and chemical ionization mass spectrometries for analysis of solids. It is convenient to view the experimental modifications which permit analysis of involatile and thermally unstable compounds by these methods as modifications which enhance vaporization of intact sample molecules.^{37,38} The remaining techniques listed in Table I—field desorption, ²⁵²Cf plasma (fission fragment induced) desorption, electrohydrodynamic ionization, and secondary ion and laser desorption mass spectrometries—differ in that ion formation almost certainly occurs at the sample-bearing surface for these techniques.

To explain the ionization processes operative in FDMS, Beckey³⁹ adapted theoretical models originally introduced by Müller⁴⁰ and Gomer^{41,42} to describe field evaporation (evaporation of emitter material itself). These models were developed to explain the ionization of single atoms in high electrostatic fields. While, in principle, theoretical treatments for molecules should be similar, the extreme difficulties encountered in adequately handling field desorption of atoms have

(24) Anderson, W. R., Jr.; Frick, W.; Daves, G. D., Jr.; Barofsky, D. F.; Yamaguchi, I.; Chang, D.; Folkers, K.; Rosell, S. *Biochem. Biophys. Res. Commun.* 1977, 78, 372.

(25) Anderson, W. R., Jr.; Frick, W.; Daves, G. D., Jr. *J. Am. Chem. Soc.* 1978, 100, 1974.

(26) Similar spectra of dimethylindol-3-ylsulfonium salts have been obtained: K. H. Park and G. D. Daves, Jr., unpublished results.

(27) Tschesche, R.; Schmidt, W.; Wulff, G. *Z. Naturforsch.* 1965, 206, 708.

(28) Reed, R. I.; Reid, W. K. *J. Chem. Soc.* 1963, 5933.

(29) Dell, A.; Williams, D. H.; Morris, H. R.; Smith, G. A.; Feeney, J.; Roberts, G. C. K. *J. Am. Chem. Soc.* 1975, 97, 2497.

(30) Ohashi, M.; Tsujimoto, K.; Yasuda, A. *Chem. Lett.* 1976, 439.

(31) Ohashi, M.; Nakayama, N.; Kudo, H.; Yamada, S. *Mass Spectrosc. (Jpn.)* 1976, 24, 265.

(32) Ohashi, M.; Yamada, S.; Kudo, H.; Nakayama, N. *Biomed. Mass Spectrom.* 1978, 5, 578.

(33) Baldwin, M. A.; McLafferty, F. W. *Org. Mass Spectrom.* 1973, 7, 1141, 1353.

(34) Hansen, G.; Munson, B. *Anal. Chem.* 1978, 50, 1130.

(35) Cotter, R. J.; Fenselau, C. C. Presented at 26th Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, Mo., May 28-June 2, 1978.

(36) Ganguly, A. K.; Cappuccino, N. F.; Fujiwara, H.; Bose, A. K. *J. Chem. Soc., Chem. Commun.* 1979, 148.

(37) The possibility that the CI and EI techniques also involve (in part) surface ionization is appealing since in common with the other techniques under discussion, they frequently exhibit ions formed by cationization^{23,38} which appears to be a facile surface ionization process (see the following discussion). It is also noteworthy that in EI-flash desorption^{24,25} MH⁺ and not M⁺ ions are observed.

(38) Daves, G. D., Jr.; Anderson, W. R., Jr. *Int. J. Mass Spectrom. Ion Phys.* 1979, 30, 385.

(39) Beckey, H. D. "Principles of Field Ionization and Field Desorption Mass Spectrometry"; Pergamon Press: Oxford, 1977.

(40) Müller, E. W. *Phys. Rev.* 1956, 102, 618.

(41) Gomer, R. *J. Chem. Phys.* 1959, 31, 341.

(42) Gomer, R.; Swanson, L. W. *J. Chem. Phys.* 1963, 38, 1613.

Table II
 Mass Spectra of Underivatized Sucrose

ionization method	special experimental parameters	characteristics of spectrum produced	ref
FDMS ^a		no. M ⁺ ; [MH] ⁺ 100%; ^b [MH ⁺ - H ₂ O] ⁺ 14%; m/z 163, ^c 57%	Moor, Waight ^f
FDMS		no M ⁺ ; [MH] ⁺ 90%; [MH ⁺ - H ₂ O] ⁺ ; [MH ⁺ - CH ₂ OH] ⁺	Schulten ^g
FDMS	1 equiv of NaI added; sucrose sample	[MNa] ⁺ 97% of total ionization	Prome, Puzo ^h
FIMS	emitter tungsten wire coated with LiI	[MLi] ⁺ very weak; all ions contain Li, much pyrolysis evident	Giessmann, Röllgen ⁱ
CI in beam		[MH] ⁺ , m/z 163, ^c fragmentation	Cotter ^j
EI in beam		[MH] ⁺ , m/z 163 ^c	Ohashi et al. ^k
EI flash desorption		[MH] ⁺ , very weak; much fragmentation and pyrolysis	Anderson et al. ^l
LDMS	CO ₂ laser	[MNa] ⁺ , [MK] ⁺ , m/z [162 ^d (180) ^e + Na(K)] ⁺	Posthumus et al. ^m
LDMS	ruby, neodymium YAG lasers	[MNa] ⁺ , [MK] ⁺	Anderson et al. ⁿ
EHDMS	solvent, glycerol; electrolyte, NaI	[MH] ⁺ ; [MNa] ⁺ , [M + glycerol + Na(H)] ⁺	Stimpson, Evans ^o

^a For abbreviations, see Table I, FIMS = field ionization mass spectrometry. ^b Percent relative intensity. ^c Mono-saccharide oxonium ion derived (formally) by protonation of product of dehydrative cleavage of interglycosidic linkage. ^d Cationized analogue of ion at m/z 163. ^e Cationized glycoside formed by cleavage of glycosidic linkage with retention of linking oxygen. ^f Moor, J.; Waight, E. S. *Org. Mass Spectrom.* 1974, 9, 903. ^g Schulten, H. R. *Methods Biochem. Anal.* 24, 313, 1977. ^h Prome, J. C.; Puzo, G. *Org. Mass Spectrom.* 1977, 12, 28. ⁱ Reference 12. ^j Cotter, R. J. *Anal. Chem.* 1979, 51, 317. ^k Reference 30. ^l Reference 25. ^m See footnotes m, n in Table I. ⁿ Reference 51. ^o See footnote e, Table I.

precluded any direct attempts to extend this theory to field desorption of molecules.⁴³ Nevertheless, it has become common practice to invoke the Müller-Gomer models in a qualitative sense in describing FDMS of even complex biomolecules.⁴³

Discrepancies between experimental results and the predictions of this theory are now evident. In 1974, Barofsky and Barofsky⁴⁴ noted that ion emission occurred in short bursts following each incremental increase in emitter heating current. In what is now a landmark paper, Holland, Soltmann, and Sweeley⁴⁵ reported a more thorough set of similar experiments which led them to the conclusion that, contrary to predictions of the Müller-Gomer-Beckey theory, desorption depended only on the emitter temperature and not on the magnitude of the electric field (within experimentally accessible limits).⁴⁶ Equally important is the nature of the ionization observed in FDMS.

There appears to be little doubt that, in accord with Beckey's model,³⁹ formation of molecular (M⁺) ions by electric field dependent ionization occurs in high-field regions at, or near, the tips of emitter microneedles. However, processes which give rise to even electron protonated and cationized molecular ions are observed with equal (or perhaps greater) frequency.

In recognition of the duality of ion-forming modes operative in FDMS, Röllgen and co-workers^{47,48} have utilized electric fields of <10⁷ V/cm to produce spectra which exhibit no evidence of radical ion formation and

consist solely of ions involving cationization. A particularly effective way of accomplishing this,⁴⁷ which we have exploited in FDMS of oligopeptides, is through use of unactivated, needleless (i.e., bare wire) emitters.⁴⁹ Needleless emitters provide more homogeneous conditions of electric field strength and temperature and, while peptide spectra obtained with activated and needleless emitters are qualitatively very similar, with needleless emitters less variation from run to run and from emitter to emitter is seen. More important for many applications is the fact that use of needleless emitters permits analysis using picogram quantities of peptide whereas nanogram amounts are required when microneedle emitters are used.⁴⁹

That ionization by cationization or protonation can occur in the absence of an electric field⁴⁵ is evident since every one of the techniques for ionization of involatile and thermally unstable compounds listed in Table I yields spectra which exhibit protonated and/or cationized molecular ions. It is significant that the seemingly quite different mass spectrometric methods (Table I) yield spectra which are strikingly similar in many important respects.^{38,50} Thus, none of the reported (Table II) mass spectra of underivatized sucrose, obtained by various techniques, exhibits a molecular (M⁺) ion; all exhibit protonated (MH⁺) or cationized (MLi⁺, MNa⁺ or MK⁺) molecular ions. A similar comparison of spectra of salts of carboxylic acids (Table III) is instructive. The dominant feature of the spectra of these and other organic and inorganic salts is a series of cluster ions of the type (A_nB_{n+1})⁺ which is independent of the mass spectrometric method used.

It is evident from consideration of these data that cationization (protonation, eq 2-4) is a general process leading to even-electron ions governed largely by sample matrix and molecular energy considerations.⁴⁵ It is

(43) For a good recent discussion see: Cocke, D. L.; Block, J. H. *Surf. Sci.* 1978, 70, 363.

(44) Barofsky, D. F.; Barofsky, E. *Int. J. Mass Spectrom. Ion Phys.* 1974, 14, 3.

(45) Holland, J. F.; Soltmann, B.; Sweeley, C. C. *Biomed. Mass Spectrom.* 1976, 3, 340.

(46) Strong disagreement with the conclusions of Holland et al.⁴⁵ has been expressed (Beckey, H. D.; Röllgen, F. W. *Org. Mass Spectrom.* 1979, 14, 188). See also the reply (Holland, J. F. *Ibid.*, 1979, 14, 291) and Professor Beckey's "Final note" (Beckey, H. D. *Org. Mass Spectrom.* 1979, 14, 292).

(47) Röllgen, F. W.; Schulten, H. R. *Z. Naturforsch.* 1975, 30, 1685.

(48) Heinen, H. J.; Giessmann, U.; Röllgen, F. W. *Org. Mass Spectrom.* 1977, 12, 710.

(49) Frick, W.; Barofsky, E.; Daves, G. D., Jr.; Barofsky, D. F.; Chang, D.; Folkers, K. *J. Am. Chem. Soc.* 1978, 100, 6221.

(50) Grade, H.; Winograd, N.; Cooks, R. G. *J. Am. Chem. Soc.* 1977, 99, 7725.

Table III
Characteristics of Mass Spectra of Carboxylate Salts

compound	ionization method	principal ions or ion series observed	ref
NaOAc	FDMS	$[(\text{AcO}^-)_n \text{Na}^+_{n+1}]^+$, $n = 1-7$	Schulten, Röllgen; ^a Wood et al. ^b
NaOAc	EI-flash desorption	$[(\text{AcO}^-)_n \text{Na}^+_{n+1}]$, $n = 1-3$	Daves, Anderson ^c
NaOAc	EIMS	$[(\text{AcO}^-)_n \text{Na}^+_{n+1}]$, $n = 1-3$	White ^d
NaOAc	LDMS	$[(\text{AcO}^-)_n \text{Na}^+_{n+1}]$, $n = 1-4$	Boerboom et al. ^e
Na(K)OBz	CI-desorption	$[(\text{benzoate}^-)_n \text{Na}^+(\text{K}^+)_{n+1}]$, $n = 1-3^+$	Hunt et al. ^f
NaOBz	EI-flash desorption	$[(\text{benzoate}^-)_n \text{Na}^+_{n+1}]^+$, $n = 1$	Daves, Anderson ^c
NaOOCR	FDMS	$[(\text{RCOO}^-)_n \text{Na}^+_{n+1}]^+$, $n = 1-3$	Wood et al. ^b
Li ⁺ , Na ⁺ , K ⁺ , Rb ⁺ RCOO ⁻	EIMS	$[(\text{RCOO}^-)_n \text{Met}^+_{n+1}]^+$, $n = 1-7$	White ^d

^a Schulten, H. R.; Röllgen, F. W. *Org. Mass Spectrom.* 1975, 10, 649. ^b Wood, G. W.; Oldenburg, E. J.; Lau, P. Y. *Can. J. Chem.* 1978, 56, 2750. ^c Reference 38. ^d White, E., V. *Org. Mass Spectrom.* 1978, 13, 695. ^e See footnote n, Table I. ^f Reference 23.

important to note, however, that the detailed dynamics of such ionizations are largely unknown and may be quite complex. This is well illustrated by recent results from our laboratory. A single aqueous solution of sucrose when analyzed by EI-flash desorption²⁵ exhibited spectra in which the ion of highest mass corresponds to MH^+ ; when analyzed by LDMS (using either a ruby or a neodymium-YAG laser) this solution yielded spectra in which MNa^+ and MK^+ ions were present but in which no MH^+ ion was observed⁵¹ (Table II). In the EI-flash desorption experiment when the ionizing electron beam was switched off MH^+ ions were absent, whereas all principal ions in the LD mass spectra (Table II) were observed irrespective of the presence or absence of an ionizing electron beam.

Ion Stabilities, Molecular Weight Determination, and Structural Studies

To date, most research in mass spectrometry of involatile and thermally unstable compounds has focussed on the production and recording of ions characteristic of compound molecular weight. In large part this emphasis results from the fact that FDMS (both in formation of M^+ and cationized molecular ions) and the other newer mass spectrometries (excluding the CI and EI methods) are "soft" ionization techniques; i.e., they produce ions possessing relatively low internal energies which undergo little fragmentation. Ions formed by alkali metal cation attachment are particularly stable because localization of positive charge on the metal cation effectively precludes the rearrangement of bonding electrons necessary for ion fragmentation.⁵² This superior stability, evident from a comparison of the various sucrose spectra summarized in Table II, makes cationization the ionization method of choice for molecular weight determination.⁵²

Now that we have increasingly reliable methods for ionization of involatile and thermally fragile compounds we can expect increased efforts toward applications of these techniques in mass spectrometric analysis of all classes of condensed matter. Increasingly, attention will be directed toward the achievement not only of molecular weight information but also of the detailed molecular structure information now associated primarily with conventional EIMS.

Because of our interest in hypothalamic hormones,^{6,8-10} we have focussed on the development of methodology for elucidating structures of oligopeptides

suitable for use when the total peptide sample available is on the order of nanograms. We initially elected a strategy in which peptide molecular weights would be determined by FDMS and amino acid composition would be obtained by gas chromatographic-mass spectrometric selected ion monitoring techniques.⁵³ At the time our research was initiated, only a few limited studies of peptide analysis by FDMS were available,^{54,55} and it appeared reasonable to expect that FDMS would provide at least limited amino acid sequence information in addition to molecular weights.⁵⁶

In our study we initially used conventional activated carbon microneedle emitters. Later we elected to use needleless emitters which significantly improved analytical sensitivity while producing spectra of tripeptides essentially indistinguishable from those produced with activated emitters.⁴⁹ A typical FD mass spectrum of a tripeptide obtained in this study exhibits approximately 20 ions. Of these, molecular weight defining ions (i.e., MH^+ , MNa^+ , MK^+) account for 70-80% of total ionization. Most of the remaining ions are doubly charged species (e.g. MNa_2^{2+}) and ions derived from the intact peptide by loss of one or more small molecules (H_2O , NH_3 , CO_2 , CONH). Finally, at most, 1-2% of total ionization is distributed among several ions indicative of (probably thermal) peptide backbone rupture. When the mass spectrum is that of a highly purified, usually synthetic peptide for which the amino acid composition (and sequence) is known, amino acid sequence defining ions can often be assigned. In a practical sense, however, we have concluded that FDMS alone is generally inadequate for elucidation of such structural features.⁴⁹

We have investigated two alternative techniques for peptide structural analysis. The first utilizes analysis by FDMS of the mixture of products obtained by peptide methylation-methanolysis.⁵⁷ Under appropriately chosen conditions, a reaction mixture consisting of a series of overlapping fragments of the original peptide is obtained and assignment of corresponding (now relatively intense) protonated molecular ions permits (with knowledge of the amino acid composition)

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a unique amino acid sequence for the original oligopeptide to be determined.⁵⁷

The second technique for peptide structural elucidation which we are studying involves EI-flash desorption mass spectrometry.^{24,25} In its present state of development, the method is relatively insensitive (requiring microgram qualities of sample), is not readily reproducible (frequent failures are still experienced), and rapidly degrades instrument performance requiring frequent ion source cleaning. However, the spectra produced in successful experiments (e.g., Figure 3) are of high quality and possess not only protonated molecular ions but also typical EI fragment ions which permit molecular structure analysis.²⁴

In other laboratories, collision-induced dissociation (CID) of ions produced by FDMS⁵⁸⁻⁶⁰ is being used to obtain spectra exhibiting structurally significant molecular fragment ions. The combination of ion formation by a "soft" ionization method and CID of the ions produced promises to be a powerful technique for structural studies.

In another important area of research in mass spectrometry, work is underway to extend the analytical mass range. Using a mass spectrometer equipped with a high field (23 kG) magnet, Dell and Morris⁶¹ have recorded ions produced by FDMS at m/z values >3000. Similarly, Winkler et al.,⁶² using special instrumentation, recorded ions of m/z >4000 during FDMS analysis of the 29 amino acid peptide glucagon. Very recently, McNeal and Macfarlane⁶³ succeeded in obtaining mass spectra of protected oligonucleotides which exhibited cationized molecular ions of m/z >5000 by PDMS and

time-of-flight mass analysis. It appears certain that mass spectrometric analyses of molecules of mass >2000 will become increasingly common.

Summary and Prospects for the Future

During the past decade, mass spectroscopists have made impressive progress in analysis of involatile and/or thermally unstable compounds. There are currently more than a half dozen techniques (Table I) which have shown significant capability for mass spectrometric analysis of these challenging compound classes. All of the techniques appear to involve, at least in part, ionization via attachment of a charged particle (e.g., a proton or cation) to a neutral molecule.^{38,50} While current research places significant emphasis on enhancement of volatility of very involatile compounds, no clear distinction has been made in all cases between gas-phase and surface ionization processes.⁶⁴

It is safe to predict that the coming years will bring: (1) ionization procedures which will permit routine production of ions characteristic of molecular weight for essentially all compounds of accessible mass range; (2) improved mass analyzers which will significantly extend the mass range from that typically available today; and (3) mass spectrometers which utilize ion activation processes (e.g., CID) to induce fragmentation of relatively nonenergetic ions arising from "soft" ionization processes to produce spectra possessing both molecular weight and molecular structure information (which is routinely available today only when the initial ionization produces ions with sufficient energy for fragmentation).

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