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MIDDLE MOLECULE MASS SPECTROMETRY (A REVIEW)

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SUMMARY

This paper reviews some recent advances in the application of mass spectrometry to the analysis of middle molecules in the mass range of 1000 to 10,000 daltons, and of very polar molecules (organic salts). The quantitative analysis of components of mixtures will be illustrated, carried out by creating a spectrum which consists primarily of molecular ion species whose relative intensities correspond to the relative molar concentrations of the various components.

In the analyses of heavy and/or polar substances, four components of the mass spectrometer must all be considered: the inlet system, ionization method, ion analyser and ion detector. Reading the spectrum also requires some special considerations, as will be seen later. Among the inlet systems, the direct probe and liquid chromatography (LC) are suitable for middle molecules or for organic salts. Suitable detectors can presently be constructed to detect ions of any size, relying, if necessary, on post acceleration or on smashing the large ions into smaller ones after separation in the analyser. All three of the most commonly used kinds of analyzers (magnetic, quadrupole and time-of-flight) have been shown to transmit ions of masses in excess of 5000 daltons; however, only magnetic analyzers can do so with unit resolution. Currently, the limiting factor in the extension of mass spectrometry (MS) to analysis of middle molecules is ionization. No ionization technique has as yet gained complete acceptance as a reliable, versatile and efficient technique for middle molecules and organic salts.

As a brief history of middle molecule MS, four specta may be mentioned. In 1966, Fales[1] published the electron impact mass spectrum of a tetrameric phosphazene of molecular weight 3628. The spectrum was obtained using a magnetic analyzer and unit resolution was achieved in the molecular ion region. The spectrum of a set of cluster ions of isopropyl alcohol formed in a high-pressure source and transmitted through a quadrupole analyzer has been measured by Beuhler and Friedman[2,3]. While the resolution is low, the clusters sixty units apart in mass may be distinguished above 8000 daltons. The spectrum of a mixture of polystyrene polymers of average mass 8500 has been published by Japanese workers[4]. This spectrum was obtained by field desorption with resolution of about 2000 using a toroidal magnetic analyzer. Ions are formed, transmitted, and detected above 10,000 daltons.

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The fourth spectrum to be mentioned in this short history is that of an oligodeoxyribonucleotide containing derivatized phosphotriesters. A peak at *m/z* 6301 corresponds to $(M + Na)^+$ ions and one at 12637 is proposed as a cationized dimer[5]. The spectrum was measured on a time-of-flight analyzer using Cf.252 plasma desorption as the ionization technique. Although the resolution of this analyzer is below 1000, centroids of the broad peaks are calculated by computer and related to average masses[5].

Thus it seems that ions in the middle mass range can be analyzed by MS. However this kind of work is not yet done routinely, and one of the main reasons is the difficulty of forming ions of heavy or polar, *i.e.* involatile, substances.

Two transformations are required initially for mass spectral analysis. Each molecule must be put into gas phase and each molecule must be ionized. The older ionization techniques for organic samples, electron impact and chemical ionization, require that vaporization precede ionization. Thus they are well suited for coupled gas chromatography–MS.

Some inroads have been made into the middle molecule mass range using electron impact or chemical ionization. The spectrum of phosphazene molecular weight 3628 mentioned above provides an early example. A most influential paper from Gif-sur-Yvette[6] reported not only the molecular weight of the nonapeptide fortuitin, but also electron-impact induced fragmentation which permitted the sequence of the peptide to be deduced. Eventually it was realized that this analysis of fortuitin was made possible because the compound was naturally derivatized. The terminal amine occurred as the amide of a long chain acyl group, and three of the nine amides in the peptide were themselves alkylated. By these means intermolecular hydrogen bonding is sufficiently reduced as to permit the vaporization required for mass spectral analysis. Derivatization has also permitted analysis of important polysaccharides in the middle molecule mass range by electron impact (permethylation)[7] and chemical ionization[8].

However, derivatization is not always desirable, and even with derivatization many molecules can not be vaporized without pyrolysis. To paraphrase Fales[9], the more important a compound is, the less likely it is to produce a molecular ion. Consequently considerable energy has, in recent years, gone into the development of new techniques to produce desorption and ionization of samples in the solid phase. These include field desorption, laser desorption, fast atom bombardment, and fast ion bombardment or plasma desorption. Work will be reviewed here from the Middle Atlantic Mass Spectrometry laboratory (an NSF Regional Instrumentation Facility) in which two of these techniques are evaluated, field desorption and fast atom bombardment (FAB). Emphasis is placed on analysis of middle molecules, organic salts and mixtures.

Fig. 1 (ref. 10) compares the electron impact mass spectrum and the field desorption mass spectrum of a mixture of soluble polystyrene oligomers of average molecular weight 1020, and dramatically makes the point that a solid phase ionization technique enhances the production of molecular ions and reduces pyrolysis. Lattimer *et al.*[11] have shown that the relative abundances of molecular ions, produced by careful field desorption of a number of oligomer mixtures of average mass above 1000, can be directly related to the relative molarities of the components of the mixture as measured by vapor pressure osmometry, gel permeation, or LC. In Fig. 2

the distribution measured by high-performance liquid chromatography (HPLC) of oligomers in a polystyrene mixture may be compared with the distribution measured by field desorption. The number average molecular weight was 811 measured by vapor pressure osmometry, 855 by LC, and 928 by field desorption MS[11]. The molecular ion distribution of a heavier polystyrene mixture is shown in Fig. 3. The number average molecular weight determined by MS was 2900 as compared with 3100 by vapor pressure osmometry[11]. These mass measurements were made on a Kratos MS-50 mass spectrometer with a special 23K gauss magnet and a Daly detector. Several scans were averaged and corrections were made for the multiplicity of molecular ions resulting from isotope abundances in these heavy hydrocarbons. This approach works best if little fragmentation or pyrolysis occur, and if there is little instrumental discrimination through the mass range.

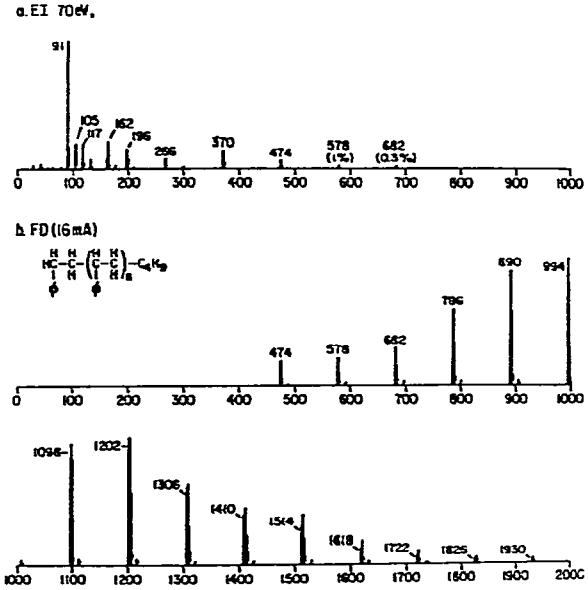

Fig. 1. Electron impact and field desorption mass spectra of a polystyrene mixture of number average mass 1020. (Reprinted with permission from ref. 10, copyright 1979 American Chemical Society.)

Lattimer and Hansen[12] have also shown that polyglycol oligomers (polyethylene glycol, polypropylene glycol and polytetrahydrofuran) can be assessed by field desorption using the high field magnet. In the cases of these more polar polymers abundances of (M + H) or (M + Na) ions were measured.

The need for corrections for multiplicity of molecular ions due to isotope abundances leads to an interesting point. The abundances of molecular species containing ^{13}C become significant in these hydrocarbon middle molecules, and the mass defect of hydrogen (and other atoms) can contribute significantly to the molecular weights observed. Thus while the nominal molecular weight for polystyrene $n = 33$, C_{276}, H_{282} is 3594, based on the convention that C = 12 daltons and H = 1, actually H = 1.0078 and 282 × 1.0078 = 284.2 daltons. In working with middle molecules we define the monoisotopic mass as the molecular weight calculated from the most abundant isotope of each atom present in the molecule, in this case C = 12.000 and H = 1.0078. Consequently, the monoisotopic mass for polystyrene $n = 33$ is 3596.2

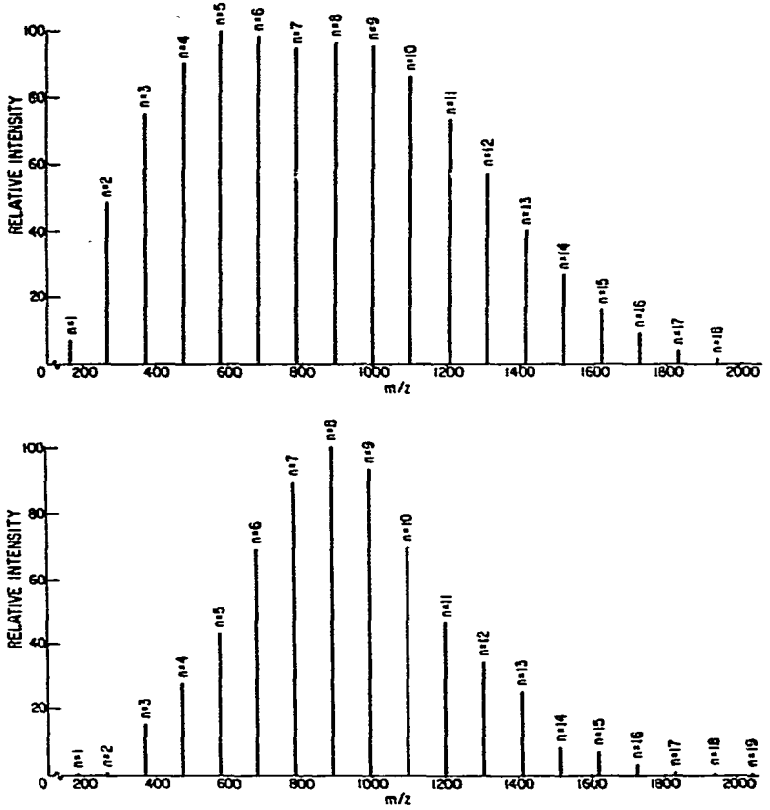

Fig. 2. Distribution of oligomers determined in a polystyrene mixture by field desorption–MS (bottom) and HPLC (top)[11].

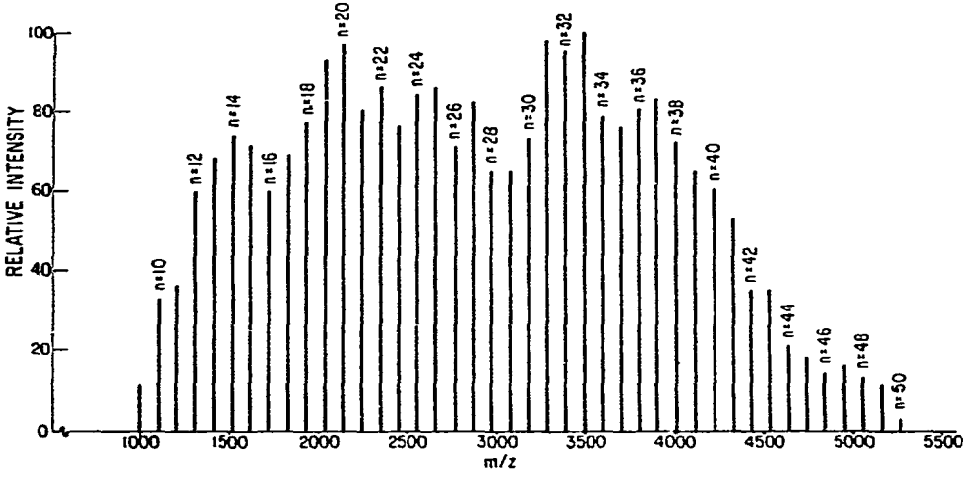

Fig. 3. Distribution of oligomers in a polystyrene mixture of number average mass 2900 determined by field desorption–MS.

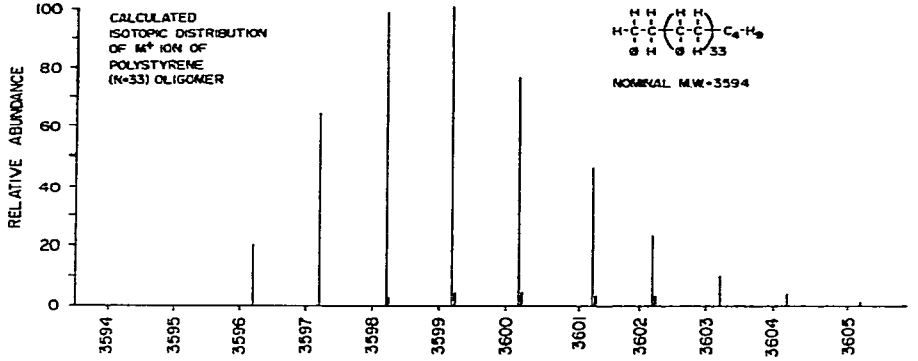

Fig. 4. Theoretical distribution of the molecular ions of polystyrene $n = 33$, generated by computer.

daltons. However, this is not the mass of the most abundant molecular ion. The abundance of ^{13}C is about 1.1% for each atom of carbon in the molecule. If the abundance of monoisotopic ions is set as equal to 100%, the relative abundance for ^{13}C, $^{12}C_{275}$, $H_{282} = 315\%$, and the most abundant ions (487%) in the molecular ion group will be $^{13}C_3$ $^{12}C_{273}$ H_{282}. The theoretical distribution of the molecular ions for polystyrene $n = 33$ is shown in Fig. 4, generated by computer and including consideration of ^2H abundances as well. The high mass end of the field desorption spectrum of trehalose octapalmitate synthesized in the MAMS laboratory is presented in Fig. 5. Here again the multiplicity of molecular ions is visible and the monoisotopic mass is indicated.

The intermittent nature of the ion flux produced by field desorption and the resultant inadequacies in ion statistics have been remarked by many workers. We illustrate the problem and our solution in Figs. 6 and 7. A single scan of the molecular ion region of polystyrene $n = 33$ is shown in Fig. 6, and a spectrum of this region compiled from 10 scans recorded and added on a multi-channel analyzer is shown in

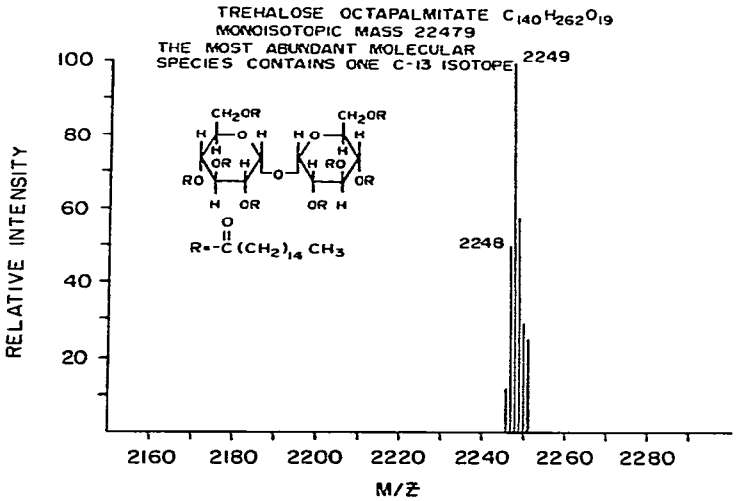

Fig. 5. Partial field desorption mass spectrum of trehalose octapalmitate.

Fig. 7. The signal-to-noise ratio is much improved in the latter, and the relative intensities correspond well to the theoretical distribution in Fig. 4. A similar set of distributions is shown in Fig. 8 for the molecule hexapus synthesized by F. Menger at Emory University. Again the single scan is visibly aberrant, while the averaged spectrum matches the theoretical distribution more closely.

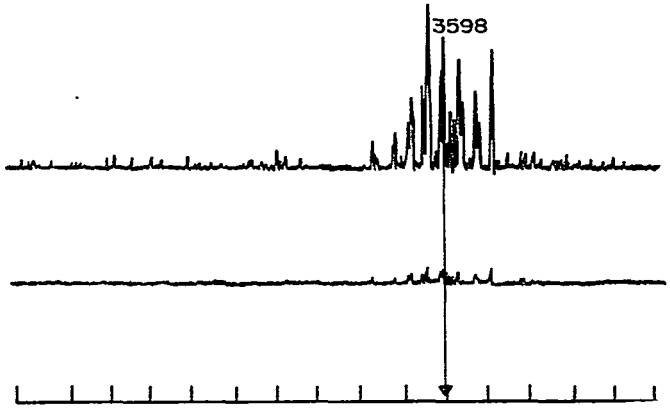

Fig. 6. A single field desorption scan of the molecular ion region of polystyrene $n = 33$. Resolution, *ca.* 4000.

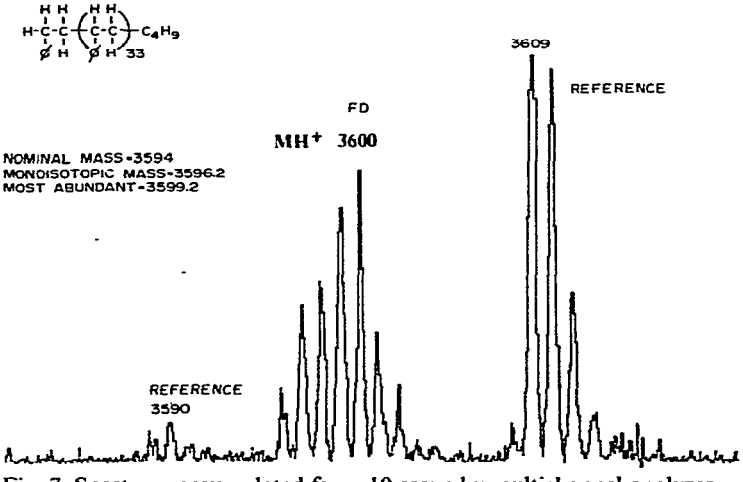

Fig. 7. Spectrum accumulated from 10 scans by multichannel analyzer.

We conclude that field desorption is a useful technique for analysis of some middle molecules. We have found it difficult, however, to obtain accurate molecular ion distributions of some more polar polymers. Thus we are currently evaluating a complementary solid phase ionization technique, called by its developers[13] Fast Atom Bombardment (FAB). The partial spectra in Fig. 9 and 10 indicate that middle molecules may be analyzed by FAB, as either positive or negative ions. In addition to the molecular ion species presented here, fragment ions are observed in the spectra of both neurotensin and γ-cyclodextrin. It should be pointed out that neurotensin con-

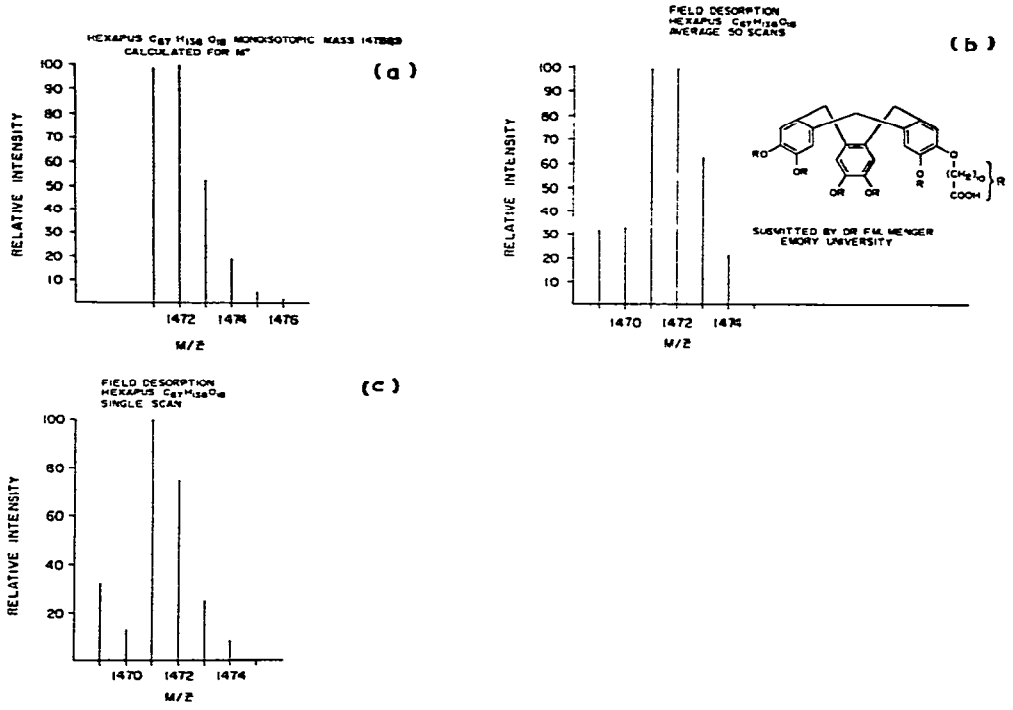

Fig. 8. Partial field desorption spectrum of hexapus, synthesized by F. Menger at Emory University. (a), Calculated; (b), averaged; (c), single scan.

tains two polar arginine residues in addition to one asparagine. FAB exhibits many of the characteristics of thermal desorption[14,15], including the fact that preformed ions, *i.e.* organic salts, are readily analyzed. An interesting example is the dimer of guanosine monophosphate crosslinked by the alkylating metabolite of cyclophosphamide, phosphoramide mustard[16]. Isolated by LC, this compound contains two quaternary ammonium cations and two potentially ionized phosphate groups. Yet it is readily

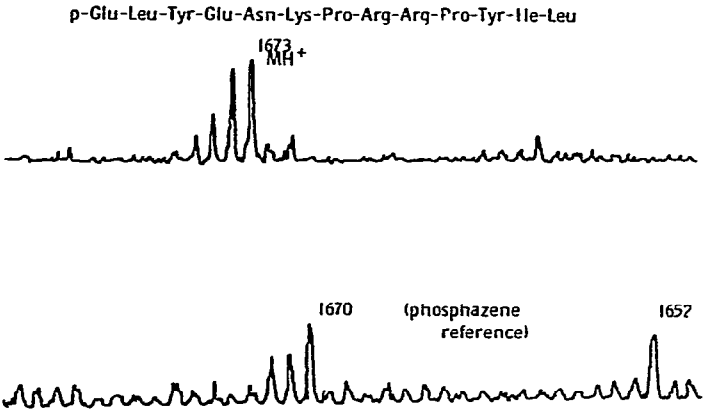

Fig. 9. Molecular ion region of the positive ion FAB mass spectrum of neurotensin.

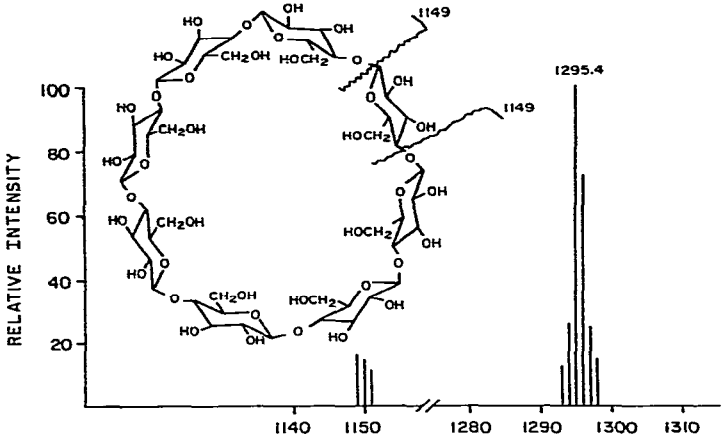

Fig. 10. Molecular ion region of the negative ion FAB mass spectrum of γ-cyclodextrin (MW = 1296.4).

analyzed by FAB, which produces a positive molecular ion species of mass corresponding to the ion shown in Fig. 11. Structurally significant fragmentation is also indicated in Fig. 11 (ref. 16).

In Fig. 12 a partial FAB spectrum is shown of a mixture of long chain alkyl quaternary amines. These are industrially important biodegradable surface active compounds (annual U.S. sales 10^8 kg/year), which occur in mixtures reflecting the origin in beef tallow of the two long chain alkyl groups. The analytical question of primary interest here is the abundance of each long chain alkyl group represented in the mixture of dialkyldimethyl quaternary amines. FAB provides per cent abundances comparable to those provided by other techniques[17]. A second question addresses the percent of trialkyl monomethyl quaternary amines present as impurities.

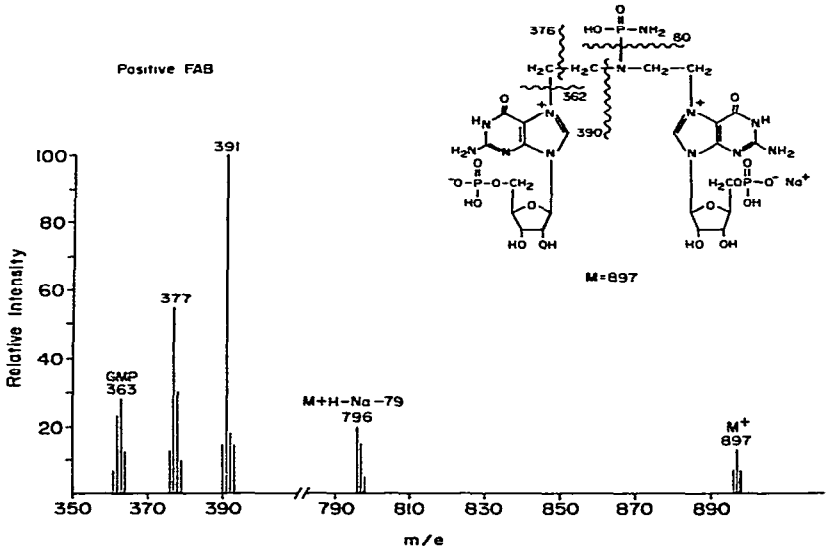

Fig. 11. Positive ion FAB spectrum of guanosine monophosphate dimer crosslinked by phosphoramide mustard[16].

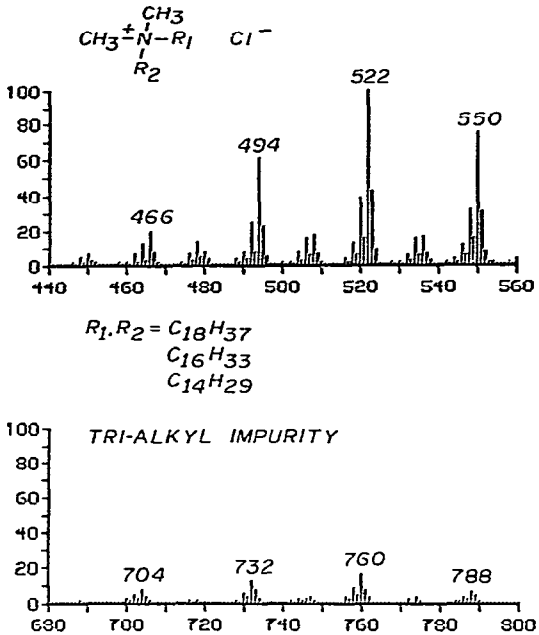

Fig. 12. Molecular ion region of the FAB spectrum of a mixture of surface active quaternary amines[17].

In this case FAB allows a value of 10% to be calculated while HPLC gives a value of 7.7% (ref. 17).

An example of the analysis of biovariability by FAB is presented in Fig. 13, where the array of molecular ion species of bovine sphingomyelin is visible[18]. This compound also contains a quaternary ammonium center and a polar phosphate group.

We suggest that FAB as well as field desorption has good potential for the analysis of mixtures based on the principle of generating an array of molecular ion species whose relative abundances correspond to the molarities of different species in the mixture.

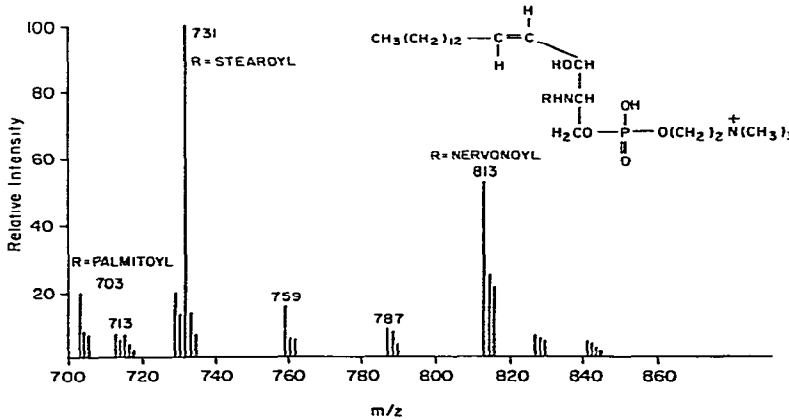

Fig. 13. Molecular ion region of the FAB spectrum of bovine sphingomyelin.

The FAB spectra shown here were measured with the Kratos FAB source on the MS-50 with the 23K gauss magnet.

Advances made in many laboratories in recent years in ionization methods for MS have brought many compounds within analytical reach which cannot be chromatographed in hot gas phase systems. If these compounds are to enjoy the considerable advantage of analysis by combined chromatography–MS, the chromatography must be in the liquid phase.

Among the solid phase ionization techniques proven for polar molecules and/or middle molecules, field desorption is unique in its incompatibility for use in on-line LC–MS. On-line coupling of plasma desorption (fast ion bombardment)[19], secondary ion MS[20], laser desorption and FAB are underway in a variety of laboratories. The thermospray method used by Blakeley *et al.*[21] for on-line LC–MS should be included as a proven technique.

ACKNOWLEDGEMENTS

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