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SURVEY OF IONIZATION METHODS WITH EMPHASIS ON LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY*

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CONTENTS

1. INTRODUCTION

The development of the combination of liquid chromatography with mass spectrometry (LC–MS) has gradually received more interest over the past decade[1] to complement the successful combination of gas chromatography with mass spectrometry (GC–MS)[2]. In the past few years especially, progress has been made in overcoming the technical problems associated with the interfacing of LC–MS[3–7]. Parallel with this development has been an explosion of new methods to generate ions from so-called "non-volatile" or thermally labile molecules. The purpose of this survey is to describe the principles of these methods and to illustrate their applicability in the analysis of compounds which are amenable to LC, according to the following sequence:

(1) Desorption chemical ionization (DCI)
(2) Laser desorption (LD)
(3) Field desorption (FD)
(4) Electrohydrodynamic ionization (EHI)
(5) ^{252}Cf plasma desorption (PD)
(6) Secondary-ion mass spectrometry (SIMS)

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(7) Fast-atom bombardment (FAB)
(8) Miscellaneous

Until now methods 1–5 and 7 have been used exclusively in the off-line mode. It has been noted[4] that within LC–MS terms this demands higher amounts of solute and the use of a conventional high-performance liquid chromatography (HPLC) detector which effectively limits the number of fractions examined further by MS. For that reason and because of the further development of LC columns which will enable to achieve fast separations accompanied by sharp narrow peaks, an on-line LC–MS system is preferred. The well known chemical ionization (CI) method with which both positive[10] and negative ions[11] can be generated (not to be described here) is at present "most easily" on-line connected with an LC system[3,6]. The LC solvent is then used as a CI reagent gas. If buffer solutions are used as LC solvents such as in reversed-phase liquid chromatography (RPLC), a modified segmented-flow extractor can be added between the liquid chromatograph and mass spectrometer to transfer the solutes into a volatile organic solvent prior to introduction into the mass spectrometer[12].

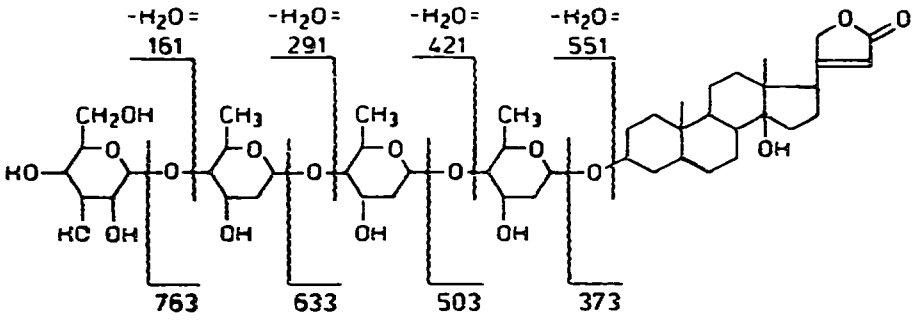
2. DESORPTION CHEMICAL IONIZATION

In this method, first introduced by Baldwin and McLafferty[13] and recently reviewed[14], the non-volatile sample is coated on a surface and directly exposed to the ion plasma in a CI source by means of an extended solid probe tip. In the original work[13], where the probe was unheated, it was found that spectra of non-volatile compounds such as underivatized tri- and tetrapeptides could be obtained at ion-source temperatures at least 150°C below those usually required. Intense $(M + H)^+$ peaks together with peaks of fragment ions, which allowed determination of the amino acid sequence, were observed in the spectra[13]. Another important observation around that time was made by Friedman and co-workers[15,16]: they found that underivatised di-, tri- and tetrapeptides, deposited on a Teflon surface, could be volatilized successfully by rapid heating which can make molecular evaporation competitive with thermal decomposition reactions.

Both coating of the non-volatile sample on the surface of an extended solid probe tip and rapid heating were applied together in many studies which followed. For example, very abundant $(M + H)^+$ ions have been generated from thermally labile creatine $[H_2N–C(=NH)–N(CH_3)CH_2COOH]$ and from an oligopeptide such as carbobenzyloxy-glycyl-prolyl-leucyl-alanyl-proline by loading them on to activated 10-μm tungsten wires, normally used in field desorption, and exposing them to the methane ion plasma with rapid heating of the wires through an electric current[17]. Incidentally, it should be noted that also essentially improved electron-impact (EI) spectra of relatively non-volatile compounds have been obtained by introducing either such wires[18] or extended solid probe tips of quartz[19,20] into an EI source very close to the electron beam. Other parameters such as the nature of the surface on which the sample is coated[21–23], the distance between the sample position and the electron beam[20,21], the heating rate[23] and the sample size[23] have been investigated. An important conclusion which has been drawn from these experiments is that the so-called non-volatile compounds are ionized in the gas phase, *i.e.* they are still volatile enough to be vaporized. This explains why spectra of good-to-excellent quality have been obtained from non-volatile and thermally labile compounds with a moving belt (polyimide) interface where the rapid evaporation technique is employed[24,25].

Recently it has been shown that steroid glycosides can be desorbed thermally from a coiled 0.2-mm platinum wire at a distance of 1 mm. from the electron beam in a CI source operating in the negative-ion mode[26,27]. Excellent OH^- negative-ion CI spectra were obtained[26,27] showing intense $(M - H)^-$ peaks and many structurally significant peaks as indicated in the structure of purpureaglycoside A [molecular weight (MW) = 926!] below:

PURPUREAGLYCOSIDE A MW = 926



3 LASER DESORPTION[28,29]

Recently a number of investigations have been reported on the use of (sub)microsecond laser pulses[30–35] with power densities of $\geq MW/cm^2$ and of a continuous wave carbon dioxide laser beam[31,36] with power densities of $10–10^4$ W/cm^2 in the analysis of non-volatile and thermally labile compounds. The method of obtaining the mass spectrum is relatively simple: the sample is coated on a metal surface such as stainless steel[30,31], copper[36] or silver[34] or on a surface of quartz[31] or Vespel[33], which are extensions of a solid probe. After introduction of the probe into the ion source the laser beam is focused onto the sample to a spot size of *ca.* 0.5 mm diameter causing a rapid heating of the sample and the surface (several thousand K/sec[29,37]). Without the help of an electron beam, abundant cationised molecules $(M + Na)^+$ and $(M + K)^+$ of systems such as oligosaccharides, glycosides, nucleotides, amino acids and oligopeptides are generated together with some fragment ions[30]. In this way the sodium cationised species of underivatized digitonin (MW = 1228) has been generated and recorded[30].

A problem is that the ion currents produced by the laser irradiation of the samples only last for periods from a few microseconds to a few seconds, depending on the pulse times of the lasers used. This requires either simultaneous ion detection[30,31] or the use of a time-of-flight mass spectrometer[32] or quadrupole instrument[36]. Recently it has been shown, however, that surface preparation by mechanical scratching can provide long-lasting ion signals in pulsed laser irradiation experiments[35]. Reasonably strong ion currents which even persisted for many minutes have been reported for LD of sucrose supported on silver foil[34], so that this ionization method becomes compatible with relatively slow-scanning sector mass spectrometers.

Laser parameters such as wavelength, pulse time and power density seem not to be critical for the production of ions[29]. In other words, the laser radiation can be best considered to be a fast heating source. This then raises the question whether the cationised molecules are formed on or close to the surface, especially because of the fact that little pyrolysis, if any, is observed for the non-volatile and thermally labile molecules studied[29,30,35,36]. In this respect it should be noted that purely thermal evaporation of intact cations of quaternary ammonium[38–40] and phosphonium[38] salts, $(M + Na)^+$ ions of benzo[15]crown-5[41,42] and of intact $[B(C_6H_5)_4]^-$ ions from a layer of $NaB(C_6H_5)_4$[42] has been reported recently. Careful studies of ion intensities as a function of irradiation time in LD recently performed at the FOM Institute in Amsterdam[37,43] have proven that cationisation of sucrose in LD is occurring by gas phase ion/molecule reactions of alkali ions and sucrose molecules evaporated from the surface. Any contribution of surface cationisation could however not be excluded[43].

4. FIELD DESORPTION

This ionization method has had an enormous impact on the analysis of thermally labile and non-volatile compounds by mass spectrometry since its introduction by Beckey in 1969[44]. It has been applied to almost all classes of organic compounds as described in an extensive review[45].

In FD, use is made of electric fields with field strengths in the order of 10^9–10^{10} V/m. These fields can be obtained with tungsten wires of 10-μm diameter on the surface of which carbon[46] or silicon microneedles[47] with lengths between 20 and 50 μm have been grown. The non-volatile or thermally labile compounds are then loaded onto these activated wire emitters from solution by using either dipping or syringe techniques[48]. After removal of the solvent in the vacuum lock system of the mass spectrometer, the sample-loaded emitter is introduced into the ion source and positioned as field anode (in the case of positive ions) at a distance of 1.5–2 mm from a slotted cathode[49]. Application of a potential difference of *ca.* 10 kV between the electrodes and heating the emitter by passing an electric current through it with an emission control device[45] results in the desorption of ions from the emitter surface. In most cases almost the whole total ion current is due to $M^{+\cdot}$, $(M + H)^+$ or $(M +$ alkali$)^+$ ions, *i.e.* fragmentation is usually negligible. The formation of $(M +$ alkali$)^+$ ions can easily be promoted by addition of a small amount of an alkali halide as NaI to the sample to be analysed[50,51]. The desorption of these stable $(M +$ alkali$)^+$ ions can even be then achieved with smooth tungsten wires[50,51], which is further facilitated by dissolving the compound and the alkali halide in a polar organic polymeric matrix[50,52].

In a similar way it has been shown possible to generate anionized molecules by attachment of Cl^- and NO_3^- ions to, for example, sugars below the onset field strength of field electron emission[53,54] which can happen under reversed polarity conditions. Landmarks in FD have been the analysis of vitamin B_{12}[55] and the generation of ions from polystyrene and polypropylene glycol with masses up to 10.000 daltons[56]. The complex guanidino-containing antibiotics bleomycin B_2 and phleomycins D_1 and E have been characterized successfully by FD[57] recently and various combined applications of (HP)LC/FD have been reported in the last few

years[58–61]. The FD method is an excellent technique for obtaining the molecular weight of many thermally labile or non-volatile compounds, notwithstanding the accompanying practical problems as experienced by the author's group where it has been used routinely and with success since 1975. For example, no special problems were encountered when obtaining the FD spectrum of the following underivatized oligopeptide with a molecular weight of 1581 at an emitter current of 16 mA: peaks were observed at m/z 1582 (100%), 1583 (83), 1584 (37) and 1585 (24)[62]: H-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH. The absence of fragmentation prevents the determination of the amino acid sequence, but this could be established by collisional activation (CA) as shown recently in an FD–CA study of some free oligopeptides[63].

Finally, it should be noted that recent work of Röllgen and co-workers[64–66] has shed much more light on the physico-chemical background of the FD method. Three significantly different mechanisms appear to contribute to the formation of gas-phase ions in FD[66]: (i) the field-ionization mechanism involving the removal of an electron from sample molecules by tunnelling, a process which occurs on the tips of field-enhancing microneedles of activated emitters and molecules may migrate to these tips either by evaporation or by surface diffusion; (ii) the field-induced desolvation mechanism which involves the extraction of ions from electrolytic solutions or from salt layers deposited on smooth 10-μm tungsten wires and is the most important mechanism for the detection of protonated or cationized molecules of thermally labile or non-volatile compounds (the reader is referred to the original paper[66] which describes in detail the successive events occurring during the desolvation process); and (iii) the thermal ionisation mechanism which is principally independent from external fields and does not contribute in general to the emission of ions with the exception of metal ions at emitter temperatures >800 K and of some organic ions mentioned under the section on LD (see above).

5 ELECTROHYDRODYNAMIC IONIZATION

This method can be used to investigate liquid solutions of non-volatile compounds as suggested by Evans and co-workers in 1974[67]. Its basic principle is the evaporation of ions (positive or negative) from a liquid which also contains an electrolyte by the application of a high electric field to the surface of the liquid. In practice this is performed by a continuous flow of a solution consisting of the non-volatile compound, sodium iodide or some other salt and of a solvent of low volatility (usually glycerol) into the ion source of a mass spectrometer through a metallic capillary needle. Application of a high constant voltage in the order of 8–10 kV to this capillary needle with respect to an extraction electrode in the close vicinity causes a strong electric field on the sharp edges of the needle. Under the action of this field, unclustered and clustered ions leave the liquid which then can be mass analysed. For example, for sucrose the $(M + Na)^+$ ions are the most abundant, but also ions of the type $Na^+.Gl_nS_m$ where $n = 0, 1, 2 \ldots, m = 0, 1, 2 \ldots$ are observed[67] (Gl = glycerol, S = sucrose).

The method has been applied both to biochemical compounds such as sugars[68], nucleosides[68] and nucleotides[69] and to inorganic compounds such as $MgCl_2$, $AlCl_3$ and $SbCl_3$[70]. Actually it is not an ionization method, but evaporation

of ions present in the solution by the high electric field[68–70]. It has been argued that the term "evaporation of ions from liquids" would describe in a better way the physical processes responsible for the observed mass spectra than the term "electrohydrodynamic ionization"[71], although the term "field-induced ion evaporation from liquid surfaces" (at atmospheric pressure) is also encountered[72]. It has been noted that FD and EHI are closely related[50,70].

6. ^{252}Cf PLASMA DESORPTION

This method was introduced in 1974[73] and uses the nuclear fission fragments from ^{252}Cf decay to effect rapid localized evaporation and ionization of non-volatile compounds from a surface. Technical details[74] and a general description of the method[75] have been given.

Of the decay of the ^{252}Cf nucleus, 3% takes place by spontaneous fission and the remaining 97% by emission of α-particles. The fission fragments have kinetic energies in the order of 80–100 MeV and each fission event produces two fragments travelling in almost exactly opposite directions. The α-particles have kinetic energies of *ca.* 6 MeV. With a thin foil behind the ^{252}Cf source a differentiation can be made between the fission fragments and α-particles for detecting them: each fission fragment passing through the foil generates about 35 times more secondary electrons than an α particle[74]. The sample is positioned in front of the ^{252}Cf source by use of a nickel support foil $1 \cdot 10^{-3}$ mm thick. Thin and uniform molecular films of the sample on the nickel foil can be obtained with the electrospray technique[74–76]. Large quantities of energy are deposited in a small area in *ca.* 10^{-12}–10^{-13} sec when the fission fragments penetrate the film. This produces a pulsed localized heating resulting in the formation and desorption of ions by a complex mechanism. The ions are accelerated and mass analysed with a time-of-flight mass spectrometer, where the time of detection of the fission fragment complementary to that causing ionization and travelling in the opposite direction (see above) is used as a zero time mark[74,75].

Both positive and negative ions are generated mainly through ion–molecule reactions or ion-pair formation[75]. Thus, molecules such as amino acids, peptides and nucleotides form quasi-molecular ion pairs $(M + H)^+$ and $(M - H)^-$ which may fragment by the loss of small molecules such as CO_2. The fragmentation of the positive ions is more pronounced than that of the negative ions and can provide structural information on the investigated molecules[75]. The proton in the $(M + H)^+$ ions can easily be exchanged with sodium or potassium cations in many cases when the Na^+ or K^+ impurity level is relatively high. The presence of these $(M + Na)^+$ and $(M + K)^+$ ions is very helpful for identification of the molecular weight of the sample molecule[75].

Another method is to form first a thin film of CsI on the nickel support foil with the electrospray technique[76] followed by a thin layer of the sample to be analysed. When a fission fragment passes through the two films, Cs^+ ions are released from the bottom layer and rapidly diffuse through the fission track to the upper layer where $(M + Cs)^+$ ions can be formed and desorb from the surface. An unambiguous molecular-weight determination is then possible on the basis of the peaks due to the $(M + Cs)^+$ and $(M + Na)^+$ ions, the latter again being due to an Na^+ impurity level. In this way the molecular weights of the very powerful toxin palytoxin[77] (MW =

2681.1 ± 0.35) and of a derivatized oligonucleotide[78] (MW = 2833.50) have been determined. PD mass spectra of other high-molecular-weight compounds have been reported[78], an impressive result being the PD spectrum of a fully protected dodecanucleotide with a molecular weight of 6277 ± 3[79]. In the latter work even an ion at m/z 12,637 (!) has been observed which has been suggested to correspond with the dimer of the fully protected dodecanucleotide plus four attached sodium atoms[79]. A recent comparison of PD mass spectra with those obtained by bombarding the sample with K^- and Cs^+ ion beams in the keV range has revealed a very high degree of similarity between the two ionization methods[80].

7. SECONDARY-ION MASS SPECTROMETRY

The application of this method to the analysis of non-volatile and thermally labile organic compounds is of recent origin[81,82]. It makes use of a 2–5-keV argon primary ion beam which bombards a layer of the sample deposited mostly on silver foil. This results in the emission of secondary ions (positive and negative) from the surface because of the large amount of energy deposited at a localized site (*cf.* LD and PD described above) which are subsequently mass analysed. A model of the dynamics of ejection of these ions which can occur within 10^{-13} sec has been described in some recent papers[83,84].

At least three distinct processes have been delineated whereby sample molecules are transformed into secondary ions[85]: (i) attachment of a cation such as a proton or metal ion to the organic molecule to give cationised species (*cf.* the methods discussed above) or attachment of a negative ion such as H^- to 9-borabicyclo[3.3.1]nonane to give the $(M + H)^-$ ion recently reported[85,86]; (ii) electron transfer between the sample molecules resulting in $M^{-\cdot}$ and $M^{+\cdot}$ ions which may fragment by unimolecular dissociations; and (iii) direct sputtering of organic cations and anions from the sample layer to the gas phase; these ions arise by transfer of momentum from the primary ion.

The efficiency of these processes are roughly in the order (iii) > (i) > (ii)[85]. The method therefore provides not only the molecular weight of the analysed molecules, but also information on their structure. It is interesting to note here that *ortho*, *meta* and *para* isomers of amino-, hydroxy- and mercaptobenzoic acids can easily be distinguished by SIMS on the basis of the $(M - H)^-/(M - H - CO_2)^-$ ratio[85,86]. Furthermore, a number of biologically important compounds have been analysed successfully with SIMS, including amino acids[81,82], peptides[81,82], vitamins[81,82], pharmaceutical compounds[81,82], nucleotides[87], mixtures of stimulants, barbiturates, opiates and amino acids[88] and the $(M - Cl)^+$ ion of choline chloride and its esters[85]. It has even been possible to identify components directly on paper chromatograms by SIMS such as the neurotransmitter acetylcholine: its SIMS spectrum taken on Whatman No. 1 paper is similar to that obtained on silver foil[85]. However, it should be noted that initially difficulties were encountered with some compounds such as with sucrose[85], but that its ionization by SIMS has been achieved recently[80,97,98]. Another problem which has been mentioned in the literature is that ion-beam bombardment can lead to a build-up of charge on insulating samples whereby the SIMS spectrum is lost[89]. This can be circumvented by use of the ionization method which will be discussed in the next section and which can be considered to be a modification of SIMS.

8. FAST-ATOM BOMBARDMENT

This method is similar to SIMS, discussed in the previous section, with the modification that not an $Ar^{+\cdot}$ ion beam, but a fast argon atom beam of 2–10 keV, is used to bombard the sample[90,91]. This argon atom beam is obtained by resonant charge exchange of 2–10 keV $Ar^{+\cdot}$ ions, generated in a discharge ion source, in a collision chamber in front of it which contains a high pressure (10^{-1}–10^{-3} Pa) of argon gas. Residual ions are removed from the beam by electrostatic deflection. The resulting argon atom beam is then directed towards the sample deposited on a copper sample stage fitted onto a direct insertion probe[91]. The angle of incidence of the atom beam is 20° and the collection angle of sputtered ions nominally 70°, both with respect to the sample surface. The sputtered ions are mass analysed to give the FAB mass spectrum. Sample preparation can occur by coating it on the probe through evaporation of a solution, but often a quite rapid decrease in the secondary ion yield is observed which is probably due to damage of the sample surface under the impact of the Ar atoms[92]. An essential improvement is obtained when the sample is mixed with a suitable non-volatile liquid such as Santovac 5 diffusion pump oil[92], or glycerol[93] prior to coating it on the probe tip. This procedure resembling those applied in FD and EHI (see above) results in a mobile sample surface and long-lasting secondary ion signals (hours).

The FAB mass spectra are characterised by relatively abundant $(M + H)^+$ ions in the positive ion spectra and $(M - H)^-$ ions in the negative ion case together with many structurally significant fragments ions[89–92]. Even metastable peaks are observed in the spectra[91]. This is an exciting feature of FAB because it permits one to obtain not only the molecular weight, but also structural information on the samples analysed.

The FAB method has already started to become very popular as can be seen from the relatively large number of papers for such a new method presented at the 29th Annual Conference on Mass Spectrometry and Allied Topics, held in Minneapolis, MN, U.S.A., May 24–29, 1981. Indeed, impressive and successful results have been obtained with FAB in the analysis of non-volatile and thermally labile compounds of biochemical interest, such as a peptide amide containing 26 amino acid units (molecular weight = 2845), vitamin B_{12} and its co-enzyme, oligosaccharides, peptide antibiotics, glycopeptides, penicillins, glycoside antibiotics, glycolipids, oligonucleotides and neurotoxins[90].

9. MISCELLANEOUS

Two ionization methods, which have not been discussed so far, are atmospheric pressure ionization (API) and the thermospray ionization technique. They will be briefly considered here.

The API method was introduced in 1973[94] and is based upon ionization of samples by the use of a radioactive ^{63}Ni (on gold foil) source. A few microlitres of a liquid sample or a solution are injected into a preheated nitrogen carrier gas stream which takes the sample molecules to a small reaction chamber (*ca.* 1 cm in diameter and 1 cm in length) containing the ^{63}Ni source. The electrons are emitted from ^{63}Ni with a mean energy of 60 keV, but are rapidly thermalised in the gas at atmospheric

pressure to generate primary positive ions of N_2^+ and primary negative ions of sample molecules either by resonance capture or by dissociative resonance capture. Both the positive and the negative primary ions can and will react further by ion–molecule reactions with eventual formation of ionized sample molecules through proton- or charge-transfer processes. The ions can then leak through a 25-μm pinhole aperture from the reaction chamber at atmospheric pressure into the high-vacuum region of the mass spectrometer to be mass analysed.

The API method, although not widely used, has been applied successfully in the negative ion mode for analysis of urine samples containing phenobarbital and metabolites[94] and of samples originating from complex biological matrices such as extracts of blood, urine, faeces and tissue[95].

The thermospray ionization technique is of more recent date[96]. It was actually discovered during work on the combination of LC–MS which operates in the following way: the effluent from the LC enters a vaporizer through a stainless-steel capillary tube (0.015 cm I.D.) which is partially immersed in a copper cylinder heated to *ca.* 1000°C by four small oxy-hydrogen flames. As a result of rapid heating, a jet of vapour and aerosol is produced near the exit from the stainless-steel tube. The jet is further heated as it passes through the 0.075-cm diameter channel in the copper. It then undergoes an adiabatic expansion and a portion passes through a skimmer to the ion source of the mass spectrometer where the beam impinges on a nickel-plated copper probe which is electrically heated to *ca.* 250°C. Both positively and negatively charged particles are produced in a direction practically perpendicular to the impinging beam and then are mass analysed.

Note that the ions are generated without any help of an electron gun. It is, however, not known where they are formed: this could be either during the adiabatic expansion or during the impingement of the beam on the nickel-plated copper probe. In any case, preliminary results have indicated that at least 80% of an involatile solute such as adenosine is transmitted to the ion source with *ca.* 5% of the solvent. Moreover, the best conditions for this new ionization technique in the preliminary study[96] involve the use of 0.2 *M* formic acid as solvent at input flow rates in the range 0.5–1 ml/min.

Primarily protonated or cationized sample molecules are observed together with a few structurally significant fragment ions for the cases studied, which are adenosine 5′-monophosphate, some underivatized dinucleotides, underivatized oligopeptides containing up to five amino acid residues, antibiotics, vitamins and fatty acids[96]. It is clear that this thermospray ionization technique is compatible with on-line LC and as noted in the original publication to which the reader is referred, it is fast, sensitive, relatively simple and inexpensive[96].

10. CONCLUSION

An impressive number of ionization methods for the analysis of non-volatile and thermally labile compounds have been developed in the past decade and especially in the past few years. It shows that mass spectrometry is a very active and dynamic field which is expected to contribute significantly to problems in fields such as biochemistry and biology, because, at present, compounds with a much higher molecular weight than a few years ago can be studied. Not all the discussed methods

are suited for coupling directly with LC, but some of them such as laser desorption, secondary ion mass spectrometry and fast atom bombardment have certainly the potential for combination directly with LC through a suitable interface (a recent publication has described already the successful coupling of LC with SIMS[9]). The ionization methods themselves, however, have still to be explored and to be developed further so that full advantage can be taken of their possibilities. This is certainly true for the thermospray ionization method which is in its infancy, but has already shown promising results for on-line LC–MS.

11. SUMMARY

Many ionization methods have been developed in the past decade for the analysis by mass spectrometry of non-volatile and thermally labile compounds with molecular weights ranging from 300 to 10.000 daltons. This process has taken place and still continues parallel with the progress made in liquid chromatography, a technique suited for dealing with such compounds. A combination of liquid chromatography and mass spectrometry is therefore a logical consequence.

In this survey the principles of the ionization methods desorption chemical ionization, laser desorption, field desorption, electrohydrodynamic ionization, ^{252}Cf plasma desorption, secondary-ion mass spectrometry, fast-atom bombardment, atmospheric pressure ionization and thermospray ionization are given together with applications reported in the literature. Some of these methods have the potential of being coupled directly to a liquid chromatograph through a suitable interface.

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