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## OPTIMIZATION OF THE INSTRUMENTAL PARAMETERS OF A COMBINED LIQUID CHROMATOGRAPH-MASS SPECTROMETER, COUPLED BY AN INTERFACE FOR DIRECT LIQUID INTRODUCTION

### III\*. WHY THE SOLVENT SHOULD NOT BE REMOVED IN LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC INTERFACING METHODS

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#### 1. INTRODUCTION

The potential interest in a reliable, sensitive and universal instrument combining both the advantages of high-performance liquid chromatography (HPLC) and those of mass spectrometry (MS) need not be emphasized. The inherent difficulties in linking such an apparently mismatched pair are also clear. Both the advantages and problems of the on-line combination of the two techniques (LC-MS) have been documented in a series of reviews<sup>1-6</sup>. During the past 7 years, the number of research laboratories and instrument companies that have devoted effort to studying LC-MS has increased steadily, and LC-MS is becoming accepted as a feasible method despite the lack of a general solution to its numerous problems.

The development of any complex instrumentation is guided in most instances by a theoretical background, even when much of the progress is made after experimental trials and errors. During the early 1970s the attempts to combine LC and MS were based on the assumption current at that time that ionization in the mass spectrometer must follow vaporization of the intact neutral molecule.

\* For Part II, see ref. 51.

During this period, active and successful research was carried out on combined gas chromatography-mass spectrometry (GC-MS). Most of the instruments used the principle of selective removal of the mobile phase from the total effluent of the GC column followed by the introduction of the solutes into the ion source block of the mass spectrometer<sup>7,8</sup>. Although improved GC techniques and better vacuum pumps have today rendered this approach obsolete, this guideline led to the commercial introduction of several well functioning GC-MS instruments.

The same philosophy underlies the development of the first LC-MS system<sup>9-11</sup>, viz.: (1) introduction of the total effluent from the HPLC column into a separator; (2) selective removal of the chromatographic carrier; (3) vaporization of the neutral analyte; and (4) ionization and mass analysis. It led to the so-called transport systems based on a moving wire or belt (all recent models take advantage of the increased sample capacity of a moving belt), for conveying the solution, then the solute, through the several stages required by the process.

From the first results, it was suggested that LC-MS could complement the GC-MS technique, which requires that the solute molecules be in the gas phase for several minutes whereas only a few seconds are allowed between thermal desorption from the belt and ionization into the ion source of the mass spectrometer. Serious efforts have been made to reduce further the time spent by the neutral molecules in the gas phase prior to ionization by letting the belt penetrate the mass spectrometer ion source block<sup>12</sup>. A discussion of the present status and future prospects of transport interfaces for LC-MS is not our aim here, but we have noted the current trend to integrate them into complex instruments in which the belt is directly bombarded with high-energy particles or radiation<sup>13-21</sup>. Even so, reports on the performance of the system as originally designed are either rare (however, see those listed in refs. 1-6) or not very optimistic<sup>3</sup>.

The idea behind the title of this paper, when it was first adopted, was to compare transport interfaces with direct liquid introduction (DLI) instrumentation. However, further consideration made us conclude that this debate would be fruitless. On the other hand, the more we investigated the different aspects of solvent introduction the more we found good reasons for not placing first the need for complete removal of the solvent under LC-MS conditions.

We present here a review of several experiments and techniques that indicate that pre-formed ions in solutions [e.g., protonated molecules,  $MH^+$ , and deprotonated molecules,  $(M-H)^-$ , from acid-base reactions, cationized molecules,  $MX^+$ , where  $X = Li, Na, K, Ag, etc.$ , and solvated ions,  $(MS_nH)^+$ ,  $[(M-H)S_n]^-$ ,  $(MXS_n)^+$ , etc.), can be vaporized directly and analysed into a mass spectrometer when enough energy is supplied to the liquid solution. In the next paper in the series, we shall review the current status and some future trends in DLI instrumentation, and discuss whether the technique is compatible with the production of gaseous ions directly from ionic or electrolytic solutions injected continuously into the mass spectrometer.

## 2. GAS-PHASE SOLUTE-DERIVED IONS FROM LIQUID SOLUTIONS

The results from several experiments that used apparently different operating principles have revealed intriguing possibilities for isolating intact sample ions in the gas phase from dilute solutions of supposedly involatile and thermally labile organic

substances. In fact, the past 5 years have seen a great number of imaginative publications on the mass spectrometry of involatile substances, already covered in comprehensive reviews<sup>22,23</sup>. However, we have focused our attention on those works which use solutions in organic solvents as test samples. The diagrams that serve for illustration were redrawn from the original papers for purposes of simplification, and the reader should consult those papers for a more complete evaluation of the different experimental set-ups.

### 2.1. *Electrospraying of a neutral solution*

Dole and co-workers<sup>24-26</sup> probably achieved the greatest mass of gaseous ions ever recorded, by electrospraying polystyrene in solution in a benzene-acetone mixture (Fig. 1). Dilute solutions of polymers with weight-average molecular weights of 51,000 or 411,000 were injected through a needle held at a high negative voltage ( $-10$  kV). Nebulization of the input solution into a spray of tiny charged droplets, followed by rapid desolvation in an evaporation chamber at atmospheric pressure, formed a beam of macro-ions that was collimated under a vacuum by a nozzle-skimmer system, and was then recorded by means of a Faraday cage.

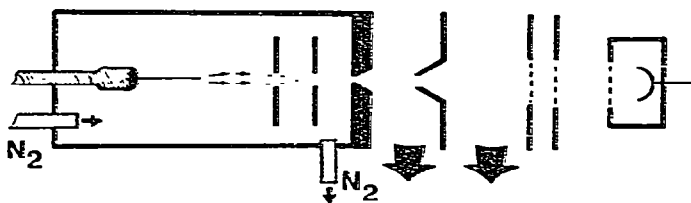
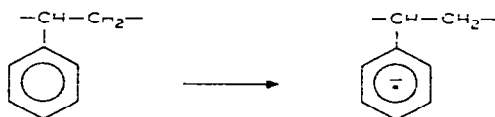


Fig. 1. Schematic principle of Dole *et al*'s electrospray. Adapted from ref. 24, by permission of the American Chemical Society.

The solvent was chosen to be not as easily ionized as the solute, so one or more aromatic rings in the polymer chain could probably capture one electron that remained on the molecule after breakage of the solvent-polymer cluster:



Despite promising results demonstrating that finite mass, charge states could be formed, the authors were unable to measure the ratio accurately. More precise measurements using a time-of-flight mass spectrometer failed because of the lack of an appropriate negative ion detector. Attempts to use a plasma chromatograph for the same purpose was unsuccessful, and the sad final conclusion<sup>26</sup> of this excellent piece of work was a disillusioned remark on the plasma chromatograph when used as a mass spectrometer, a conclusion already drawn by Keller and Metro<sup>27</sup> several years previously.

Mass separation and ion detection were more difficult to achieve than the formation of macro-ions from neutral polymers. This evidence prompted several authors to take recent patents on this mode of ionization although no experimental results were presented<sup>28,29</sup>.

## 2.2. Electrohydrodynamic ionization

Electrohydrodynamic (EHD) ionization, mainly developed by Evans and co-workers<sup>30-33</sup>, also uses a high electric field and solutions in an organic solvent to produce gaseous ions from involatile samples. The experimental set-up (Fig. 2) bears some similarities to the previous system as it also uses a needle held at high voltages (although here at positive values), and the organic solutions are injected through the needle. However, the entire system is under a vacuum, liquid droplets and rapid solvent evaporation must not occur, and electrical conduction through the organic liquid is a prerequisite. Thus what Evans and co-workers call the "host fluid" must meet the following requirements: low vapour pressure for operation under a vacuum, minimal corrosive behaviour, high viscosity to avoid loss of solution through droplet formation, and high solvation capability for both inorganic salts that increase the electrical conductivity (e.g., sodium iodide, lithium chloride) and the investigated organic samples. All conditions suggest the choice of glycerol at room temperature, or a related polyol for experiments at higher temperatures. Note that the same "host fluid" plays a key role as "the matrix" in another technique to be examined below (see section 2.5).

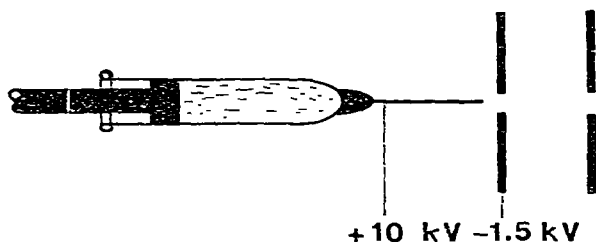


Fig. 2. Schematic principle of Evans and co-workers' experimental set-up for electrohydrodynamic ionization. Adapted from ref. 30, by permission of Elsevier Scientific Publishing Company.

Under the conditions of EHD ionization, the recorded mass spectrum is composed predominantly of a series of clusters of an intact sample molecule with several glycerol molecules and a proton or a metal cation attached<sup>30-34</sup>. Ions derived from sample fragmentation are rare or even absent. Metastable transitions indicate that high-molecular-weight clusters with up to nine glycerol molecules are extracted from the liquid and they decompose rapidly by losing one or several molecules of solvent<sup>30,31</sup>. The use of an acidic electrolyte such as mixed lithium chloride-hydrochloric acid was shown to enhance and to stabilize the ion current for the protonated solvated molecule from such basic samples as pyrimidines, purines, nucleosides and nucleotides<sup>32</sup>. Poor sample solubility was accounted for the poor mass spectra, and the problem could be partly solved by changing the pH of the medium. The authors concluded that "the pH of the medium can affect the sample ion information which results in electrohydrodynamic ionization"<sup>32</sup>.

The electrohydrodynamic disintegration of the liquid film deposited on to a tungsten wire emitter for field desorption (FD) has been observed directly under a microscope by Giessmann and Röllgen<sup>34</sup> in order to make a comparison between EHD and FD. As in early experiments by Evans and co-workers<sup>30-33</sup>, a sucrose solution in glycerol containing sodium iodide was deposited on to the emitter, which was held at high positive voltage and gradually heated by a current through the

emitter. Various stages of disintegration of the liquid layer were distinguished, but the final and almost stationary state corresponding to the maximum emission of protonated sample molecules was obtained when the temperature of the wire reached a value equal to the best anode temperature (BAT), that is, the optimal experimental condition for recording a good quality FD mass spectrum. The result suggests that a common principle may act under the conditions of EHD ionization or FD.

### 2.3. Field desorption

This technique is generally considered as a soft surface ionization technique and no organic solvent is present during the recording of the mass spectrum. The experimental set-up, which uses a heated emitter covered with fine whiskers held at high voltage, is well documented<sup>35,36</sup> and does not need to be presented here. The theory of FD is still a hotly debated topic<sup>37,38</sup>, and we, not being experts, do not propose to make any assumptions on the validity of either the "classical" FD model<sup>34-37</sup>, which suggests that the electrical field plays a major role in the desorption process, or the "non-classical" FD model<sup>38-41</sup>, which suggests that ions could be formed from a field-independent thermal desorption process. However, we believe that the following quotations from the paper of Giessmann and Röllgen<sup>34</sup> back up our assertion on the prime role of ions pre-formed in solution before their vaporization. They wrote that, "if high field induced ionization of neutral molecules cannot be denied for volatile substances, field induced desorption of ions from electrolytic solutions or salt layers at the surface of FD emitters is the most important mechanism of FD. Protonated molecules of involatile substances can be formed only via this mechanism". They also recalled that a proton-donating agent such as tartaric acid, when deposited on the FD emitter, is effective in increasing the yield of  $(MH)^+$  for polar non-acidic compounds.

Of course, we could interpret these observations in the light of other reports<sup>41-43</sup>, and argue that they do not preclude purely thermal desorption from the heated emitter as claimed by "non-classical" FD model supporters. So we would rather leave the FD controversy and next examine the results from another experimental set-up in which a heated plate appears as the only ionizer, and real organic solutions are injected into the device.

### 2.4. Thermospray ionization

The ionization process in the prototype instrument presented in 1980 by Vestal and co-workers<sup>44-46</sup> for on-line LC-MS purposes received the tentative name of thermospray ionization a year later<sup>47,48</sup>. It seems to us that it bears a resemblance to the spray impact detector designed by Mowery and Juvet<sup>49</sup> in 1974 as an HPLC detector, except that in this instance only the total ion current for both the solvent and the solute was recorded. These authors had already noted that the phenomenon of asymmetric charge generation by mechanical disintegration of a liquid surface into an aerosol had previously been known for many years by an assortment of names such as waterfall electrification, balloelectricity and spray electrification<sup>49</sup>.

Vestal and co-workers' experimental set-up shown in Fig. 3 has been already modified by replacing the four oxy-hydrogen torches that heat the copper cylinder by electrical cartridges, but the remainder of the apparatus is unchanged. According to the authors' description<sup>46</sup>, the rapid heating of the liquid input produces a jet of va-

pour and aerosol, which then undergoes an adiabatic expansion under a vacuum, and a portion of the jet passes through a skimmer and impinges on a nickel-plated copper probe which is electrically heated *ca.* 250°C. About 95% of the liquid is vaporized, and involatile samples appear to be carried preferentially by the remaining solvent in aerosol particles. These have masses of about  $10^{-7}$  g and each carries a charge of *ca.*  $5 \cdot 10^{-12}$  C. The baseline current that they produce is about  $10^{-8}$  A, a value already reported for the experiments of Mowery and Juvet<sup>49</sup> and also measured from the droplet spray produced by a diaphragm DLI probe<sup>50,51</sup>.

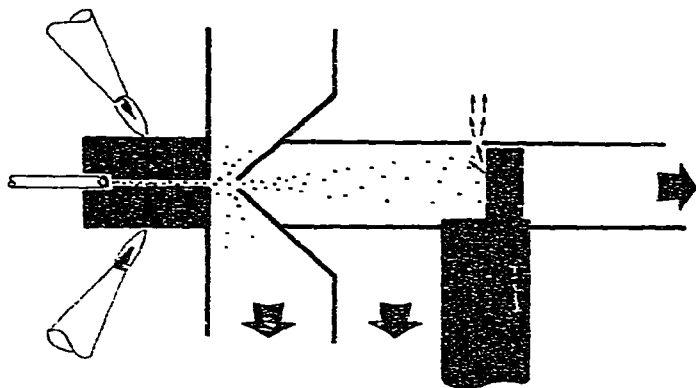


Fig. 3. Schematic principle of Vestal and co-workers' thermospray. Adapted from ref. 46, by permission of the American Chemical Society.

Vestal and co-workers assume that when a charged particle undergoes a high-energy impact with the heated probe, it is wholly or partially vaporized and some of the resulting molecules are ionized<sup>46</sup>. In fact they are very cautious about the exact process, stating<sup>47</sup> that "the mechanism of the ionization process is not yet fully known", but they conclude that "ions are produced by very rapid heating of the sample solution and final vaporization of the aerosol on the heated metal plate". Thus thermal energy transfer would appear to be an important factor, a hypothesis that agrees well with some recent hypotheses concerning laser ionization desorption (LD)<sup>41</sup>.

Maybe it is worth emphasizing that Vestal and co-workers' sample molecules, including a series of mono- and dinucleotides, generally bear strong proton-attracting sites at a nitrogen atom, and that a 0.2 M formic acid solution is used as the solvent. Hence, the final impact of the charged particles on the heated probe may just serve the purpose of freeing the pre-formed protonated molecule from its solvent molecule cluster. On the other hand, if the ionization process in the thermospray is assumed to be similar to that of the spray impact detector, then it should be recalled that the receiving target in the later instrument was a glassy carbon or a gold rod kept at nearly ambient temperature.

### 2.5. Fast atom bombardment

Fast atom bombardment (FAB) emerged in 1981<sup>52-61</sup>, and is sometime hailed as a revolutionary new solid-phase ionization technique. However, "solid phase" may

not be appropriate and a closer examination appears to bring additional support to our proposal.

The ionization of such involatile substances<sup>52-55</sup> as series of underivatized oligopeptides and oligonucleotides, vitamin B<sub>12</sub> and several other polar substances of biological importance was easily effected using the experimental set-up shown in Fig. 4. A primary beam of ions with a kinetic energy of 2-8 keV is readily transformed into a beam of neutral atoms with nearly identical kinetic energy after resonant charge-exchange neutralization in a gas of the same species as the incoming ions. Argon or xenon is generally used. The unexchanged ions are removed and the neutral beam impinges on the sample, at a low angle of *ca.* 20°, in the ion source block of a double-focusing magnetic sector mass spectrometer.

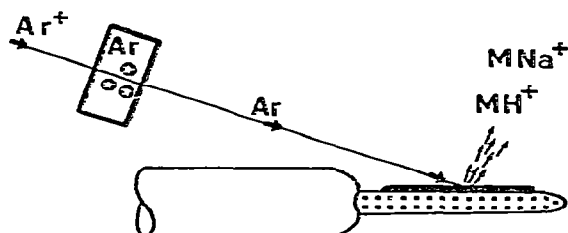


Fig. 4. Schematic principle of the experimental set-up for fast atom bombardment ionization

The results are impressive, showing intense peaks for the protonated or cationized molecule and little or no fragmentation from polar samples with molecular weights well above 1000 daltons. Equally important is the fact that the peak bearing intact sample molecule information can be observed for several minutes, which contrasts with the transient appearance of similar ions under FD or thermal desorption conditions. Thus the instrument parameters can be optimized, and complementary techniques such as collision-induced dissociation and combined mass spectrometry-mass spectrometry can be applied on the flight<sup>56</sup>. Such good results raise a number of questions.

First, a beam of neutral atoms is important only when using a magnetic sector mass spectrometer in which the ion source block held at 4-8 keV would oppose a beam of positively charged ions. In addition, charge accumulations inside the source and high voltage may also cause problems. On the other hand, equally good results have been obtained using high-energy ions and a quadrupole mass spectrometer on samples prepared in the same way as for FAB experiments<sup>57</sup>. Thus neutral atoms or positive ions of same kinetic energy appear to make no difference to the ionization process.

Second, FAB's excellent results contrast sharply with poorer results from established solid-phase ionization techniques such as organic secondary ionization mass spectrometry (SIMS)<sup>58</sup>, which uses an ion beam in a quadrupole mass spectrometer, or molecular beam solid analysis (MBSA)<sup>59</sup>, which uses instrument conditions nearly identical with those used in FAB. In these two solid-phase ionization methods, peaks for sample ions often vanish rapidly, and mass spectra are often characterized by a dearth of intact molecule information when analysing involatile substances.

The trick lies in the sample preparation, which requires a solution of the sample in an organic solvent, or, speaking in the latest fashion, by placing the sample molecule in a (poorly defined) "matrix". The original paper shadows this aspect<sup>52</sup> but information released later<sup>53</sup> emphasized the importance of good solubility of the sample in the matrix. For reasons not very different from those advanced by Evans and co-workers<sup>30-33</sup>, glycerol containing ionic salts (*e.g.*, sodium iodide or ammonium chloride) is used to prepare a solution of the sample. Barber (see ref. 56) believes that the role of the matrix is to provide a surface mobility and "healing" properties, and Sedgwick (see ref. 60) assumes that presenting the sample in a viscous fluid support medium causes a constantly renewed source of the sample to be exposed to the atom beam. They rather underestimate the possible preforming of the ions in solution which could be "knocked-off, peeled-off, or heated-off" by the neutral beam, or by any other source of energy. An acidic medium was found to enhance the intensity of the  $(M + H)^+$  ion when a sample of vitamin B<sub>12</sub> was loaded into a matrix of glycerol acidified with dilute hydrochloric acid<sup>61</sup>. The addition of oxalic acid to the matrix was found to produce up to a 10-fold increase in  $(M + H)^+$  ion current for some underivatized oligopeptides<sup>57</sup>. The surprising concept of reverse derivatization has been introduced: it implies that making the sample more polar through chemical derivatization in order to increase its solubility in a liquid matrix should be recommended to obtain better quality mass spectra from apolar involatile substances.

Finally, it was found later that under the experimental conditions of ionization techniques other than FAB, for instance thermal desorption inside a chemical ionization (CI) plasma (*i.e.*, in-beam CI<sup>25</sup>, surface CI<sup>62</sup>, direct-exposure CI<sup>63</sup>, or direct CI<sup>64</sup>, etc.), adding glycerol or polyglycol ether with some ionic salts dissolved in it, permits the observation of protonated or cationized molecules from samples that resisted previous investigations in the absence of a liquid matrix<sup>65</sup>.

### 2.6. Other methods

We lack the space to review other experiments in which ionization assistance through solvent effects in the liquid phase can be suspected: Day<sup>66</sup> reported that under LD conditions, carbohydrates as large as 800 a.m.u. were easily cationized when mixed with methanol and sodium chloride. Tsuchiya and co-workers, using a method they call "liquid ionization"<sup>67</sup> and which bears similarities to some of the methods reviewed in this paper, have also shown that acids or bases, depending on the samples, added to the liquid solution increase the abundance of the  $(M + H)^+$  ions<sup>68</sup>.

More examples need not be added as enough have been provided and tentative conclusions can be drawn.

## 3. CONCLUSION

During the preparation of this review, it was not difficult to compare two or more ionization techniques and find differences or resemblances between them<sup>69</sup>. However, all methods have in common (1) similar or identical mass spectra for a given sample; (2) major intact molecule-derived ions are the protonated molecule, or the cationized molecule; (3) these ions are stable and do not fragment extensively



unless they are activated, for instance by collision with a neutral gas; and (4) non-polar substances exhibit poor mass spectra unless their solubility in the liquid medium is increased, using either solvent modification (pH or ionic strength adjustment, addition of complexing reagents) or chemical "reverse" derivatization.

The conclusion has already been drawn by Busch *et al.*<sup>70</sup>, in their paper on mechanisms in molecular SIMS and other forms of desorption ionization. They state that "when the sample itself is precharged, then the desorption efficiency and both specificity and sensitivity are optimized. . . . cationization is an ionization process. . . . The recurrent similarity in spectra of complex organic molecules obtained by SIMS, FD, PD, LD and EHD, suggests close relationships between them". and, what is probably the best temporary conclusion, ". . . Practically, then, for these methods of desorption ionization, the question is not *how* applied energy acts to desorb ions, but rather *how much* energy is applied, and with what spectral effects".

For those among us who have attempted to defend the case of LC-MS since its beginning, these statements are encouraging. Up to now, we were embarrassed to answer such frequently asked questions from potential LC-MS users: how do you deal with polar molecules?; can you accommodate salts, buffers or ion-pairing reagents which improve our HPLC separations (because they often help in dissolving the sample in the mobile phase)?; can you introduce polar solvents? From now on, we may have to answer that these are the conditions we like best. Besides, we may even be in the position to advise biochemist colleagues that they should offer more polar molecules by derivatizing them before we investigate their problems. Those who have worked with a GC-MS instrument will surely appreciate the change.

If direct desorption of pre-formed ions is possible, then a liquid solution is probably a very convenient medium for preparing the ions. Vaporization is replaced by desorption from the solvent matrix. We may look forward to future developments. Mass spectrometry has been a tool of utmost importance for the understanding of gas-phase chemistry because unimolecular decomposition of a "naked" ion, or reactions between ions or between ions and molecules, in the absence of solvent cages, could be observed directly. The results, sometime in contrast with those of equivalent reactions under "wet" organic chemistry conditions, have provided indirect evidence of some previously unsuspected influences of solvents on ion reactivities. One of the results of future LC-MS experiments and ionization enhancements through solvent effects could be the direct monitoring of solvent influences on some organic reaction mechanisms, strengthening the current assertion that considers the modern mass spectrometer as a complete chemical laboratory.<sup>71</sup>

The remaining question is whether advantages for handling involatile substances through direct ion desorption are taken when using a direct liquid inlet interface for LC-MS. It is well known that some, if not all, of the solvent introduced through the DLI probe must be vaporized and eliminated by the vacuum system<sup>72</sup>. *When* should the solvent be vaporized, *how much* energy is needed for desorbing the pre-formed ions from the liquid droplets and *when* should this energy be applied are the new questions which need to be answered. Attempts to do so will be made in the next paper.

Our hypothesis may turn out to be untrue; however, it may serve as a useful guide for future instrumental developments.

## 4. ABBREVIATIONS

CI	Chemical ionization
DLI	Interface for direct liquid introduction
EHD	Electrohydrodynamic ionization
FAB	Fast atom bombardment
FD	Field desorption
GC-MS	Combined gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
LC-MS	Combined liquid chromatography-mass spectrometry
LD	Laser desorption
MBSA	Molecular beam solid analysis
SIMS	Secondary ionization mass spectrometry

## 5. ACKNOWLEDGEMENTS

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## 6. SUMMARY

Recent advances in the application of mass spectrometry to the analysis of involatile samples in solution in organic solvents are reviewed. It appears that ions pre-formed in solution by protonation or cationization of the sample molecule can be released in the gas phase by supplying energy to the solution. The results could suggest new directions for liquid chromatographic-mass spectrometric instrumentation.

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