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Use of non-volatile ion-pairing agents for liquid chromatographic–mass spectrometric analyses with a moving-belt interface

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ABSTRACT

Micro-membrane suppressor systems were employed to allow non-volatile ion-pairing agents to be used with combined liquid chromatography–mass spectrometry (LC–MS). This enabled the technique to be used with reversed-phase gradient chromatography for the determination of both anionic and cationic species from a range of compounds. The membrane suppressors remove the ion-pairing agent by counter-current flow regeneration. The determination of cationic species is demonstrated with the use of pyridinium and imidazolium iodides. Hexanesulphonic acid was used as the chromatographic ion-pairing agent and tetrabutylammonium hydroxide as the regenerant. The determination of anionic species is demonstrated with carboxylic and sulphonic acids, which were chromatographed as the tetrabutylammonium hydroxide ion pair using sulphuric acid as the regenerant. The system described has been used successfully with a moving-belt interface, enabling spectra to be acquired in both electron impact (EI) and chemical ionization (CI) modes, with a magnetic sector instrument. The system is in essence compatible with any LC–MS interface, although some modification may be required if the interface generates a significant back-pressure, *e.g.*, thermospray.

INTRODUCTION

Liquid chromatography (LC) is often used, in preference to other analytical techniques, for the determination of polar compounds such as acids and amines. However, in order to obtain a chromatographic separation of these compounds, it is necessary to reduce their polarity. This is usually achieved with the addition of ion-pairing agents to the LC solvent system.

Careful choice of the ion-pairing agent can enhance difficult separations by providing additional selectivity. However, many ion-pairing agents are non-volatile, and the majority of LC–mass spectrometry (MS) interfaces are not designed for use with these types of reagents. This then precludes the advantages of ion-pair chromatography from many LC–MS applications, if non-volatile ion-pairs are required.

The use of volatile buffer systems has been investigated and shown to be readily used, particularly with thermospray (TSP) interfaces [1,2]. There is, however, a very limited range of volatile ion-pairing agents. The use of non-volatile ion-pairing agents

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requires post-column removal systems, which can allow the use of other types of LC–MS interfaces, *e.g.*, moving belt, ion spray–atmospheric pressure ionization (API) and particle beam. Ion-pairing agents have previously been removed with the use of a column-switching technique [3] and a two-phase extraction system [4]. Column switching can enable high sensitivities and selectivities to be obtained, but the system is discontinuous and therefore more suited to target analyte analysis, rather than routine LC–MS applications. Post-column ion-pair extraction will remove the "excess" of reagent, but it will also allow analyte/ion-pair species to enter the ion source, which can result in contamination and a poor MS response. The sensitivity achieved is determined by the extraction and phase separation efficiencies obtained.

The use of a counter-current membrane system permits complete removal of the ion pairs. In the work described here, the use of Dionex micro-membrane systems was investigated. These are available in two forms, anionic (AMMS) and cationic (CMMS), which remove cations and anions, respectively, when used with a suitable regenerant. These systems are designed for use in ion chromatography (IC) to remove background buffer ions and enhance the conductimetric detection of inorganic ions. They have been used with IC–API-MS for the determination of quaternary ammonium compounds [5] and IC–TSP-MS for carbohydrate determinations [6]. The work reported here illustrates that their use can be extended to reversed-phase ion-pair chromatographic systems, allowing LC–MS analysis of a wide range of cationic and anionic organic compounds.

EXPERIMENTAL

Reagents

Solvents and ion-pairing agents. The following were used: Acetonitrile, far-UV grade (Romil Chemicals), hexane sulphonic acid, 40% (Dionex), Tetrabutylammonium hydroxide, 40% (Dionex) and Sulphuric acid, 20 m*M* (FSA Laboratory Supplies). Water was purified with a Millipore Milli-Q system.

Standard materials. Phthalic acid, *p*-methoxybenzoic acid, *p*-chlorobenzoic acid and *p*-toluenesulphonic acid were obtained from BDH, *p*-chlorophenoxyacetic acid and N-methylimidazole from Aldrich and alkylated imidazolium iodides from BP Chemicals.

Equipment

A schematic diagram of the apparatus used is shown in Fig. 1.

Chromatography. The liquid chromatograph consisted of two Gilson Model 303 pumps with an Apple II microcomputer for gradient elution control. UV detection was achieved with Philips LC–UV and Kontron 720LC detectors. Injection volumes of 50 μl were made with a Valco C6W injection valve. The separations were achieved with 250 × 4.6 mm I.D. columns, packed with 5-μm BDS octadecylsilane material (Shandon Southern) and 5-μm PLRP-S, 100Å polystyrene–divinylbenzene polymeric reversed-phase material (Polymer Labs).

The micro-membrane suppressors used were the CMMS and AMMS obtained from Dionex. These were used with $^1/_{16}$ in O.D. × 0.010 in I.D. and $^1/_{16}$ in O.D. × 0.020 in I.D. PTFE tubing for the column eluent and regenerant streams, respectively. The sulphuric acid and tetrabutylammonium hydroxide regenerants were prepared as aqueous solutions as shown in Table I.

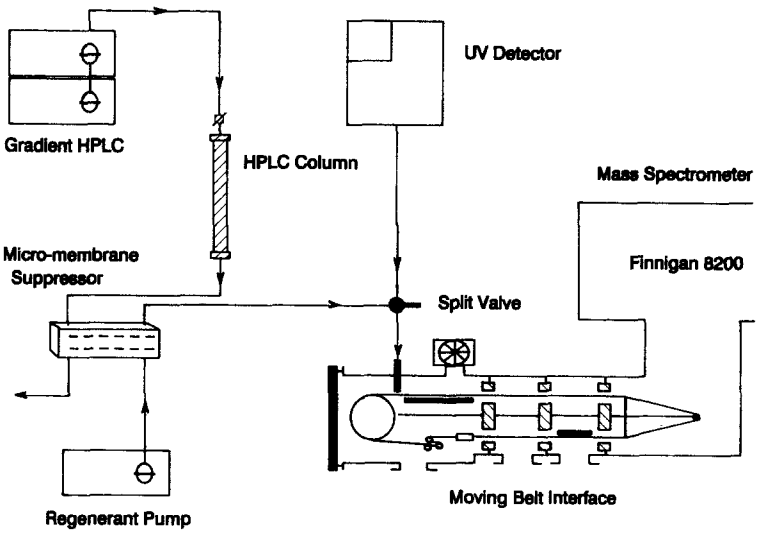


Fig. 1. Schematic diagram of the non-volatile ion-pair removal system for LC–MS.

Mass spectrometry. The analyses were performed on a Finnigan Model 8200 magnetic sector instrument operating in a low resolution/high intensity mode. Data were acquired using both electron impact (EI) ionization at 70 eV and chemical ionization (CI) with either ammonia or isobutane as the reagent gas. The instrument was scanned over the mass range m/z 50–800 at the rate of 2 s per scan.

The LC eluent was divided by a stainless-steel stream splitter with *ca.* 80% being passed to the UV detector and 20% to the mass spectrometer via a Finnigan moving-belt interface. The interface was operated with belt speed 3.5 cm/s, solvent evaporator temperature 180°C, sample tip heater power 8 W and clean-up heater 75%. The ion source of the spectrometer was maintained at 230°C.

Analysis conditions

The chromatographic conditions used are summarised in Table I.

RESULTS AND DISCUSSION

The basis of the suppressors is an ion-exchange resin membrane, which can be used with buffer concentrations of up to 100 mM. This concentration range encompasses that required for effective ion-pair chromatography, which is normally of the order of 3–10 mM. Within this concentration range, a combination of ion-pair adsorption (with ion exchange) and ion-pair partition mechanisms effects the separation [7]. However, the choice of the ion pair and the regenerant is critical for compatibility with LC–MS analyses, and as such the acid and hydroxide counter ions should be used.

The choices of reagents cited here have ensured that the ion-pair counter ion, which is replaced by the regenerant, produces water in the eluent stream. The pyridi-

TABLE I

LC CONDITIONS

Analyte	Separation conditions[a]	Regenerant[a]
Carboxylic acids	A = 5 m*M* aqueous tBAH B = ACN–H_2O (80:20) containing 5 m*M* tBAH 1 ml/min 10–100% B in 30 min BDS and PLRP-S columns	20 m*M* H_2SO_4 2 ml/min
Imidazolium iodides	A = ACN–H_2O (5:95) containing 5 m*M* HSA 1 ml/min B = ACN–H_2O (80:20) containing 5 m*M* HSA 0% B for 10 min 0–60% B in 30 min PLRP-S column	0.1% (v/v) tBAH 2 ml/min
Pyridinium iodides	ACN–H_2O (10:90) containing 5 m*M* HSA 1 ml/min	0.1% (v/v) tBAH 2 ml/min

[a] ACN = acetonitrile; H_2O = deionized water; HSA = hexanesulphonic acid; tBAH = tetrabutylammonium hydroxide.

nium and imidazolium sulphonate ion pairs are converted to the hydroxides, whilst the carboxylic acid tetrabutylammonium ion pairs become the free acids. Thus the processes result in column eluent streams which are totally compatible with the MS interface.

The extraction efficiency of the Dionex membranes has been shown to be >99.9% [5]. Fig. 2. shows the effectiveness of removing an ion pair (*e.g.* the tetrabutylammonium cation, *m/z* 242) by monitoring the 242 ion in the eluent with and

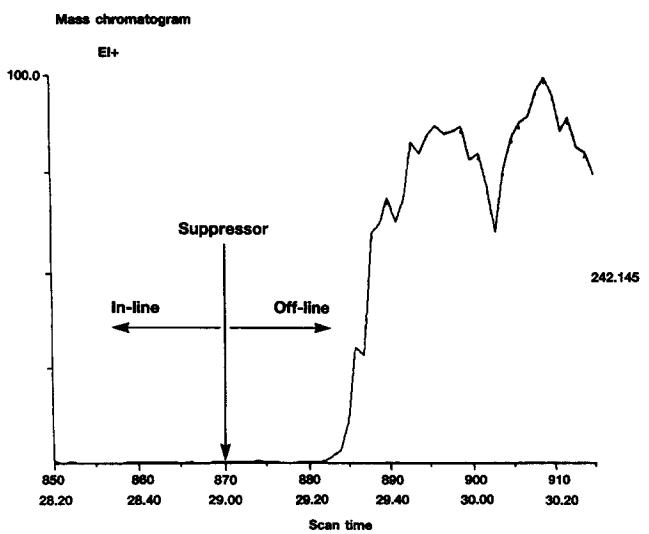


Fig. 2. Single ion chromatogram of *m/z* 242 (tetrabutylammonium ion) with and without the membrane suppressor in-line. Time in min.s.

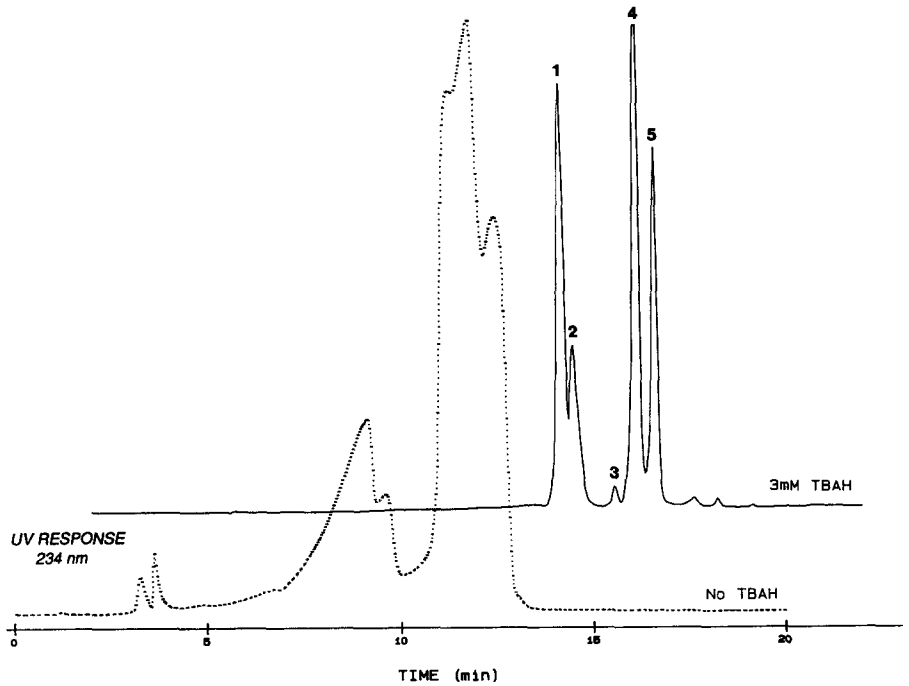


Fig. 3. Effect of the ion pair on the separation of carboxylic acids. Peaks: 1 = phthalic acid (96 mg/l); 2 = *p*-methoxybenzoic acid (432 mg/l); 3 = *p*-chlorobenzoic acid (746 mg/ml); 4 = *p*-toluene sulphonic acid (502 mg/l); 5 = *p*-chlorophenoxyacetic acid (502 mg/l).

without the membrane in-line. With the membrane in place, the ion current obtained is equivalent to that of the baseline without the ion pair in the solvents. The minimum concentration for the regenerant solutions was found to be 0.1%. Below this level the extraction efficiency was decreased, particularly with mobile phases containing high (>50%) concentrations of organic modifier.

The chromatographic separation of polar compounds and ionic compounds (both acidic and basic) by reversed-phase LC without the use of an ion pair or a buffer system, results in poor component resolution and peak shapes. This is due to polar interactions with the residual silanol groups on the column, which is in competition with the non-polar interactions with the bonded-phase ligands. The formation of the ion pair effectively decreases the analyte polarity and hence the interactions with the silanol groups. This greatly increases the chromatographic integrity of the separation, and this is clearly illustrated in Fig. 3. The upper trace was obtained with a solvent system containing 3 m*M* tetrabutylammonium hydroxide and the lower trace without the ion-paring agent. The use of the BDS column provided a more efficient separation, with better resolution of the acids, than the PLRP-S column. However, the apparent pH of the mobile phase, which as measured at 9.4, is above the working range of these columns (quoted by Shandon Southern to be pH 2–8). The column could therefore only be used continuously for 1 week before dissolution of the silica base caused significant deterioration of the peak shapes. The PLRP-S column, whilst

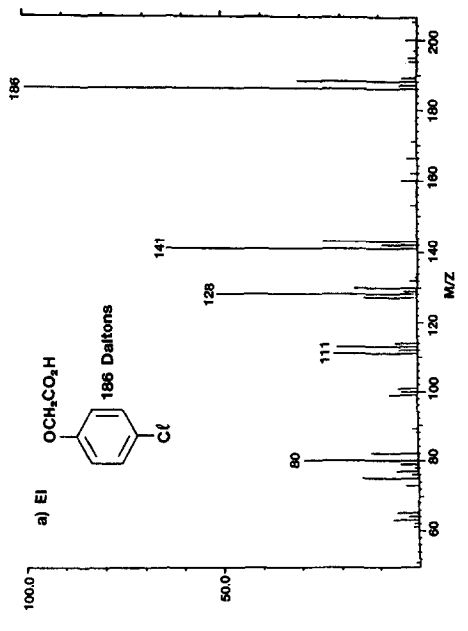


Fig. 4. (a) EI and (b) CI mass spectra of *p*-chlorobenzoic acid (peak 3, Fig. 3).

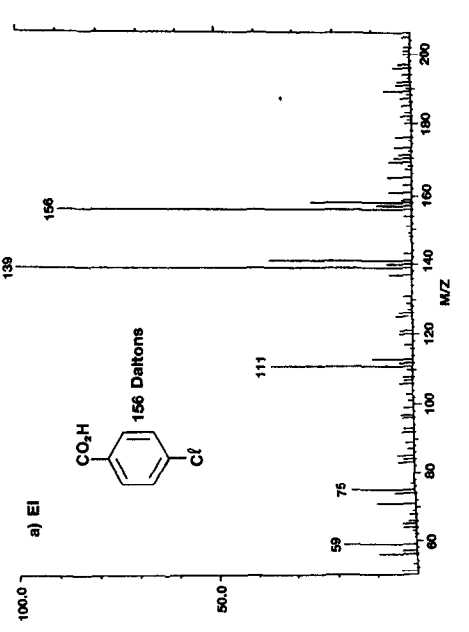


Fig. 5. (a) EI and (b) CI mass spectra of *p*-chlorophenoxyacetic acid (peak 5, Fig. 3).

being resistant to high pH values, does not provide an equivalent separation efficiency. The efficiency of these polymeric reversed-phase materials has been shown to be very dependant on the solvent system [8], but alternative mobile phases were not investigated in this study.

Examples of the EI and isobutane CI mass spectra of the acidic components are shown if Figs. 4 and 5. Mass spectral library searches identified these components correctly as *p*-chlorobenzoic acid and *p*-chlorophenoxyacetic acid. The CI spectrum of *p*-chlorophenoxyacetic acid (Fig. 5) shows only very weak pseudo-molecular ions, *m/z* 187/189, and it is dominated by fragment ions (*m/z* 141/143) corresponding to loss of H_2CO_2 from the protonated molecule. *p*-Toluenesulphonic acid gave an extremely poor response in both ionization modes, which appears to be due to decomposition of the compounds on the belt, and phthalic acid was observed only in the CI mode as the 149 dalton ion.

Fig. 6. shows the total ion current (TIC) and UV traces for the isocratic separation of a mixture containing 1-methyl-4-ethylpyridinium iodide (peak 1) and 4-

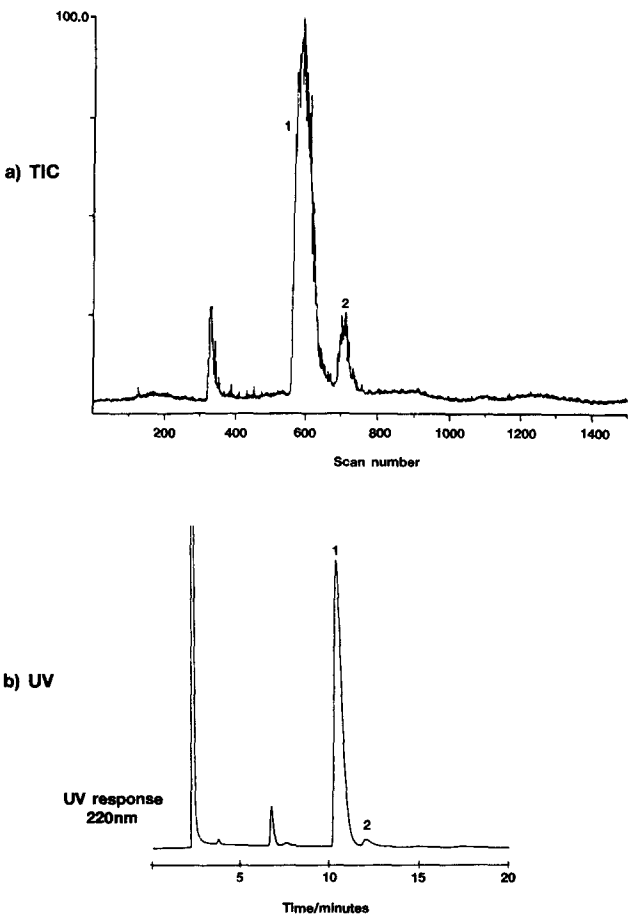


Fig. 6. TIC and UV traces from the LC–MS analysis of the pyridine–pyridinium iodide mixture.

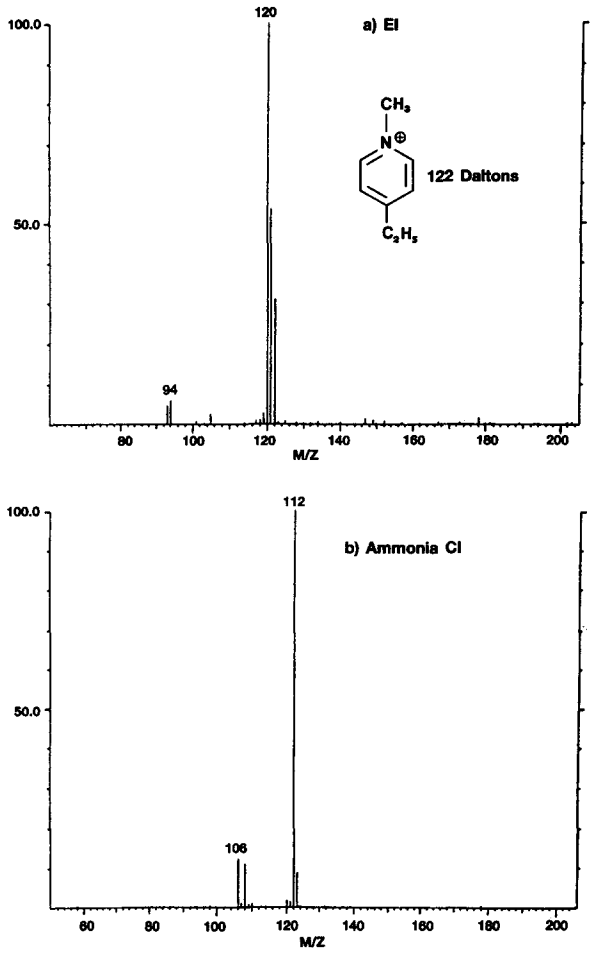


Fig. 7. (a) EI and (b) CI mass spectra of the ethylmethylpyridinium cation (peak 2, Fig. 6).

ethylpyridine (peak 2). The EI and CI mass spectra of the 1-methyl-4-ethyl pyridinium cation are shown in Fig. 7. The spectra obtained show no evidence for the presence of the hexanesulphonate ion pair (*m/z* M + 165), with prominent molecular ions for the cations evident in both EI and CI modes.

The ion-pair separation, obtained with gradient elution, for alkylimidazolium iodides is shown in Fig. 8. The AMMS micromembrane suppressor was again completely effective in removing the hexanesulphonate ion pair. When octanesulphonic acid was used as the ion-pairing agent, some breakthrough was observed with increasing acetonitrile content in the mobile phase, but this system was not studied further or optimized.

Typical spectra obtained from this separation are shown in Figs. 9 and 10. The major CI ions observed correspond to the imidazolium ions (*m/z* 97, 111 and 139) and ions at 14 mass units lower. These undoubtedly arise from thermal dequaternization

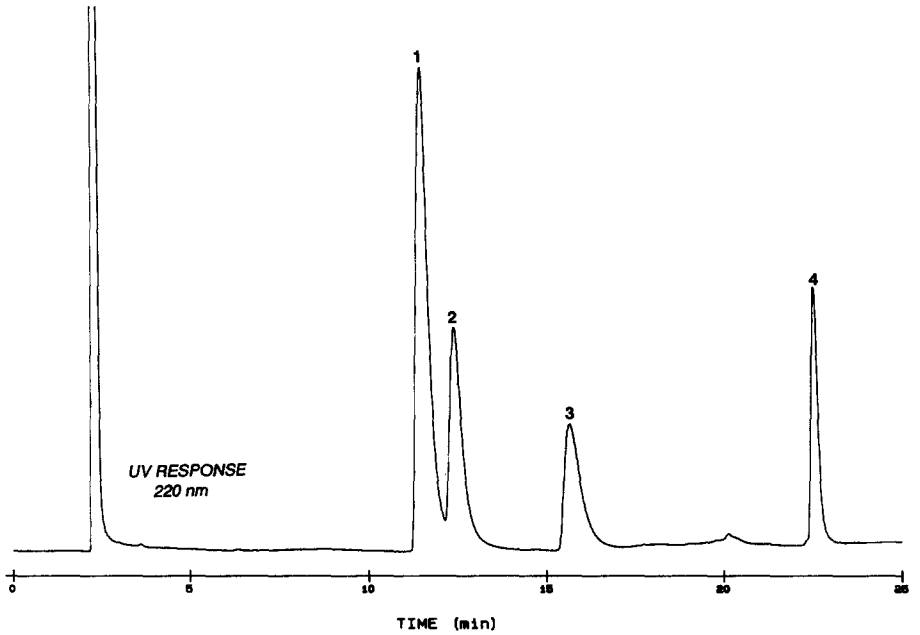
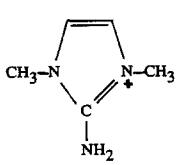


Fig. 8. Reversed-phase separation of methylimidazole and methylated imidazolium iodides. Peaks: 1 = N-methylimidazole (503 mg/l); 2 = N,N-dimethylimidazolium iodide (456 mg/l); 3 = 1,2,3-trimethylimidazolium iodide (301 mg/l); 4 = pentamethylimidazolium iodide (205 mg/l).

followed by re-protonation of the neutral imidazole. Such thermal degradation is well known, *e.g.*, with alkylpyridinium halides [9] and pyridinium N-oxides, which show variable intensity [M–O] ions depending on the thermal conditions employed [10–12]. Whereas quaternary salts are considered to be involatile, lower molecular weight compounds do volatilize directly (both with and without rearrangement), such as N-methyl-3-pyridinium oxide [13–15]. The "volatility" of these compounds is likely to be affected by the counter ion involved, which, after passage through the micromembrane suppressor, is effectively OH^-. It has certainly been shown that the counter ion is important in establishing the fragmentation pathway of quaternary species, *e.g.*, pyridinium salts [16].

Imidazolium compounds not substituted in the 2-position show adduct ions at M + 15 (*e.g.* *m/z* 112 for dimethyl imidazolium), which is postulated to be the result of replacement of H with NH_2 for in the 2-position to give the ion shown. Imidazolium compounds with alkyl substitution in the 2-position did not produce these adduct ions. Adduct ions derived from C-alkylation have been observed in the methane CI spectra of pyrroles, where M + C_2H_5 result from substitution at the C-3 position [17].



m/z 112

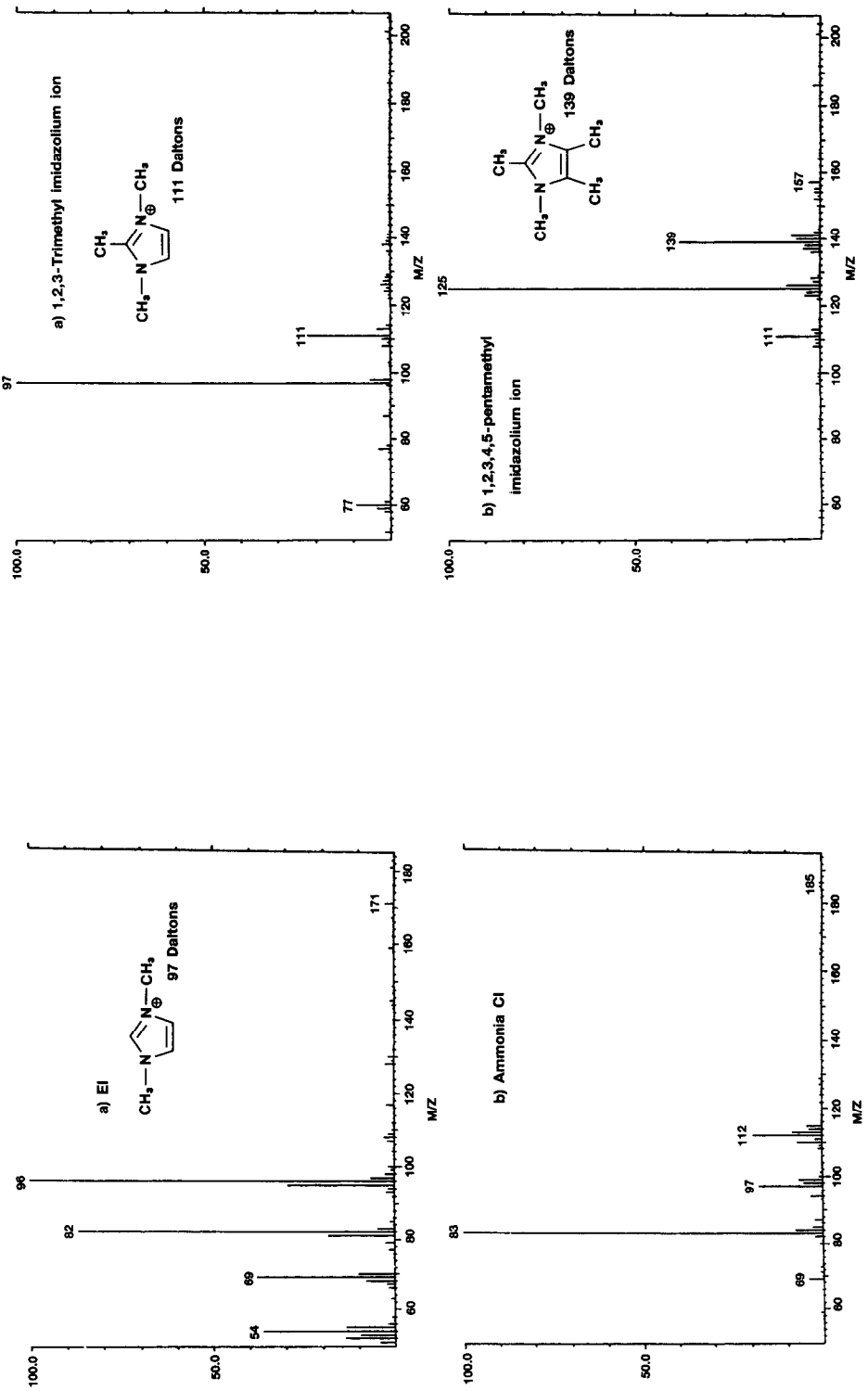


Fig. 9. (a) EI and (b) CI mass spectra of the dimethylimidazolium cation (peak 2, Fig. 8).

Fig. 10. Ammonium CI mass spectra of (a) the trimethyl- and (b) the pentamethylimidazolium ions (peaks 3 and 4, Fig. 8).

As would be expected, a degree of variability in both the EI and CI mass spectra of these quaternary compounds was observed, arising from thermal degradation. This might reduce the scope of the technique for these types of compounds, particularly for more complex mixtures with higher molecular weight quaternary species. The range of applications for the technique might be usefully extended with the use of fast atom bombardment (FAB) ionization combined with the moving-belt interface [18], but this has not yet been studied.

The use of the micromembrane suppressor has, however, considerable potential for the analysis of polar compounds, both acidic (as in the example detailed here) and basic which require ion-pairing agents to effect good separations and where the low volatility of such agents rapidly interferes with the operation of the MS interface. The use of the micromembrane suppressor should also be easily adaptable to other LC–MS interfaces.

CONCLUSIONS

The Dionex micromembrane suppressors can be readily used with a moving-belt interface, thus enabling polar organic acidic and basic compounds to be characterized by LC–MS. The system described is suitable for reversed-phase ion-pair chromatography with gradient elution. This provides a much wider range of ion-pairing agents, from which a suitable one can be chosen for a particular application. The ion-pairing agent and the regenerant can be chosen such that the composition of the solvent stream is compatible with the MS interface. The range of analytes and ion-pairing agents which can be used with the suppressors are currently being studied. A limitation of the current suppressor design is their low back-pressure tolerance, *ca.* < 300 p.s.i. Thus, without any modifications, this limits their applicability to LC–MS interfaces that do not generate any significant back-pressure.

It is apparent that no single LC–MS interface is universal in its application; *e.g.*, thermal degradation of analytes is one disadvantage of the moving-belt system. The use of FAB ionization with the moving-belt interface may overcome some of the thermal degradation problems, but recent developments in capillary zone electrophoresis –MS may provide a more suitable system, particularly for the analysis of higher molecular weight ionic compounds. Also, if the limitations of the membrane suppressors are established and overcome, then other modes of liquid chromatography (*e.g.*, ion-suppression, ion-exclusion and ion-exchange) can be used routinely with many LC–MS systems.

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