CHROM. 21 502

Note

Effects of repeller position and voltage in thermospray mass spectrometry

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Chromatography coupled directly with mass spectrometry (MS) provides the analyst with a uniquely powerful combination, allowing rapid, efficient separation of complex mixtures with accurate and sensitive characterization of the individual components. Gas chromatography–mass spectrometry (GC–MS) has been in widespread use for more than two decades. It has proved so successful that GC–MS has had a major impact on mass spectrometer design and the majority of mass spectrometers are now purchased specifically for that purpose.

By contrast, and despite substantial effort by both academic and industrial researchers, the linking of high-performance liquid chromatography (HPLC) and MS has only recently become routine with the development of the thermospray ion source. Unlike most of the alternative LC–MS interfacing methods, thermospray does not compromise the chromatography significantly as it allows operation at high flow-rates (1 cm³/min) using reversed-phase chromatography with high aqueous content mobile phases containing volatile buffers.

Thermospray provides a soft ionization method yielding predominantly protonated or other cationated molecular ions and generally giving little ion fragmentation. It involves pumping the liquid eluent through a heated capillary tube from which it sprays into a separately heated expansion chamber. This is evacuated by a high capacity mechanical pump, causing solvent molecules to evaporate rapidly, clusters of solute molecules or ions being broken up by collisions. Ion production is enhanced by the use of a buffer such as ammonium acetate. A proportion of the ions formed are drawn into the analyzing region of the mass spectrometer through an aperture in a sampling cone. This aperture is small enough to maintain differential pumping, which allows the mass spectrometer to operate effectively.

Most commercial designs incorporate a repeller electrode in the expansion chamber to enhance fragment ion production, but the precise role of the repeller has been subject to controversy, as some users report little enhancement of fragmentation. Several factors influence thermospray spectra[2], including mobile phase composition, flow-rate, nozzle temperature, chamber temperature, applied voltage on any repeller electrode, and the use of a filament or discharge as a secondary ionization method[3]. The use of a gas phase ion/molecule reaction model can explain the observ-

ed ionization phenomena[4], whereby a basic molecule (B) in dilute aqueous ammonium acetate gives species such as $[BH]^+$ and $[BNH_4]^+$. The observation that fragment ions can be enhanced by the use of a repeller electrode down-stream of the sampling cone has been explained by collisional dissociations[5], the retarded ions being impacted by the high thermal energy molecules streaming through the expansion chamber. The effects of repeller voltage and vaporizer temperature on protonated and other cationated molecular ions as well as on fragment ion production have been described[3,6]. Lower voltages favor ammonium adduct ions, whereas intermediate voltages favor protonated molecular ions and high voltages favor fragment ions. Temperature effects are less clear-cut as they are compound-dependent, but higher temperatures discriminate against ammonium adducts compared with protonated molecular ions, and they tend to favor fragment ion production.

EXPERIMENTAL

This work was carried out on a VG 12/250 quadrupole mass spectrometer (VG MassLab, Altrincham, U.K.), operating under computer control with a standard VG thermospray source having no filament or discharge facility. The temperatures of the nozzle, the expansion chamber and the ion source were 190, 270 and 200°C respectively. The repeller electrode height was varied in the range 0–7 mm in 1-mm steps. (For simplicity the data reported here correspond only to heights of 2, 4 and 6 mm, but this is sufficient to give an accurate picture of the overall behaviour.) The height of the expansion chamber is 10 mm and the sampling cone protrudes into this by 4 mm. The repeller electrode comes into the chamber from the opposite side, so when it is at a height of 6 mm its tip is level with the aperture in the sampling cone.

At each height the voltage was varied between 0 and 340 V. The eluent was 0.1 M ammonium acetate (Analar BDH) in water prepared through a Milli-Q system (Millipore), delivered by a DuPont 870 pump at 1 cm^3/min. Furosemide was obtained from Hoechst and was introduced by direct injection in 0.3 mM aqueous solution through a Rheodyne valve with a 20-μl loop. The data presented here are averages of results obtained on two separate occasions several months apart.

RESULTS AND DISCUSSION

In this work a commercial thermospray source was used having a rod-shaped repeller electrode in the expansion chamber approximately 3 mm downstream of the sampling cone. The source design allows for easy dismantling and cleaning but the support arrangement for the repeller does not guarantee reproducibility in the repeller height settings on reassembley. It was noted that the repeller characteristics were
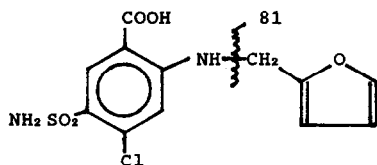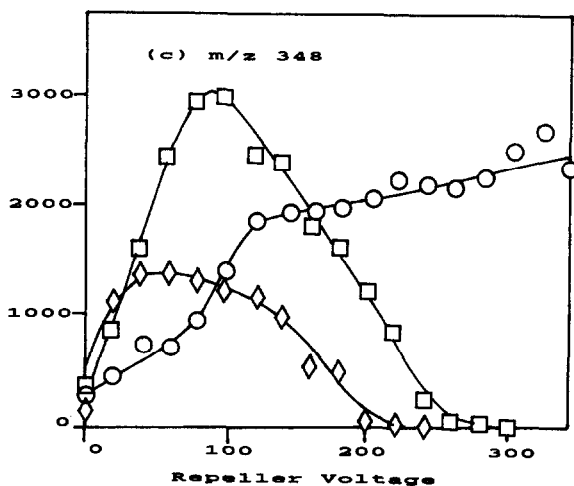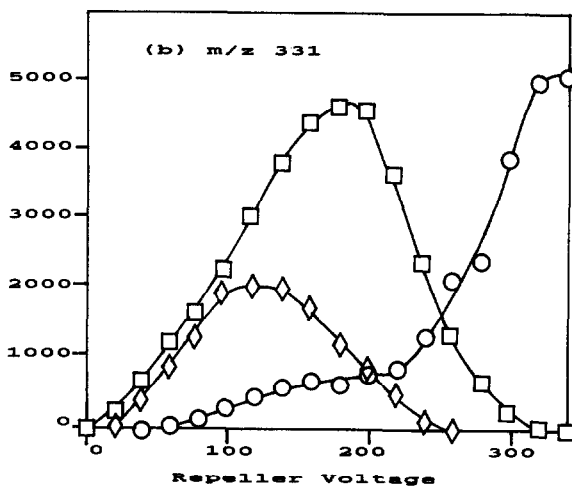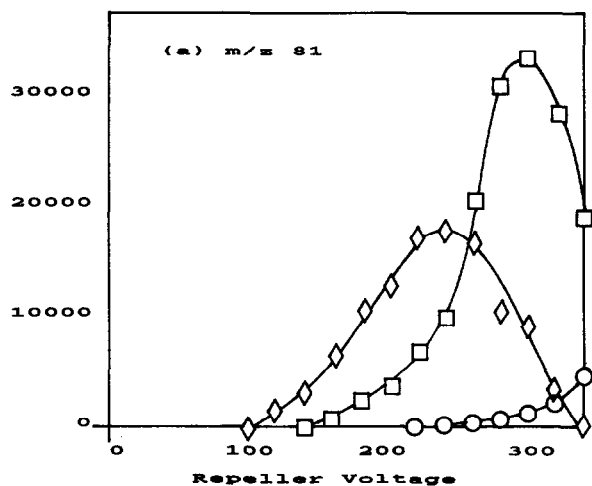

1 (M_R =330)

Fig. 1. Ion current (arbitrary units) *versus* repeller voltage for (a) *m/z* 81; (b) *m/z* 331; and (c) *m/z* 348 for furosemide at repeller height settings of 2 (○), 4 (□) and 6 (◇) mm.

often different after the source had been dismantled, and it was decided to investigate the effect of varying the repeller height. The diuretic drug furosemide (1) was used as a model compound, variations in the intensities of m/z 348 ($[M+NH_4]^+$), 331 ($[M+H]^+$) and m/z 81 (fragment ion) being monitored as functions of both height and voltage. The data are shown in Fig. 1a–c.

Fragment ion intensity

The retarding effect is at a minimum when the electrode height is only 2 mm. Under these conditions almost no fragmentation occurs to give m/z 81, whatever voltage is applied. At 4 mm a substantial signal is obtained for the fragment ion when a high voltage (*ca.* 300 V) is applied to the repeller, and the maximum is seen to shift to lower voltages as the height is increased to 6 mm. This behaviour is consistent with the observations of Bencsath and Field[5] and suggests that the fragmentation is not unimolecular but results from collisional activation. When the repeller height is set too low to retard the ions there is no fragmentation and whatever the height, a retarding voltage of at least 100 V is required to produce any fragmentation at all.

The origin of the m/z 81 ion was investigated as it could correspond either to the furfuryl group or to the protonated sulphonamide moiety. Protonation at the sulphonamide seemed unlikely as such groups are somewhat acidic whereas the secondary amine function is basic. This was confirmed by thermospray analysis from ^2H_2O: the protonated molecular ion increased in mass by 5 u and the ammonium adduct by 8 u whereas the fragment ion mass was unaltered, confirming its identity as the furfuryl cation.

Cationated molecular ions

The application of an appropriate voltage to the repeller gives increased intensity for both m/z 331 and 348 over the full range of repeller heights studied. At 2 mm the ion current *versus* voltage functions are still rising at the highest voltage available, 340 V, whereas the curves for 4 and 6 mm pass through maxima and the ion currents fall to zero before the maximum voltage is attained. The ion current maxima shift to lower voltages as the repeller height is increased. The ammonium adduct ion m/z 348 is favoured by lower voltages compared with the protonated ion m/z 331, which is consistent with the theory that much of the protonated molecular ion current arises through dissociation of the ammonium adduct[4].

CONCLUSION

The evidence concerning the effects of ion repeller voltage on fragmentation in thermospray spectra has frequently been contradictory. This study shows that there may indeed be little effect if the repeller is set too low, *e.g.* not even the maximum applied voltage gives significant fragmentation when the repeller height is only 2 mm.

For the particular design of source used in this work it is essential to take steps to ensure reproducibility in setting the repeller height after dismantling the source for cleaning and maintenance. Even then it may be necessary to select a height that optimises performance for a particular ion type at the expense of others; fragment ions are favoured by high repeller settings (4–6 mm) with high voltages (200–250 V) whereas these same settings will supress the ammonium adducts. It is noteworthy that

when the repeller height and voltage are optimised for fragmentation, the apparent ion current at m/z 81 is six times greater than that observed for the optimised protonated molecular ion. This casts doubt on the description of thermospray as a soft ionisation method, although as already explained most fragment ions arise by collisional dissociation and it must also be appreciated that there is significant discrimination against higher mass ions in quadrupole mass spectrometers.

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