Journal of Chromatography, 394 (1987) 109–116
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 434

STUDIES WITH A DUAL-BEAM THERMOSPRAY INTERFACE IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

L. BÜTFERING, G. SCHMELZEISEN-REDEKER and F. W. RÖLLGEN*
Institut für Physikalische Chemie, Universität Bonn, Wegelerstrasse 12, D-5300 Bonn (F.R.G.)

SUMMARY

A dual-beam thermospray (TSP) interface has been constructed to provide a mode of ionization which is almost independent of the operating conditions for high-performance liquid chromatography. In this interface, one capillary is used for vaporization of the eluate, *i.e.*, sample solution while the other capillary provides the reagent ions for ion formation by vaporization of an appropriate electrolyte solution. Accordingly, mass spectra are obtained from compounds dissolved in an electrolyte-free non-aqueous mobile phase. For water-soluble compounds the dual-beam technique yields a slightly higher sensitivity and lower level of fragmentation than the conventional single-beam TSP technique. For optimization of the ionization efficiency, the setting of the vaporizer temperature of the sample solution is much less critical in a dual-beam than in a single-beam TSP interface. The compatibility of the dual-beam TSP interface with low mobile phase flow-rates as are typical with microbore columns is also demonstrated.

INTRODUCTION

The thermospray (TSP) technique[1–3] has the advantage of combining an interface for on-line high-performance liquid chromatography–mass spectrometry (HPLC–MS) with soft ionization of the solute. However, the use of a conventional single-beam TSP system also has disadvantages: not every mobile phase can be applied but only those comprised of a solvent of high dielectric constant[4] such as water and an electrolyte to provide the reagent ions for ionization of the solute. Accordingly, samples insoluble in such solvents are not amenable to this mode of ionization, and, furthermore, the sensitivity for samples dissolved in solvents other than pure water is typically low. Since a minimum flow-rate ($\geqslant$ 0.5 ml/min for a capillary of 0.1 mm I.D.) is required for an high ion yield and stable ion currents by vaporization of an electrolyte solution, a single-beam TSP interface is not compatible with microbore column HPLC.

Some of the limitations of a single-beam TSP interface have been overcome by post-column addition of the electrolyte[5] and by ionization of molecules in the jet chamber via electron impact ("filament on") ionization or a gas discharge[6]. In another approach we have constructed a dual-beam TSP interface employing two in-

0021-9673/87/$03.50

dependently heatable capillary vaporizers[7]. One capillary vaporizer is used for introduction of the eluate, *i.e.*, the sample solution, into the ionization chamber of the mass spectrometer, while the other generates the reagent ions by vaporization of an appropriate electrolyte solution. This interface provides a mode of TSP ionization which is almost independent of the HPLC operating conditions such as the mobile phase, presence of electrolyte and flow-rate.

We have studied the properties of our present dual-beam TSP interface in respect of the following points: comparison of a dual-beam with a single-beam system for ionization of water-soluble compounds, ionization of water-insoluble compounds and application of low flow-rates. The results are reported in this paper.

EXPERIMENTAL

The dual-beam TSP interface is shown schematically in Fig. 1. Two stainless-steel capillaries of 0.1 mm I.D. and 0.5 mm O.D. terminate 1 mm apart from each other in the jet chamber. The capillaries are directly heated[8]. For vaporization of the electrolyte solution and the sample solution respectively, the capillaries are independently heated by a d.c. current over a length of 180 mm. The vaporizer controller regulates the heating currents for constant temperatures measured about 40 mm apart from the end of the capillaries by thermocouples. Thermal connections between both capillaries and between the capillaries and the heated jet chamber exist at the end of the capillaries. The temperature of the jet chamber and, as indicated in Fig. 1, the temperature of the gas near the orifice to the quadrupole mass analyser can also be measured.

In the present experiments a 0.01 *M* aqueous solution of ammonium acetate was exclusively applied as the electrolyte solution and passed at a flow-rate of 1 ml/min through the capillary vaporizer. The temperatures of the two capillaries and

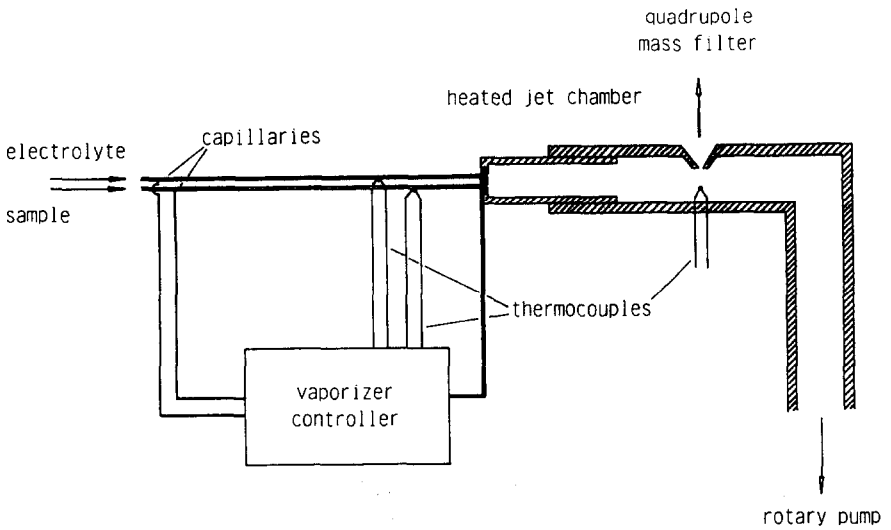

Fig. 1. Dual-beam thermospray (TSP) interface employing two directly heated capillary vaporizers for the sample and electrolyte solution, respectively.

the jet chamber were chosen to achieve a maximum total ion current (TIC = sum of ions in the mass spectrum). After optimization of the TIC, the vaporization temperature of the sample solution was found to have a much less critical influence on the ionization efficiency of solute molecules than in a single-beam TSP system. Typical operating temperatures were about 170°C for the capillary providing the reagent ions at a constant flow-rate of 1 ml/min and about 280°C for the jet chamber. The temperature of the capillary used for vaporization of the sample solution was, of course, very much dependent on the solvent and flow-rate applied. For an aqueous solution and flow-rate of 1 ml/min the capillary temperature was near 170°C. The pump-out-line gas pressure could be regulated and was maintained in the range of several millibar for optimum TSP performance[9]. In the experiments with a single-beam TSP vaporizer, only one of the two capillaries was used.

The ions leaving the jet chamber through an orifice (diameter 0.5 mm) were analysed by a Finnigan 400 quadrupole mass filter with a mass range of 1–420 a.m.u. The spectra were recorded by signal accumulation employing a multichannel analyser (Tracor NS 570 A).

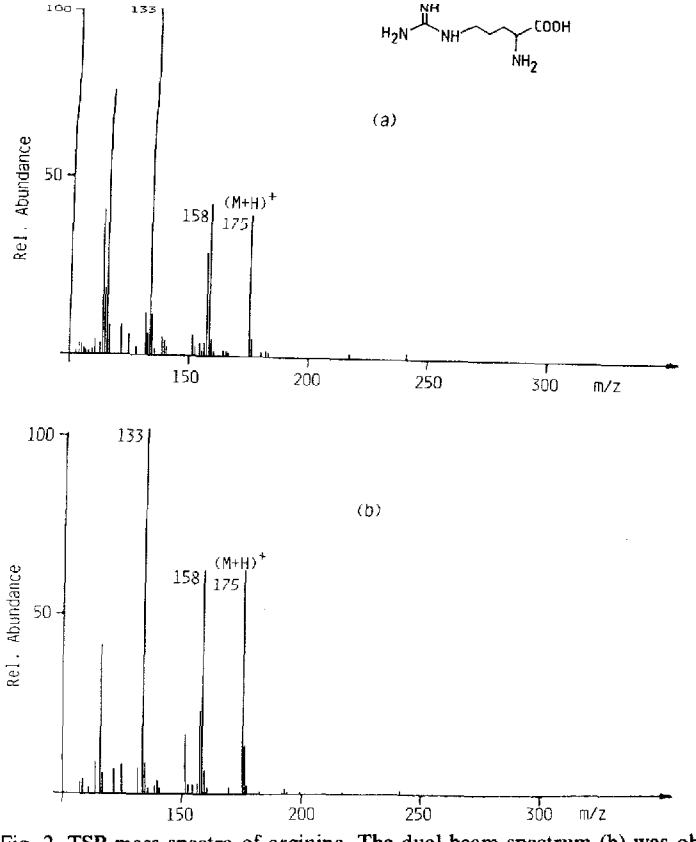

Fig. 2. TSP mass spectra of arginine. The dual-beam spectrum (b) was obtained by vaporization of an aqueous 10^{-3} *M* solution of arginine and an aqueous 0.01 *M* solution of ammonium acetate. The single-beam spectrum (a) was obtained by vaporization of an aqueous solution of 10^{-3} *M* arginine and 0.01 *M* ammonium acetate. The flow-rates were 1 ml/min.

All experiments were performed without an LC column by injecting the sample solutions in a carrier solvent. The two HPLC pumps were obtained from Knauer (Berlin, F.R.G.). The degree of purity of the samples was about 99%. Accordingly, small peaks from impurities were frequently observed in the spectra.

RESULTS AND DISCUSSION

For ionization of sample molecules the sensitivity difference between a dual-beam and a single-beam TSP interface is of main interest. We have measured this difference in sensitivity for some water-soluble compounds which are amenable to both modes of TSP ionization and also searched for dissimilarities in the fragmentation of these molecules. Since the mass range of our quadrupole filter was rather limited, only test compounds of lower molecular weight were chosen such as arginine, chloramphenicol and sucrose.

Figs. 2 and 3 show the mass spectra of arginine and sucrose, respectively, obtained from aqueous solutions (a) in the single-beam and (b) in the dual-beam

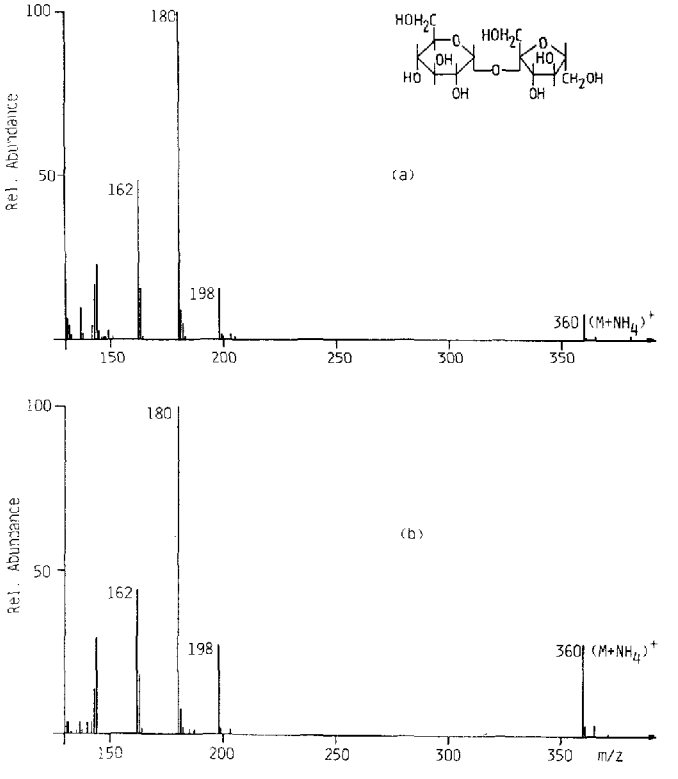

Fig. 3. TSP mass spectra of sucrose. The dual-beam spectrum in (b) was obtained by applying an aqueous 10^{-4} *M* solution of sucrose to the one and an aqueous 0.01 *M* solution of ammonium acetate to the other capillary vaporizer. The single-beam spectrum in (a) was obtained by applying an aqueous solution of 10^{-4} *M* sucrose and 0.01 *M* ammonium acetate to one capillary vaporizer. The flow-rates were 1 ml/min.

mode of TSP ionization. In the single-beam mode only one of the two capillaries was used for volatilization and ionization of sample molecules. The temperatures of the capillary vaporizer in (a) and of the vaporizer of the electrolyte solution in (b) were set near 170°C to achieve a maximum TIC. The experimental conditions were not optimized regarding a low level of fragmentation. The base peak at *m/z* 133 in Fig. 2 can be attributed to the fragmentation of arginine by hydrolytic cleavage of the NH_2NHC–NHR bond in the guanidine moiety. The fragment of *m/z* 180 in Fig. 3 arises from a glycosidic bond rupture plus water elimination and ionization by attachment of NH_4^+. It is seen that the mass spectra obtained by the two modes of TSP ionization are very similar. A small difference is the lower level of fragmentation in the dual-beam mass spectra. This effect was found to be independent of which of the two capillaries was selected for the single-beam mode of TSP operation and also of the diameter of the capillary used for the interface. In previous experiments, capillaries of 0.2 mm I.D. were examined but were replaced by the present capillaries which provided more stable ion currents, particularly at lower flow-rates (about 1 ml/min).

The difference in sensitivity between the dual-beam and single-beam TSP interfaces is displayed in Fig. 4 for the $(M + NH_4)^+$ molecular ions of chloramphenicol and sucrose. In the whole concentration range the dual-beam is somewhat more sensitive than the single-beam technique. The same conclusion can be drawn for the sum of the molecular and fragment ions because the level of fragmentation remains

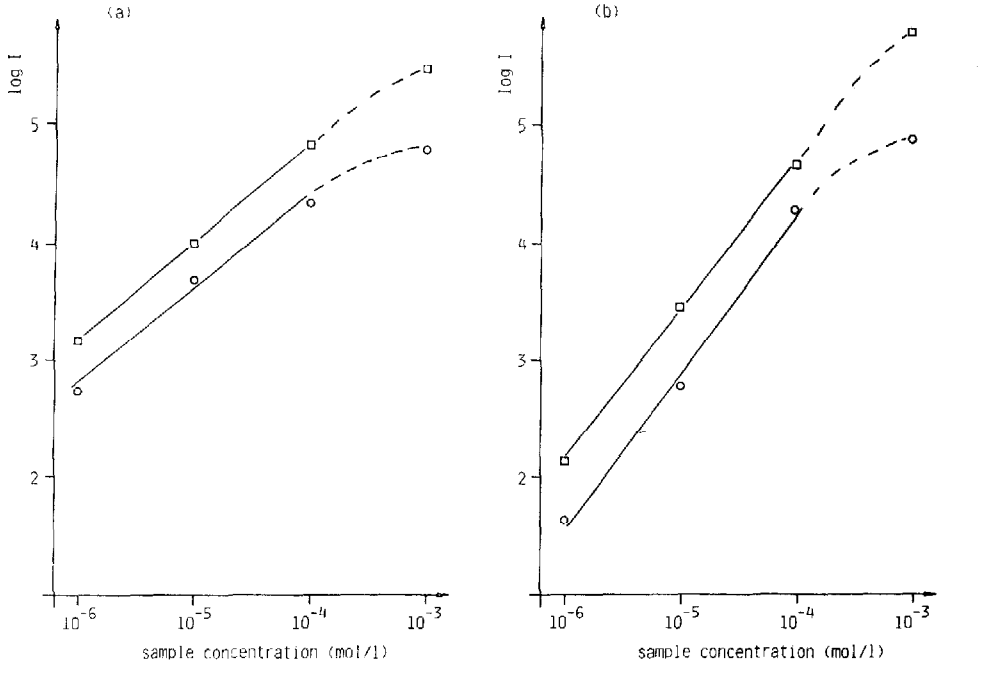

Fig. 4. The $(M + NH_4)^+$ intensity of chloramphenicol (a) and sucrose (b) obtained in the dual-beam (□) and in the single-beam (○) modes of TSP ionization as a function of the sample concentration. Aqueous solutions were applied at a flow-rate of 1 ml/min. The concentration of ammonium acetate was 0.01 *M*.

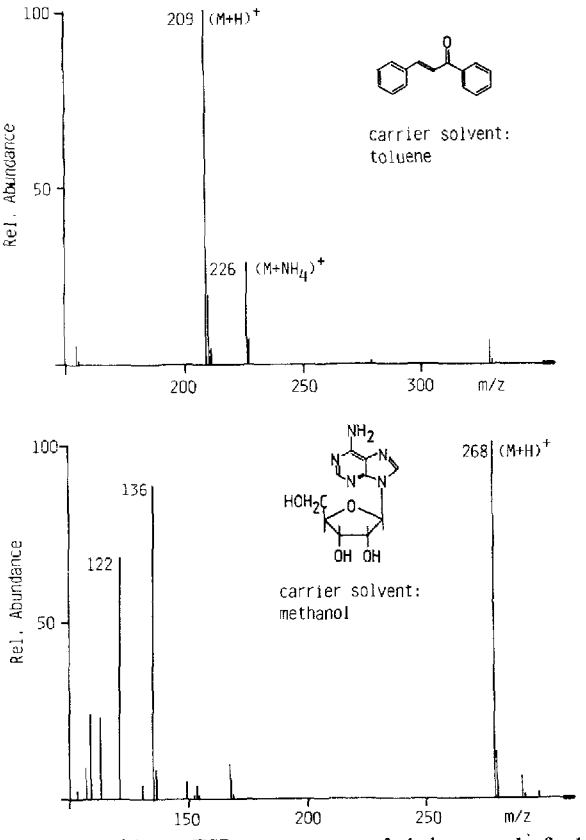

Fig. 5. Dual-beam TSP mass spectra of chalcone and of adenosine obtained by applying toluene and methanol as the solvents and sample concentrations of 10^{-4} *M*. An aqueous solution of 0.01 *M* ammonium acetate was used as the electrolyte solution.

nearly constant. The deviation from a straight line at higher concentrations can be attributed to the effect of clustering of sample molecules in the desolvation process[9,10].

Dual-beam TSP mass spectra of two compounds introduced into the jet chamber by carrier-solvents other than water are shown in Fig. 5. For adenosine, which is also soluble in water, and for sucrose, which is soluble in water and methanol, no significant difference in sensitivity could be found between the TSP ionization of these compounds from a water and methanol solution, respectively, within the experimental inaccuracies. For compounds insoluble in water as chalcone or hexamethylbenzene whose dual-beam TSP spectrum was reported previously[7] no such comparison is possible. As expected, in the ionization of water-insoluble samples we found a strong dependence of the molecular ion yield on the difference in proton affinity between the sample and the solvent and a smaller effect from the proton affinity of ammonia. Accordingly, no detectable molecular ion signal could be obtained from phenanthrene with toluene as the carrier-solvent, while hexamethylbenzene could be ionized with this carrier-solvent.

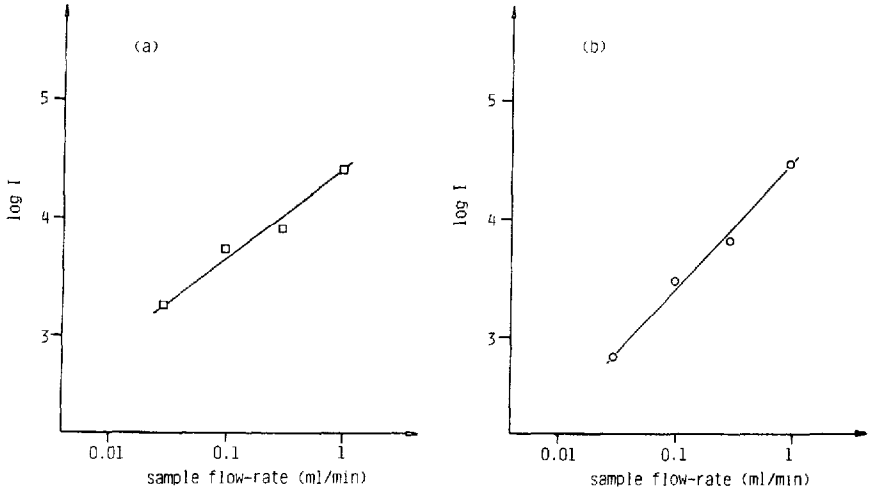

Fig. 6. The $(M + NH_4)^+$ intensity of sucrose (a) and chloramphenicol (b) as a function of the flow-rate. Aqueous 10^{-3} *M* solutions of the samples were applied. The electrolyte solution was an aqueous 0.01 *M* solution of ammonium acetate.

Finally, we have performed experiments to investigate the dependence of the molecular ion intensity on the flow-rate at which the sample solution is passed through the capillary vaporizer while the flow-rate of the electrolyte solution is kept constant. Results for aqueous 10^{-3} *M* solutions of sucrose and chloramphenicol are shown in Fig. 6. With decreasing sample flow-rate the temperature of the capillary vaporizer was reduced from about 170 to about 90°C to adjust it to an optimum yield of molecular ions. In these experiments the lowest flow-rate was 30 μl/min and only limited by the minimum flow-rate of the HPLC pump. The application of flow-rates down to a few μl/min appears possible for this interface, *i.e.*, the dual-beam TSP interface is compatible with the use of microbore columns.

CONCLUSIONS

The results indicate the potential of a dual-beam TSP system as a combined LC–MS interface and ionization technique: for water-soluble compounds the sensitivity is at least as high as that obtained by a single-beam interface and the level of fragmentation is virtually the same for the two modes of TSP ionization. The dual-beam interface is compatible with polar and non-polar solvents and solutes, although the ionization efficiency of solute molecules depends on the proton affinity which should be higher than that of the solvents and of water. Furthermore, mobile phase low flow-rates as are typical with microbore columns can be applied. Another interesting feature of the dual-beam technique is its capability of derivatization in the jet chamber and selective ionization of solute molecules. This has not yet been explored and will be the subject of further research.

The close similarity between the mass spectra of thermally labile compounds obtained by the single-beam and by the dual-beam modes of TSP ionization suggests

no significant difference in the ion formation mechanisms, in particular regarding the "softness" of molecular ion formation. Since the dual-beam technique favours the separate desolvation of molecules and ions with subsequent ion–molecule reactions in the gas phase, the formation of ions in the single-beam technique can be assumed to arise from a similar mechanism, *i.e.*, the desolvation of molecular ions or molecules and ions without a significant contribution from a field-induced ion-evaporation mechanism as discussed in the current model of TSP ionization[11]. This conclusion is supported by previous observations that, at low concentrations ($< 10^{-3}$ *M*), cations of salts are considerably desolvated from small droplets containing solvent molecules only[12] and that at a concentration of 0.1 *M*, ions are preferentially formed by decomposition of solid particles[10]. A detailed discussion of ion formation in TSP will be presented elsewhere[13].

ACKNOWLEDGEMENTS

Support of this work by the Deutsche Forschungsgemeinschaft and the Fonds der Deutschen Chemischen Industrie is gratefully acknowledged.

REFERENCES

1 C. R. Blakley, J. J. Carmody and M. L. Vestal, *J. Am. Chem. Soc.*, 102 (1980) 5931.
2 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750.
3 M. L. Vestal, *Science* (Washington, D. C.), 221 (1984) 275.
4 D. J. Liberato and A. L. Yergey, *Anal. Chem.*, 58 (1986) 6.
5 R. D. Voyksner, J. T. Bursey and E. D. Pellizzari, *Anal. Chem.*, 56 (1984) 1507.
6 C. H. Vestal, D. A. Garteiz, R. Smit and C. R. Blakley, *Proc. 33rd. Ann. Conf. Mass Spectrom. Allied Topics, San Diego, 1985*, p. 771.
7 L. Bütfering, G. Schmelzeisen-Redeker and F. W. Röllgen, *J. Chem. Soc., Chem. Commun.*, (1986) 579.
8 M. L. Vestal and G. J. Ferguson, *Anal. Chem.*, 57 (1985) 2373.
9 G. Schmelzeisen-Redeker, M. A. McDowall, U. Giessmann, K. Levsen and F. W. Röllgen, *J. Chromatogr.*, 323 (1985) 127.
10 G. Schmelzeisen-Redeker, L. Bütfering and F. W. Röllgen, *Adv. Mass Spectrom.*, 10 (1986) 631.
11 M. L. Vestal, in A. Benninghoven (Editor), *Ion Formation from Organic Solids*, Springer, Heidelberg, 1983.
12 G. Schmelzeisen-Redeker, F. W. Röllgen, H. Wirtz and F. Vögtle, *Org. Mass Spectrom.*, 20 (1985) 752.
13 G. Schmelzeisen-Redeker, L. Bütfering and F. W. Röllgen, *Int. J. Mass Spectrom. Ion Processes*, in preparation.