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STUDIES WITH A LABORATORY-CONSTRUCTED THERMOSPRAY LIQUID CHROMATOGRAPH–MASS SPECTROMETER INTERFACE

G. SCHMELZEISEN-REDEKER, M. A. McDOWALL, U. GIESSMANN, K. LEVSEN and F. W. RÖLLGEN*
Institute of Physical Chemistry, University of Bonn, Wegelerstr. 12, D-5300 Bonn (F.R.G.)

SUMMARY

The influence of a series of "thermospray electrolytes" (ammonium acetate, chloride, bromide and iodide and potassium acetate) on the characteristic mass spectra of saccharides and diquaternary ammonium salts was investigated with a laboratory-constructed thermospray (TSP) liquid chromatograph–mass spectrometer interface. The observed dependence of mass spectral fragmentation and total ion current on the concentration and thermal stability, or temperature of volatilization, of TSP electrolytes indicates a significant role for solid particles in TSP ionization. The observed fragmentation is suggested to be related to the formation and decomposition of solid particles and to ion chemistry in the desolvation of molecules rather than to a "purely" thermal effect. A strong influence of the thermospray operating temperature on the relative fragment ion intensities could only be observed for diazo dyes.

INTRODUCTION

In the thermospray (TSP) technique pioneered by Vestal and co-workers[1,2] for on-line liquid chromatography–mass spectrometry (LC–MS) coupling, soft ionization of molecules and direct introduction of the high-performance liquid chromatographic (HPLC) eluate into the ion source of a mass spectrometer is combined in a unique way. The high liquid flow-rates (*ca.* 1–2 ml/min) that can be accommodated in this mode of ionization, with detection sensitivities for thermally labile and involatile compounds in the sub-nanomole range, in addition to the mechanical simplicity of TSP interfaces, suggests that this technique is the most promising for the development of analytically useful LC–MS systems.

In spite of an increasing number of applications of TSP LC–MS[3–6], the present understanding of the physical and chemical processes involved in the formation of molecular and fragment ions by thermospray ionization and of the many parameters that affect the ion yield is poor. Nevertheless, a first approach to a theory of TSP ionization has been presented by Vestal[7,8]. Moreover, commercially available TSP interfaces may not provide optimum conditions with respect to sensitivity, thermal stress to sample molecules and reproducibility of spectra. Therefore, we have con-

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structed a TSP system in order to study in more detail the dependence of ion formation on various parameters. This paper is concerned with the influence of TSP electrolytes and temperature on the characteristic mass spectra of saccharides and diquaternary ammonium salts.

EXPERIMENTAL

The TSP–ion source and LC–MS interface assembly was constructed for use with a Finnigan 400 quadrupole mass filter (mass range 1–420 a.m.u.)[9]. The system consists of a stainless-steel capillary (80 μm I.D.), which is brazed at one end into an electrically heated copper block. The copper block vaporizer is connected to a stainless-steel jet chamber that is also electrically heated. The jet chamber contains a conical exit aperture that is sited so as to impinge on the TSP jet. Ions generated in the thermospray pass through the orifice (500 μm diameter) of the sampling cone and into the mass analyzer. The jet chamber is connected to a rotary pump via a cold trap. The inclusion of a throttle valve between the ion source and the rotary pump is most advantageous[10]. In our experience, the ability to regulate the pressure in the jet chamber whilst maintaining a constant thermospray temperature and solvent flow-rate is essential if optimal TSP performance is to be attained.

All spectra were recorded in the off-line LC–MS mode by injecting sample solutions into an aqueous carrier solvent of flow-rate 1 ml min^{-1}. The TSP ion source conditions were as follows, unless otherwise stated: vaporizer temperature, 250°C; jet-chamber temperature, 180°C; and pump-out line pressure, 8 mbar. When operating this system we noted that under certain conditions the thermospray becomes unstable, which is reflected in rapid periodic (*ca.* 10–100 msec) fluctuations observed whilst recording mass spectra. Typically when optimizing the TSP vaporizer temperature for a given set of LC parameters one observes an increase in total ion current (TIC) intensity with increase in vaporizer temperature until a maximum is obtained[2]. Increasing the vaporizer temperature beyond this point causes a decrease in TIC intensity to occur.

We found that the thermospray is stable until a temperature has been reached close to that required to produce the TIC maximum. Operation at higher temperatures leads to destabilization of the thermospray. The temperature at which the onset of this instability is noted is well defined and reproducible to within *ca.* ± 3°C for a given set of HPLC conditions. The TSP destabilization temperature is determined by the solvent composition and flow-rate of the mobile phase. Therefore, in cases where the optimum vaporizer temperature for organic sample ionization exceeds that of TSP destabilization, one is faced with the dilemma of producing either a stable thermospray or optimal sample ionization efficiency from an unstable thermospray.

RESULTS AND DISCUSSION

As shown for glucose in Figs. 1 and 2, the degree of mass spectral fragmentation increases with increase in "TSP electrolyte" concentration. Similar observations were made for sucrose[9]. We have also noted that ammonium bromide and iodide "TSP electrolytes" promote a greater degree of fragmentation than ammonium chloride. The reported decomposition temperatures of the first two salts are

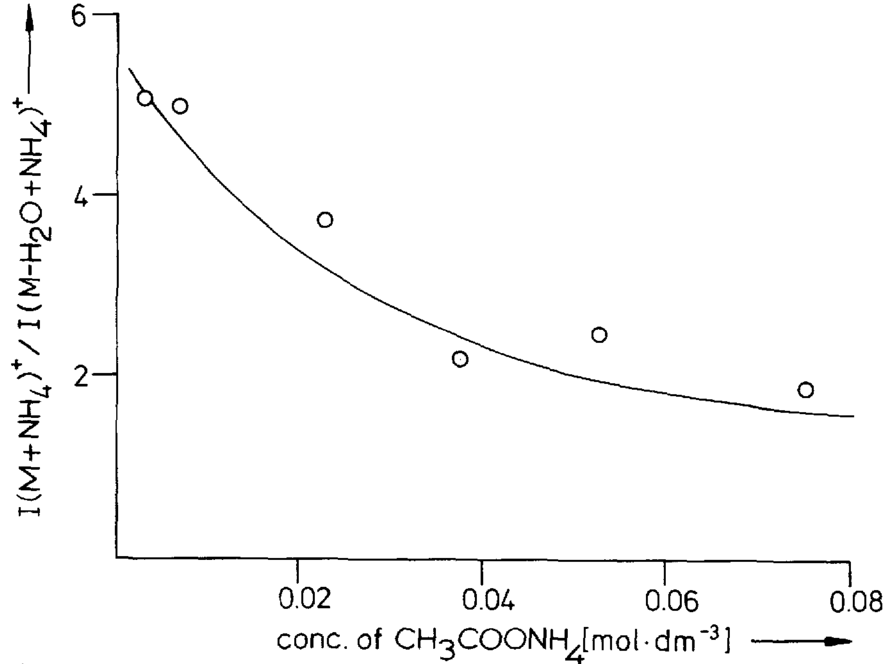

Fig. 1. Intensity ratio of the $[M + NH_4]^+$ molecular ion and the $[M - H_2O + NH_4]^+$ fragment ion of glucose as a function of ammonium acetate concentration.

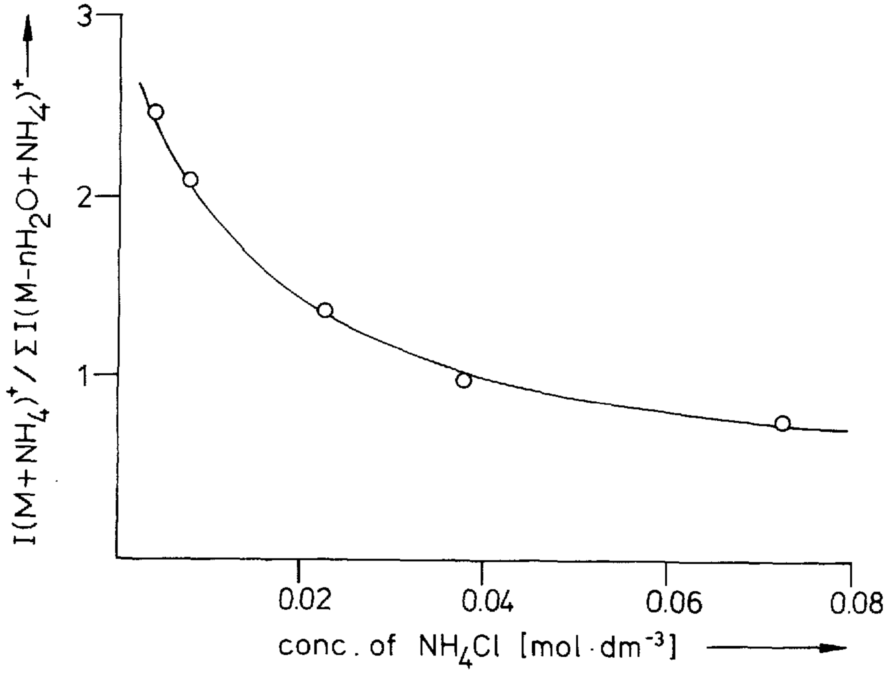

Fig. 2. Intensity ratio of the $[M + NH_4]^+$ molecular ion of glucose and its water elimination products $[M - nH_2O + NH_4]^+$ (n = 1-3) as a function of ammonium chloride concentration.

higher than that for ammonium chloride[11]. These observations point to the effect of decomposing solid particles on the fragmentation of saccharides. The presence of solid particles in the jet is independently indicated by a higher noise level at the detector when instead of ammonium acetate less volatile electrolytes such as ammonium bromide or iodide, potassium acetate or alkali metal halides are utilized. The less volatile salts and in particular the alkali metal halides yield a weaker TIC than ammonium acetate. They decompose or evaporate at much higher temperatures than ammonium acetate and therefore solid particle formation is more favourable when they are employed. Iribarne and Thomson[12] showed that in electrospray ionization the formation of non-decomposing solid charged particles becomes significant compared with ion evaporation from charged liquid droplets at sample concentrations above about 10^{-4} mol dm^{-3}.

The fragmentation reactions of glucose as induced by the thermal decomposition of solid particles are still unknown. Nevertheless, as the same fragments are formed in TSP if instead of an ammonium salt acids such as acetic or hydrochloric acid are employed, it is reasonable to assume that ion chemistry is involved in these fragmentation reactions.

The effect of the "TSP electrolyte" on ionic compounds such as diquaternary ammonium salts was also investigated. In a previous paper[13] we reported the observation of very clean spectra from a number of diquaternary salts ($Cat^{2+}2I^{-}$) in which the Cat^{2+} ions formed the base peaks with only a few weak fragment ions. The TSP spectra were found to resemble the electrohydrodynamic ionization spectra of the same compounds using glycerol as solvent[14,15]. Differences in the spectra obtained from the two modes of ionization can be attributed to the difference in solution chemistry between water and glycerol[15]. For recording the initial TSP spectra of the dications no further electrolyte was added to the aqueous mobile phase. However, as shown in Fig. 3, the addition of ammonium acetate again gives rise to an increase in the relative intensities of the fragment ions and of the noise level. As dications, when kept apart from counter ions, are thermally very stable the fragmentation has to be attributed once again to the formation and decomposition of solid particles in the TSP jet.

We observed that our TSP system suffers from strong memory effects after analysing either mono- or diquaternary ammonium salts. We are unable to explain these effects exclusively in terms of dead volumes contained within the LC–MS interface. We consider that these memory effects are caused by the adsorption of ammonium cations on the wall of the heated jet chamber with subsequent slow desorption of these species. This hypothesis is consistent with recent results from independent experiments providing evidence for the stability of adsorbed tetraalkyl ammonium cations up to temperatures of about 1000°C[16].

The dependence of relative fragment ion intensities on the operating temperature of the TSP system was found to be weak for the saccharides and other compounds examined. An exception is shown in Fig. 4. The mass spectral fragmentation of diazo dyes is very sensitive to change in TSP vaporizer temperature between 200 and 250°C. Unfortunately, the structure of the fragment ion at *m/z* 241 could not be elucidated.

From our experience with TSP LC–MS, we suggest that the observed fragmentation of molecules is more likely to be a consequence of ion chemistry in the

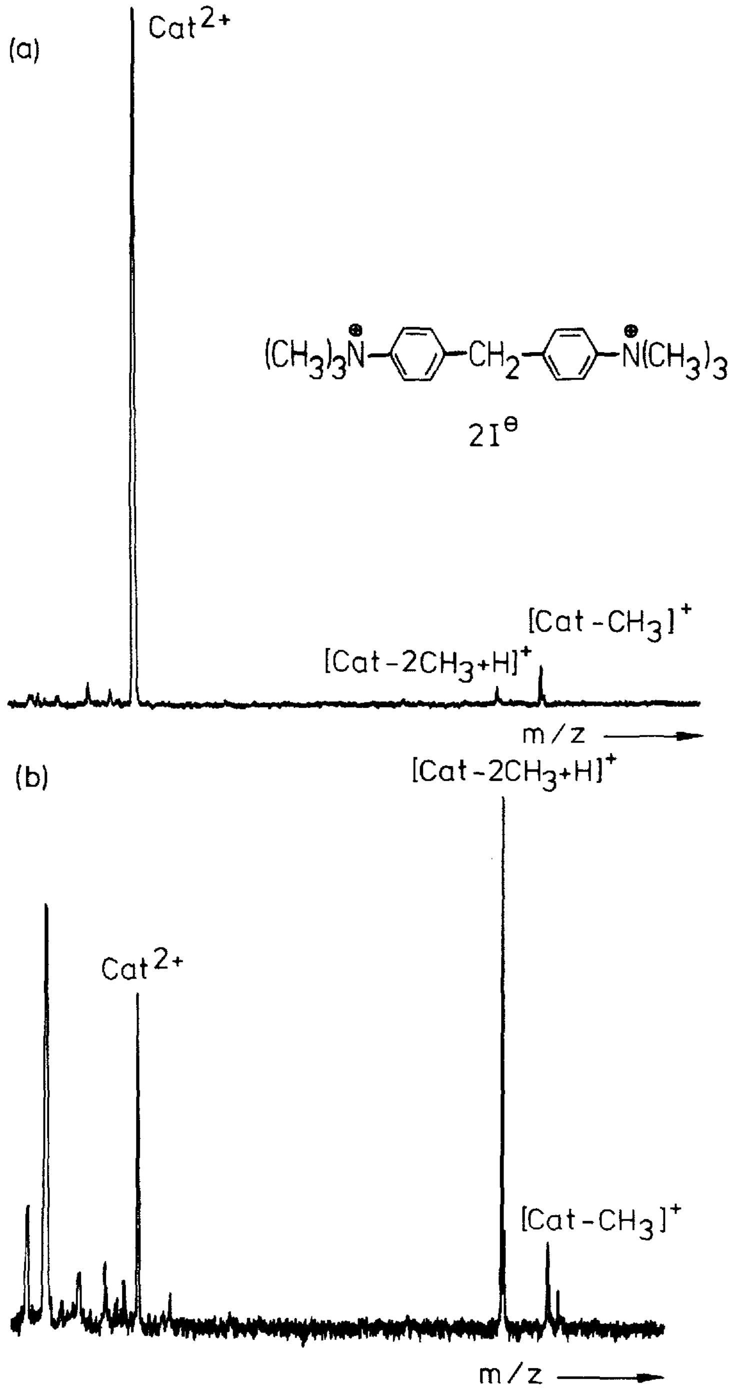

Fig. 3. Influence of the "TSP electrolyte" ammonium acetate on the mass spectral fragmentation of N,N'-bis(dimethyl)-4,4'-methylenedianiline diiodide: (a) without ammonium acetate; (b) with 0.05 mol dm^{-3} ammonium acetate solution.

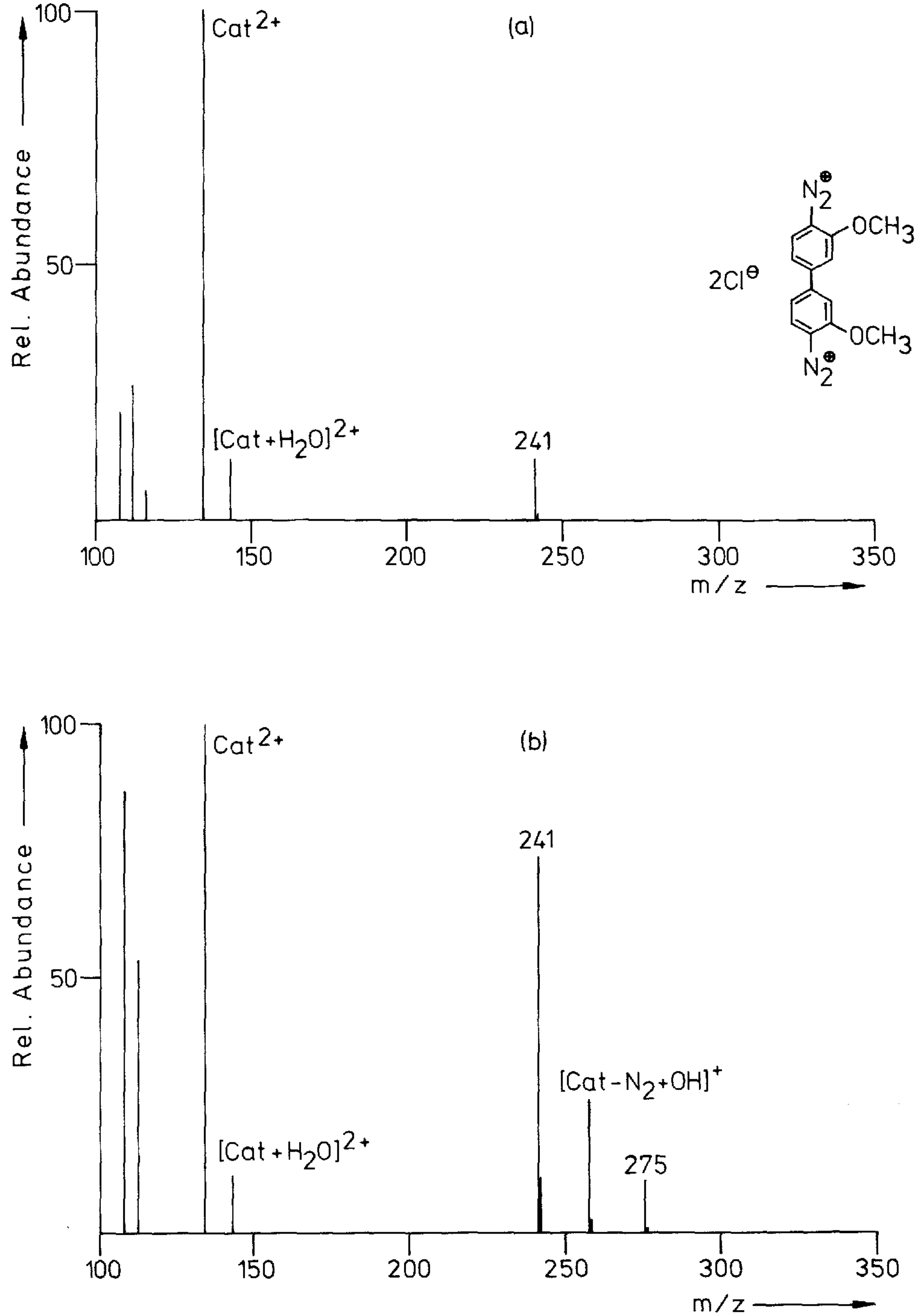

Fig. 4. Influence of vaporizer temperature on the mass spectral fragmentation of the dye tetrazotized *o*-dianisidine. Vaporizer temperature: (a) 200°C; (b) 250°C.

desolvation of molecules and/or of the formation and decomposition of solid particles, rather than a purely thermal effect. The clean TSP spectra of peptides that have been obtained[4] with a very low level of fragmentation point to a high thermal stability of molecules and molecular ions in the gas phase and to the use of an "inert" mobile phase. Further, the use of "TSP electrolytes" for the analysis of ionic compounds is found to be disadvantageous.

Further investigations are required if an adequate understanding of TSP ion formation and fragmentation is to be gained.

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