

Applications of dual-beam thermospray liquid chromatography–mass spectrometry

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ABSTRACT

A dual-beam thermospray system has been constructed, by which the requirements of the electrolyte ion evaporation process have been decoupled from those of the separation and volatilization of the analytes. The dual-beam system has a high flexibility with regard to mobile phase polarity, flow-rate and eluent vaporizer temperature. Results are presented from applications of dual-beam thermospray in the coupling of organic gel permeation chromatography with mass spectrometry, and in gradient elution liquid chromatography. In a comparison of the dual-beam with the single-beam thermospray technique for two thermolabile compounds, the mass spectra obtained with the dual-beam system show much less degradation.

INTRODUCTION

Coupled liquid chromatography–mass spectrometry (LC–MS) has emerged as an important analytical tool since the introduction of the thermospray LC–MS interfacing technique several years ago [1]. Thermospray is compatible with standard reversed-phase liquid chromatographic (RPLC) methods employing aqueous mobile phases, provided that they do not contain non-volatile buffer salts. Ionization of the sample is effected by means of a volatile electrolyte added to the LC eluent, or, alternatively, by chemical ionization with reagent ions which are generated by means of electron bombardment of the solvent vapour, in a “filament-on” or a “discharge” mode of operation. Although somewhat more universal, the last two ionization methods are often not preferred because of their tendency to induce solvent–analyte and fragmentation reactions that complicate the spectra obtained and hamper their interpretation.

A number of limitations of the thermospray

technique have to do with conflicting requirements of electrolyte ion evaporation, on the one hand, and of analyte separation and/or volatilization on the other. The vaporizer temperature window for optimum electrolyte ion evaporation is generally narrow. In gradient-elution LC, this necessitates the programming of the temperature according to the varying eluent composition, which implies the determination of the optimum vaporizer temperature at two points along the gradient at least [2]. Eluents with a high water content require a high vaporizer temperature, which may be detrimental to the analysis of compounds that are susceptible to thermal degradation. Furthermore, in our experience, thermospray has serious limitations with regard to chromatographic methods employing non-aqueous mobile phases. In this case, the addition of electrolytes as ionizing agents poses solubility problems. “Filament-on” ionization does not produce sufficiently high ion yields in most cases, while in “discharge” ionization mode the discharge electrode tends to become rapidly contaminated by a carbonaceous deposit produced from the organic solvent. Probably because of such problems, very few reports have appeared

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in the literature on the application of thermospray in combination with non-polar mobile phases [3,4].

The flexibility of the thermospray technique with regard to mobile phases and vaporizer operating conditions can be significantly enhanced by decoupling the ion evaporation process from the analyte volatilization process in a "dual-beam" arrangement with two independently heated vaporizers, in which the eluent is carried by one vaporizer and the electrolyte solution that is used as the ionizing agent is pumped through a second vaporizer (Fig. 1). This dual-beam concept was pioneered by Bütfering *et al.* [5,6] several years ago, but did not find analytical application at that time. An alternative, recently reported [7], approach is based on the introduction of a chemical ionization gas in the thermospray source in combination with filament-on or discharge ionization. We have constructed a dual-beam thermospray source with the initial aim of coupling organic gel permeation chromatography (GPC), with tetrahydrofuran as solvent, to mass spectrometry. Today, the dual-beam system is also used in the majority of all standard reversed-phase LC–MS applications in our laboratory. This paper presents a few examples of applications of dual-beam thermospray in combination with non-polar mobile phases, in gradient-elution LC, and in the analysis of thermolabile compounds.

EXPERIMENTAL

Liquid chromatography

The liquid chromatographic system consisted of a Waters 600MS multisolvent delivery system, and a Waters 490MS multiwavelength UV detector connected in series with the mass spectrome-

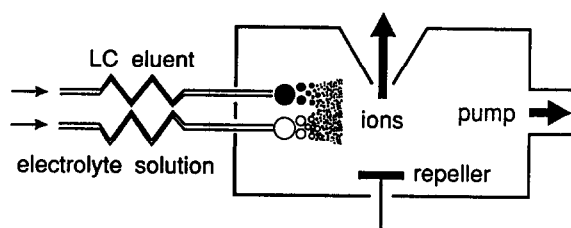


Fig. 1. Diagram of the dual-beam thermospray system.

ter. For GPC, a 60-cm-long, 100 Å pore size, PL gel column (Polymer Laboratories) was used, with tetrahydrofuran (Merck Lichrosolv HPLC grade) as the mobile phase, at a flow-rate of 1.0 ml/min. Reversed-phase separations were performed with a Waters Novapak C₁₈ column, 15 cm × 3.9 mm I.D., and a mobile phase consisting of a mixture of water (purified by a Millipore purification system) and acetonitrile (Rathburn HPLC grade), either in a constant 1:1 composition ratio or in a gradient from 100% water to 100% acetonitrile in 40 min. In both cases, the flow-rate was 1.0 ml/min.

Mass spectrometry

A Finnigan 4500 triple-stage quadrupole (TSQ) mass spectrometer with a Finnigan INCOS data system was used. The data were obtained in full-scan mode, with a typical scan range of m/z 150–850, at a rate of 1 scan/s. The UV detector signal was fed into the data system through an auxiliary input.

Dual-beam thermospray

A Finnigan MAT 4500 thermospray source was modified into a dual-beam system. The only modification of the source itself relates to the original Vespel[®] vaporizer plug, which was changed for a similar plug with two parallel holes, 3.0 mm apart, to accommodate the two vaporizer capillaries. The second vaporizer was fed into the vacuum chamber through a port, which in the original design was connected to a vent valve. Both vaporizers were of the original Finnigan MAT type, with sapphire tip. Electrical connections to the second vaporizer were made through the spare pins on one of the electrical feedthrough plugs. The two independent vaporizer temperature-control circuits were similar to the control system that has been described before [8]. In this system, the original time-proportional control circuit has been replaced by one based on phase-angle control, which yielded a much better vaporizer temperature stability. The LC eluent vaporizer was held at a temperature of 65°C, typically. A 0.1 M ammonium acetate (Baker reagent grade) aqueous solution was pumped through the second vaporizer at a flow-rate of 1.2 ml/min by a Waters 510 pump. The

temperature of this electrolyte vaporizer was set at 140°C for maximum background ion intensity. For single-beam thermospray operation the ammonium acetate solution, at a flow-rate of 1.0 ml/min, was added post column to the LC eluent. The optimum vaporizer temperature in this case was determined to be 124°C.

RESULTS AND DISCUSSION

Gel permeation chromatography–mass spectrometry

The combination of GPC and MS is a particularly attractive one, because GPC is a relatively fast and easy technique with regard to method development, but it has only limited separation efficiency. In GPC–MS, overlapping elution profiles may be deconvoluted into selected ion chromatograms. Furthermore, mass spectrometry provides GPC with an accurate mass scale calibration in applications where appropriate calibration standards are not available. Fig. 2 shows results of a GPC–MS analysis of a polystyrene A300 standard calibration mixture. The upper five traces represent ion chromatograms at five selected m/z values, corresponding to the ammoniated polystyrene oligomers $n = 3$ to $n = 7$. The bottom trace shows the UV absorbance chromatogram. The mobile phase, tetrahydrofuran, would be incompatible with the ionizing agent, ammonium acetate, in a single-beam thermospray system. Using the dual-beam system, the elution profiles of polystyrene oligomers from $n = 7$ down to $n = 3$ are easily observed. Remarkably, ions corresponding to poly-

styrene monomers and dimers were not detected. Apparently, these two compounds have a lower ammonium affinity than the higher polystyrene oligomers.

Gradient elution

In the dual-beam system, only the operating conditions (flow-rate, temperature) of the electrolyte vaporizer are critical. The eluent vaporizer conditions are much less critical. The eluent flow-rate and the vaporizer temperature may be varied over a wide range without an appreciable change in sensitivity. On the other hand, the temperature may be kept constant during a change in solvent composition. This is illustrated on an LC–MS analysis of a mixture of polyethylene glycol (PEG400) and alcohol ethoxylate (octanol to dodecanol ethoxylates). For reversed-phase separation, a linear solvent gradient was applied from pure water to pure acetonitrile in 40 min. The eluent vaporizer was set at 65°C and was kept constant. Fig. 3 shows the total ion current trace (bottom) and three mass spectra, the first obtained at high water content of the eluent (summed spectrum of PEG400), and the other two spectra at low water content (spectra of nonanol and undecanol ethoxylates).

Thermolabile compounds

Bütfering *et al.* [6] compared mass spectra obtained by single-beam thermospray and by the dual-beam system, for a number of thermally labile compounds. They reported very similar mass spectra and sensitivities for the two different thermospray modes. However, they used the same vaporizer temperatures in both modes. We find that in the dual-beam set-up, a much lower eluent vaporizer temperature can be applied, without an appreciable loss of sensitivity. We compared the performance of the dual-beam thermospray system, operating at a low eluent vaporizer temperature, with that of the standard single-beam system on two thermolabile compounds, shown in Fig. 4. The first compound, the 2,4-dinitrophenylhydrazone of mesityl oxide, was produced by derivatization of mesityl oxide (4-methyl-3-penten-2-one), an industrial solvent, with 2,4-dinitrophenylhydrazine. This selective UV labelling of aldehydes and ketones by the

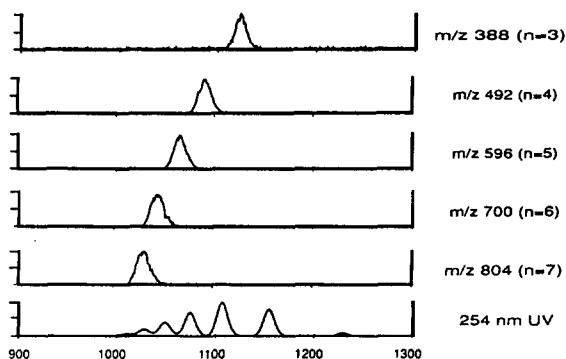


Fig. 2. GPC–MS analysis of a polystyrene A300 sample.

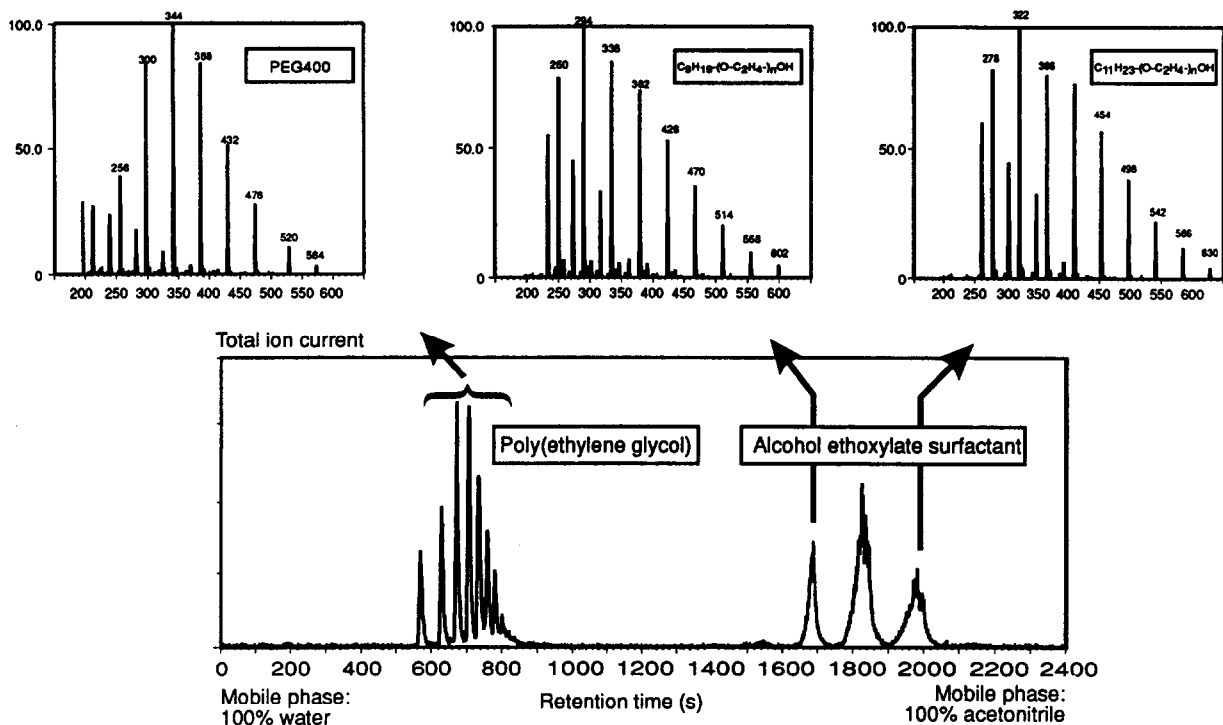


Fig. 3. Gradient elution LC-MS analysis of a mixture of polyethylene glycol (PEG400) and an alcohol ethoxylate surfactant.

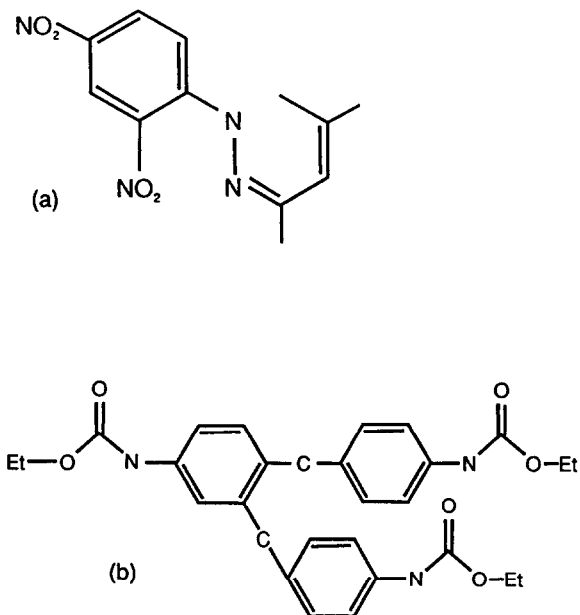


Fig. 4. Structures of (a) 2,4-dinitrophenylhydrazone of mesityl oxide (molecular mass 278) and (b) an urethane prepolymer (molecular mass 519).

reaction with dinitrophenylhydrazine is a well-known method in liquid chromatography. The urethane prepolymer is a product used for the manufacture of polyurethane materials.

Fig. 5 shows the single-beam (top) and the dual-beam (bottom) mass spectra of the first compound. The single-beam spectrum was recorded at a vaporizer temperature of 124°C. Apart from the protonated molecular ion peak at m/z 279, a large number of decomposition product peaks are observed in the spectrum, corresponding to losses of 30 (NO), 32 (O_2) and 46 u (NO_2) and so on. The dual-beam thermospray spectrum was obtained at an eluent vaporizer temperature of 65°C. In contrast to the single-beam spectrum, only two significant peaks are observed, corresponding to the protonated molecular ion and to a loss of 30 mass units (NO) from the molecular ion. Fig. 6 shows the single-beam (top) and the dual-beam (bottom) thermospray mass spectra of the urethane prepolymer. In this example, the difference between the two systems is even more dramatic. Whereas in the dual-beam spectrum fragment ion inten-

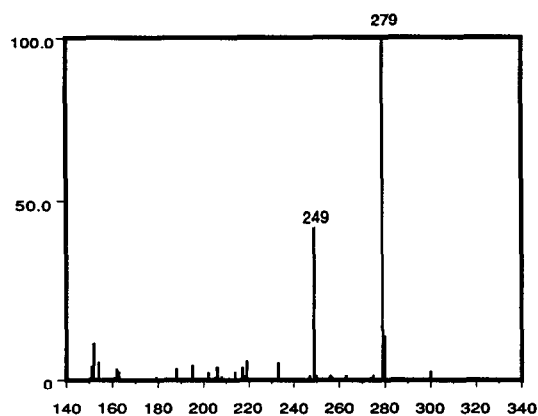
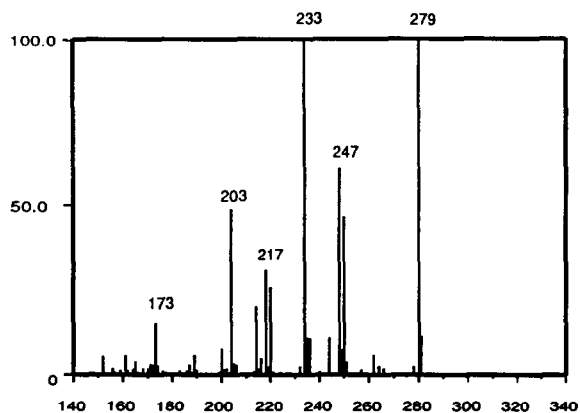


Fig. 5. Comparison of the single-beam (vaporizer 124°C) (top) and dual-beam (vaporizer 65°C) (bottom) thermospray mass spectra of the dinitrophenylhydrazone of mesityl oxide.

sities are less than 15% of the ammoniated molecular ion intensity (m/z 537), in the single-beam thermospray mass spectrum this ammoniated molecular ion peak is hardly observed.

CONCLUSIONS

Applications of dual-beam thermospray in the areas of organic GPC-MS, gradient elution LC and thermolabile compounds have been shown. They highlight the advantages of a dual-beam arrangement over the standard single-beam thermospray system. Much higher flexibility is obtained with regard to mobile phase polarity and flow-rate, and with regard to vaporizer temperature. The dual-beam system enables the soft electrolyte ionization method to be used in combination with non-polar mobile phases, it

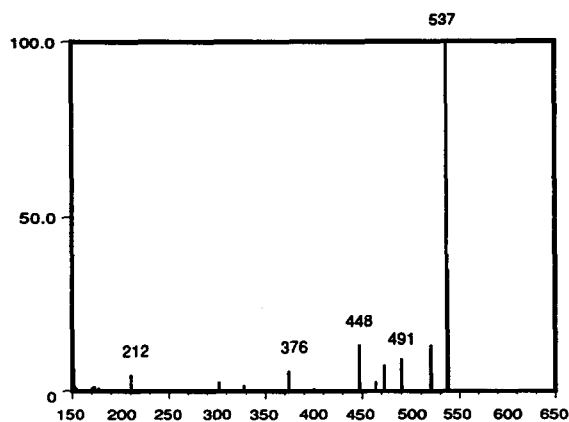
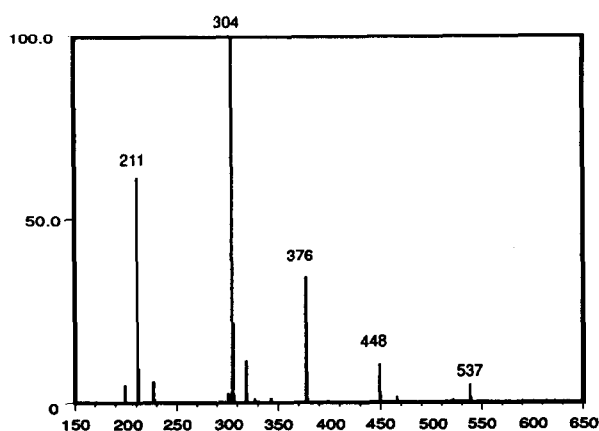


Fig. 6. Comparison of the single-beam (vaporizer 124°C) (top) and dual-beam (vaporizer 65°C) (bottom) thermospray mass spectra of an urethane prepolymer.

eliminates the need for vaporizer temperature programming in gradient elution LC and it has a superior performance in the analysis of thermolabile compounds.

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