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# New Developments in Thermospray Liquid Chromatography/Mass Spectrometry

# INTRODUCTION

Liquid chromatography (LC) is a separation technique generally applied to analytes that are not amenable to gas chromatographic analysis because of their involatility, polarity, or thermal instability. At the Shell research laboratories in Amsterdam, liquid chromatographic methods are widely applied in support of chemical product and process research in many different areas, such as resins and other polymers, oil and polymer additives, agrochemicals and detergents.

A universal detector, such as the flame ionization detector in gas chromatography (GC), is not available in LC. The most widely used detection technique, UV-absorbance detection, can only be applied for compounds that contain a chromophore. i.e. a UV-light absorbing functional group. Refractive index (RI) detection is a more universal detection technique, but it has only a moderate sensitivity and cannot be used in separation systems where the mobile phase composition is varied ("gradient elution").

Mass spectrometry (MS) not only has the potential to be a truly universal detection technique for LC, but could also provide useful information on the structure of eluting compounds. However, while gas chromatography/mass spectrometry (GC/MS) has been a widely used, routine analytical technique for over a decade now, the coupling of LC with MS (LC/MS) has until recently been much less common, due to the conflicting operation conditions of the two techniques: the LC eluent flow into the MS high vacuum, and the low volatility of LC analytes versus the requirement to volatilize the sample in the MS ion source. Several different LC/MS coupling approaches have so far been proposed and realized with varying degrees of success, the most successful one being the "Thermospray" technique.

Thermospray was developed by Vestal and co-workers several years ago, after they had accidentally observed gas-phase ions in a jet emerging from a heated capillary tube into the vacuum, without applying any external means of ionization [1]. These gas-phase ions appeared to be produced from buffer ions in the eluent through a mechanism called "ion evaporation" [2], which is explained as follows. In the heated capillary the eluent is partially evaporated, and this high-pressure solvent vapour nebulises the remaining liquid at the capillary exit. Ions, if present in the eluent, will be statistically distributed over the small droplets produced, and when these droplets get smaller by subsequent evaporation of the solvent, any surplus of positive or negative charge in a droplet will lead to an increasing columbic force. Eventually, the repulsive forces will exceed the surface tension, and a charged particle will be expelled.

Thus, ions present in solution (positive and negative) are directly evaporated as ions. Other polar compounds can be ionized by adding a volatile electrolytes to the LC eluent, which effects ionization of the sample molecules by ion-molecule reactions in the gas phase. This "electrolyte ionization" method is very soft (no fragmentation of the ions produced) but not universally applicable to all compounds of interest. Therefore, other ionization methods have been devised, in which compounds are chemically ionized by reagent ions, which are generated by means of electron bombardment of the solvent molecules. These "filament-on" and "discharge" ionization techniques are somewhat more universal in that moderately polar compounds, too, are efficiently ionized in many cases.

At the Shell laboratories in Amsterdam, a thermospray system has been available for almost five years now. During this period, a development programme has been carried out to adapt the thermospray system to problems encountered in petrochemical research, and to optimize its performance in these applications. Two main modifications have been made to the original thermospray design: improvement of the temperature control system, and construction of a "dualbeam" thermospray source for nonaqueous mobile phases. These modification will be described below, together with a typical application of thermospray LC/MS in the analysis of epoxy resin products.

#### INSTRUMENTAL

A Waters 600MS multisolvent delivery system, equipped with a special flow-rate stabilization system, is used as the LC pump. A Waters 490MS multiwavelength UV-absorbance detector, with high-pressure flow cell, is provided between the LC column and the thermospray interface. The output from the detector is fed into the Finnigan INCOS data system of the mass spectrometer via an auxiliary input. In electrolyte ionization mode, a 0.1 M ammonium acetate aqueous solution is added post-column (or via the second vaporizer in the dual-beam set-up; cf. section 5) by a Waters 510 pump.

Fig. 1 shows a schematic drawing of the Finnigan MAT thermospray interface. The LC eluent flows through a resistively heated vaporizer, consisting of 0.13 mm ID stainless-steel tubing, and emerges as a fine spray at the exit of the capillary. Ions are sampled into the mass spectrometer through a 0.5 mm diameter hole in a small sampling cone. A repeller is placed opposite this cone to enhance the



Fig. 1. A schematic drawing of the Finnigan MAT thermospray LC/MS interface

extraction efficiency in the higher mass range. The large volume of evaporated solvent is pumped off by a cold trap connected to a Busch rotary pump.

The vaporizer tip in the Finnigan interface is crimped to obtain a good spray. To prevent plugging, an SSI 0.5  $\mu$ m high-pressure column prefilter with a replaceable filter disc is mounted directly in front of the vaporizer capillary. It was found that buffer salts or other materials that cause vaporizer blocking by precipitation can usually be re-dissolved by operating the vaporizer at a low temperature for some time. Following this procedure, vaporizers can be operated for several months without plugging problems. Signal fluctuation were frequently observed, and were found to be mostly due to poor vaporizer temperature stability. Therefore, the original vaporizer temperature control circuit has been replaced with a Eurotherm system (cf. section IMPROVEMENT OF SIGNAL STABILITY).

The thermospray LC/MS work has been performed on a Finnigan 4500 TSQ (triple-stage quadrupole) mass spectrometer. Changing from GC/MS to LC/MS and vice versa implies the simple exchange of complete ion source flanges, which usually takes less than one hour. In normal thermospray operation, the pressure in the analyzer region is typically  $2 - 4 \times 10^{-3}$  Pa. The quadrupole scan rate was 1.0 scans/s.

#### IMPROVEMENT OF SIGNAL STABILITY

The vaporizer temperature of the thermospray interface has to be controlled very carefully to maintain a constant evaporation rate and a stable spray. Small variations in the vaporizer temperature, or in the eluent flow rate, have a large effect on ion current stability [3,4]. The time-proportional control system applied in the Finnigan MAT interface tends to induce vaporizer temperature variations due to the pulsed mode of heating, which allows the vaporizer capillary to cool down between successive pulses, and due to electrical feedback of the heating current pulses through the thermocouple spot weld. The rapid ion intensity fluctuations resulting from these variations hamper the optimisation of thermospray operating conditions, and make mass calibration and quantification from thermospray data even more problematic.

Experiments performed on our Finnigan MAT thermospray interface, using various vaporizer temperature control systems, have shown that the temperature stability could be significantly improved by applying phase-angle control instead of time-proportional control. We have implemented such a phase-angle control system [5], made up of an 818P programmable controller and a 425 phase-angle thyristor unit (both from Eurotherm, Worthing, U.K.). Fig. 2 shows the old control circuit (a) and the new one (b), together with their power control characteristics. In the test set-up the Waters 600MS multisolvent delivery system, provided with a flow rate stabilization option, is used to pump a 50/50 methanol/water, 0.50 M ammonium acetate electrolyte solution with 100 ppmv PEG (polyethylene glycol) 400 added, at a flow rate of 1.5 ml/min. The thermospray mass spectrum of this tuning mixture is shown in Fig. 3. From this spectrum, the peak at m/z 344, corresponding to the ammonium ion adduct of the n = 7 oligomer, is selected for the ion current stability tests. The quadrupole filter is manually set at m/z 344, and the vaporizer temperature and ion current are recorded simultaneously on a two-pen recorder.

A typical result is shown in Fig. 4. The first part (A) of the figure was obtained using the time-proportional control, without flow rate stabilization. The vaporizer temperature, optimized for maximum m/z 344 ion current, rapidly oscillates between 107 and 110°C, and the ion current trace shows corresponding fluctuations. Part B was recorded using the new phase-angle control system and without flow rate stabilization. The high-frequency variations in temperature and ion current are much smaller than in A, and the slow variations on an approximately 10-second time scale, observed in both traces, are exactly in phase with the piston movements of the solvent pump. This result illustrates the inadequacy of piston pulse-damping devices in normal LC pumps for thermospray LC/MS work, at least when they are operating in column by pass mode, or as a post-column electrolyte addition pump. In part C, using phase-angle temperature control combined with solvent flow rate stabilization, this slow variation is eliminated, and the vaporizer temperature is stable to within 0.1°C from the set value. The corresponding ion current trace shows the remaining thermospray noise, probably originating from the process of partial evaporation and break-up of the eluent flow into small droplets.

#### LC/MS ANALYSIS OF LIQUID EPOXY RESINS

Epoxy resins based on diphenylolpropane (DPP) and epichlorohydrin (ECH) generally consist of a mixture of diglycidylether homologues, together with molecules containing other endgroups, and/or branched structures. These components largely originate from side reactions in the manufacturing process. The major resin components have been identified in the past by LC analysis using UV



Fig. 2. (a) Modified Finnigan time-proportional vaporizer temperature control circuit; (b) The new phase-angle control circuit



absorbance detection, and by comparison of retention times of unknown components with those of reference materials. However, some trace impurities can have a deleterious effect on the performance of the resins in certain critical end-uses, and since these components are only present at concentrations in the order of 0.01%. identification can only be achieved by a combination of liquid chromatography with mass spectrometry. Thanks to the support from LC/MS to LC method development, the analysis of liquid epoxy resins by liquid chromatography has now progressed to a stage where virtually all important resin components can easily be quantified.

As a typical LC/MS result, Fig. 5 shows the total ion current chromatogram (in discharge mode) and UV chromatogram of a Shell "Epikote" liquid epoxy resin sample. This sample has been selected for its relatively high level of impurities; recent progress in process technology now enables the manufacture of high-purity grade resins with much lower concentrations of impurities. Fig. 5 illustrates the chromatographic integrity of the thermospray interface: the resolution observed in the mass spectrometric trace is only slightly less good than with the UV trace. Almost all the components observed in the UV trace also show a clear response in the MS trace. The large peak observed at a retention time of 750 s in the UV trace is a marker that was added to the mixture and which has a molecular mass below the scan range of the mass spectrometer (150–900 amu).

Figs. 6a and 6b show the electrolyte ionization (top) and discharge ionization (bottom) thermospray mass spectra of two epoxy components, a chlorine-containing impurity and a regular epoxy resin component. "Filament-on" ionization did not yield any complementary information, producing spectra similar to discharge ionization, but with much lower intensities. Electrolyte ionization spectra of the resin components all show abundant ammonium adduct ion peaks, from which the molecular masses of the compounds are easily deduced. Additions of one or two water molecules to these adduct ions are sometimes also observed. Acetonitrile or water addition, and water loss from the protonated molecular ion are frequently observed in the discharge mass spectra, complicating molecular mass determina-



Fig. 4. Vaporizer temperature and m/z 344 ion current trace as a function of time Λ: timeproportional heating system, B: phase-angle heating system, C: phase-angle heating system + flow rate stabilization







Fig. 6. Electrolyte ionization (a) and discharge ionization (b) mass spectra of two epoxy resin components

tions. For many other epoxy components, the discharge ionization mode does not produce molecular mass information at all. All epoxy compounds typically show intense fragment ion peaks at m/z 191 and/or m/z 209 in discharge mode (indicating a rupture of the phenol-phenolpropane bond in the DPP-moiety) and some other fragment ions useful for structure elucidation. Total ion yields were generally 5–10 times higher in discharge ionization than in electrolyte ionization mode.

## **DUAL-BEAM THERMOSPRAY**

The thermospray LC/MS interface has so far been very successful in interfacing standard "reversed phase" liquid chromatography (RPLC), employing polar (aqueous) solvent systems, to mass spectrometry. Although RPLC is still by far the most important LC technique, several recent chromatographic developments have caused renewed interest in less polar mobile phases. The availability of stable, chemically bonded, polar stationary phases makes normal phase liquid chromatography (NPLC) an attractive alternative for RPLC in specific cases. Also, decreasing pore sizes of gel permeation chromatography (GPC) columns extend the workable mass range of GPC down to molecular masses in the order of 100 amu, well within the reach of mass spectrometry. However, the thermospray technique is not easy to use in combination with nonaqueous solvents. The addition of electrolytes as ionizing agents generally poses solubility problems, while in "discharge" mode of ionization the discharge electrode is rapidly contaminated by carbon deposit produced from the organic solvent.

Solubility problems might be avoided by mixing electrolyte ions with the sample in the gas phase instead of in the liquid phase, using a dual-beam thermospray system. The dual-beam concept was reported in the literature a few years ago [6], but has never been exploited for analytical purposes. We therefore modified our Finnigan 4500 thermospray source into a dual-beam system [7]. Fig. 7 shows the set-up schematically. The nonpolar LC eluent enters the ion source through one heated vaporizer capillary, while an aqueous ammonium acetate solution is pumped through the other capillary.

Results of a GPC/MS analysis of a polystyrene standard mixture, as normally used for GPC mass scale calibration, are shown in Fig. 8. The upper five traces represent ion chromatograms at five selected m/z values, corresponding to the ammonium adduct ions of the polystyrene oligomeres n = 3 to n = 7. The bottom trace shows the UV absorbance chromatogram. A PLgel column, 60 cm long, 10 mm pore size was used for separation. The mobile phase, tetrahydrofuran, would be incompatible with the ionizing agent, ammonium acetate, in a single beam thermospray system. Using the dual-beam set-up, the elution profiles of polystyrene oligomers from n = 7 down to n = 3 are easily observed. Ions corresponding to polystyrene monomers or dimers were not detected, probably due to insufficient ionization efficiency.



Fig. 7. A schematic drawing of the dual-beam thermospray ion source



Fig. 8. GPC/MS analysis of polystyrene A300: traces of selected ion currents corresponding to the n = 3 to n = 7 oligometers

## CONCLUSION

The work described in this article illustrates that Thermospray LC/MS is a powerful technique for the analysis of compounds that are not amenable to gas chromatography because of their involatility, polarity and/or thermal instability.

Initial signal instability problems have been traced back to an inadequate vaporizer temperature control circuit, which can be remedied through the use of phase-angle control instead of time-proportional control. Also, a good stability of the LC eluent flow rate is essential to obtain a stable spray.

As for the ionization methods explored, electrolyte ionization and discharge ionization have been found to be useful in different applications, depending on the polarity of the compounds to be ionized. Filament-on ionization has no advantage over discharge ionization, as it produces similar spectra, but of a much lower intensity. Negative ionization can be profitably applied in cases where the negative ions produced are more stable than their positive counterparts.

The sensitivity depends very much on the type of compounds to be analyzed. In this study, the sensitivity required for the identification of trace impurities in liquid epoxy resins at the 100 ppm (1 ng) level, was easily met. However, the ionization efficiencies for less polar compounds may be several orders of magnitude lower than for these impurities, which all contain epoxy groups.

The lack of structural information in thermospray mass spectra hampers the structure elucidation of totally unknown species. Buffer ionization almost only produces molecular (protonated or ammonium adduct) ions. Discharge ionization usually induces some fragmentation, but it also tends to promote rearrangement and ion adduct formation reactions, which often make discharge spectra difficult to interpret. The "repeller-induced" dissociation method, reported in the literature as a means to produce additional structural information [8], has not been successful in our attempts.

The GPC/MS results demonstrate the usefulness of the dual-beam approach to circumvent solubility problems. In practice, the dual-beam thermospray source appears to offer a very high flexibility, not only with regard to mobile phase systems, but also with regard to thermospray operating conditions. The possibility to apply much lower eluent vaporizer temperatures, compared to the conventional system, might also prove useful in the reversed phase LC/MS analysis of thermolabile compounds.

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