Microcolumn size-exclusion chromatography coupled with electrospray ionization mass spectrometry[1]

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Abstract

Microcolumn (250×0.5 mm I.D.) size-exclusion chromatography was implemented for the separation of polydisperse mixtures prior to electrospray ionization (ESI) mass spectrometric detection. An improved separation, compared to conventional-bore SEC, was demonstrated upon coupling with ESI quadrupole ion-trap mass spectrometry and a Fourier transform ion cyclotron resonance instrument for the separation of individual oligomers present in octylphenoxypoly-(ethoxy)ethanol. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

A number of analytical methods have been developed to characterize oligomeric and polymeric mixtures. There has been a steady growth in the use of mass spectrometry for polymer analysis [1–3]. Today's mass spectrometers are equipped with a variety of sample volatilization and ionization techniques that provide intact oligomer or polymer ions with a minimal number of fragment ions. Under ideal conditions, these techniques preserve molecular mass information and allow the determination of absolute molecular mass distributions. Electrospray ionization (ESI) mass spectrometry has been found suitable for the characterization of several synthetic polymers in terms of the monomer mass(es), chemical composition, endgroups and molecular mass distribution [4]. However, the sample should be of relatively low molecular mass with narrow molecular mass distribution, and/or also of relatively simple chemical composition due to an excessive overlap of multiply-charged ions generated from polydisperse mixtures. Multiple charging may also complicate or preclude interpretation of complex mass spectra of polymers upon using low-resolution mass spectrometry [5], and require ultrahigh resolution [6,7] and/or chromatographic fractionation before ESI. Discrimination effects due to specific conditions of the electrospray or the mass analysis should also be considered [4,8,9].

Chromatographic methods are among the most

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prevalent techniques used for the analysis of polymer composition and for the determination of the molecular mass distribution. Size-exclusion chromatography (SEC) has been the method of choice to characterize polydisperse samples and to obtain average molecular masses [10]. However, molecular mass information obtained from the chromatography is highly dependent upon the accuracy of the calibration procedure. Well-characterized oligomer and polymer calibrants of similar chemical composition and narrow molecular mass distribution provide the most accurate results. Such calibrants are usually unavailable, and narrow molecular mass polystyrene standards are often used [11]. The mechanism of separation in SEC may involve solute–solvent–packing interactions that are not strictly dependent on molecular size [12] and such interactions lead to systematic errors in estimation of the molecular mass upon using calibration curves obtained by polystyrene standards when measuring polymers other than polystyrene. The SEC analyses of oligomeric mixtures may suffer the most from structure-dependent interactions, and the ideal conditions that the retention of sample constituents should only be governed by the principle of size exclusion are seldom met.

Coupling SEC with ESI-MS has been a successful approach to overcome various problems associated with the use of MS or SEC alone [13–16]. Upon using commercially available, conventional-bore (7.8 mm I.D.) SEC columns, only a small fraction ($<1\%$) of the effluent enters the ESI source of the instrument. Microbore size-exclusion chromatography (μSEC) offers various advantages, such as low eluent consumption, low cost per column, reduced maintenance requirement, ability to interface to other chromatographic techniques (multidimensional LC) and coupling to the ESI source without the need for flow splitting [17–20].

In addition, better chromatographic performance may be achieved with microcolumns for SEC when compared to conventional-bore systems, which enables an improved separation of sample constituents or significantly reduced time of analysis. Coupling of μSEC with matrix-assisted laser desorption/ionization (MALDI) MS by using a robotic interface has been recently reported [21]. Our paper describes an on-line μSEC–ESI-MS ''hyphenation''.

2. Experimental

2.1. Conventional size-exclusion chromatography

Conventional SEC was carried out by using a 300×8 mm I.D. PLGel Mixed-E (3 μm) column (Church Stretton, Shropshire, UK). The tetrahydrofuran (THF) mobile phase was delivered by a Spectroflow 4000 solvent delivery system (Kratos Analytical, Manchester, UK) at 1.0 ml/min flowrate. Preformed ions were obtained by dissolving $5\cdot10^{-5}$ to 10^{-4} M sodium iodide in the THF mobile phase, or a post-column salt addition was applied. The oligomer mixture of octylphenoxypoly-(ethoxy)ethanol was supplied by the Marshall R&D Laboratory (E.I. duPont de Nemours, Philadelphia, PA, USA), and dissolved in the mobile phase ($\approx$0.5% w/v) before analysis. The sample solution was injected by using a Rheodyne 7125 valve (Cotati, CA, USA) equipped with a 20-μl loop. Effluent splitting was achieved with a T-junction (Valco, Houston, TX, USA) that supplied only about 8–10 μl/min flow to the mass spectrometer through a $\approx$25 cm long fused-silica capillary (50 μm I.D., SGE, Ringwood, Australia).

2.2. Microcolumn size-exclusion chromatography

PLGel Mixed-E (3-μm) porous styrene–divinylbenzene particles (Polymer Labs.) were slurry-packed into 25 cm×0.5 mm I.D. glass-lined stainless steel columns (SGE) according to a procedure adapted from the literature [20]. The slurry (20 ml THF/g of packing) was prepared, then allowed to swell for at least 2 h before packing. The packing adapter supplied by the column manufacturer was used. The particles were suspended in the medium by ultrasonication for 5 min before introducing into the slurry reservoir. An ISCO (Lincoln, NE, USA) 260D solvent delivery system was used to pack the columns at constant pressure (170 atm.).

For the μSEC solvent delivery, the ISCO 260D syringe pump (operated at 4 μl/min flow-rate) or a flow splitting arrangement (similar to the post-column effluent described in Section 2.1) was used to reduce the flow of the THF mobile phase delivered by a conventional pump at 0.2 ml/min to 3–4

μl/min. A Hewlett-Packard (Palo Alto, CA, USA) Model 1050 pump was used in the latter case. A Valco (Houston, TX, USA) CW4I valve equipped with 0.1-μl internal loop was applied as an injector. The column effluent was either supplied directly into the ESI source of the mass spectrometer by a 50-μm I.D. fused-silica capillary tubing (≈50 cm long), or an in-line UV detection was used. The Applied Biosystems (Foster City, CA, USA) Model 759A detector was fitted with a capillary flow cell supplied by LC Packings (San Francisco, CA, USA) and operated at 254 nm.

2.3. Mass spectrometry

Two types of mass spectrometers were used in this study: an ion-trap and a Fourier transform ion cyclotron resonance (FT-ICR) instrument. The ion-trap mass spectrometer was an LCQ (Finnigan, San Jose, CA, USA) equipped with the manufacturer's ESI source which was operated under the following conditions: spray voltage, 3.5 kV; capillary temperature, 200°C; capillary voltage, 3 V and sheath gas flow, 30 (arbitrary units). The instrument was operated and the spectra were processed by Finnigan's NAVIGATOR (version 1.1) data system. Full-scan mass spectra were acquired from m/z 500 to 2000. Ion collection was set for three total microscans with a maximum injection time of 200 ms.

The FT-ICR mass spectrometer was a 3-Tesla

Finnigan FTMS (Madison, WI, USA) system fitted with an Ultrasource I ESI interface. The spraying voltage was 2.8 kV, the capillary temperature was 215°C, and the capillary voltage was 30 V. Mass spectra were collected every 5 s, and the data were processed by a Finnigan FTMS Odyssey data system.

Sheath liquid (100 μM or 1 mM NaI solution in methanol, when applied) was delivered at 2–3 μl/min flow-rate to the ESI interface by a syringe pump which was either built into the mass spectrometer (LCQ), or a Cole-Parmer (Chicago, IL, USA) 74900 pump was used.

3. Results and discussion

Figs. 1 and 2 show representative chromatograms and mass spectra obtained during μSEC–ESI-MS analysis performed by using a quadrupole ion-trap and an FT-ICR analyzer, respectively, of a commercial oligomeric surfactant, octylphenoxypoly(ethoxy)ethanol, whose structure is given below:

$$C_8H_{17} - \bigcirc\!\!\!\!\bigcirc\!\!\!\!\bigcirc - O(CH_2CH_2O)_n CH_2CH_2OH$$
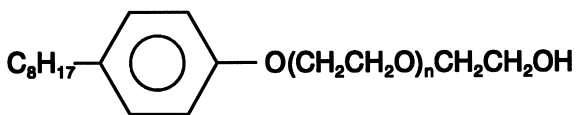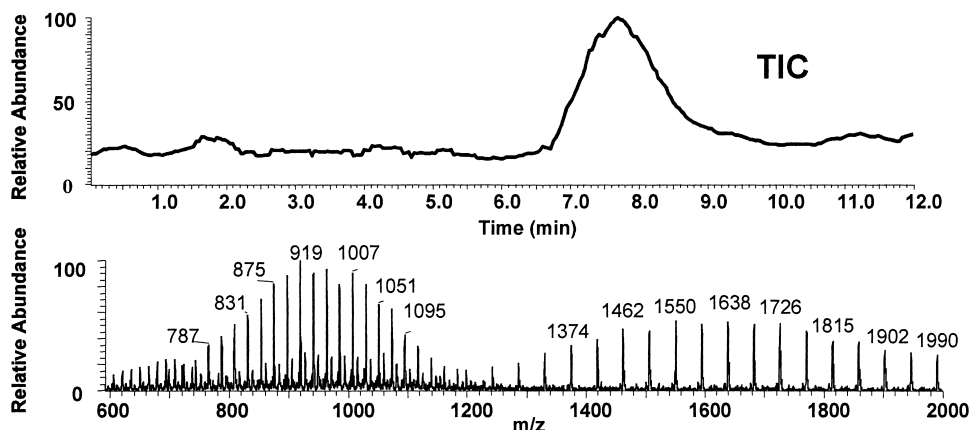
This oligomeric mixture, separated here by using a

Fig. 1. μSEC–ESI-MS analysis of octyloxypoly(ethoxy)ethanol. Top trace: TIC chromatogram, bottom trace: averaged mass spectrum between 6.9 and 9.2 min. Solvent delivery: syringe pump, 4 μl/min; Mass spectrometer: Quadrupole ion trap (LCQ).
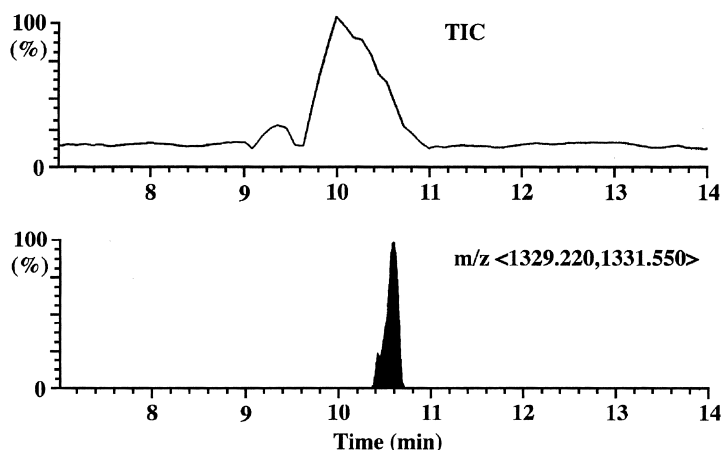
Fig. 2. μSEC–ESI-MS analysis of octyloxypoly(ethoxy)ethanol. Top trace: total ion current (TIC) chromatogram, bottom trace: reconstructed ion chromatogram for an oligomeric species ($n = 25$, $[M+Na]^+$) between m/z 1329.220 and 1331.550. Solvent delivery: conventional HPLC pump, flow splitting to ~3 μl/min; mass spectrometer: FT-ICR instrument (3-Tesla, Finnigan FTMS Newstar).

μSEC column packed with 3-μm mixed-bed particles, has been studied earlier by SEC–ESI-MS using a three-column (1000, 500 and 100 Å pore-size, 300×7.8 mm I.D.) system for chromatographic separation and a quadrupole mass analyzer [13]. ESI conditions have been adapted from this earlier studies; the choice of cationization (Na^+) versus protonation to form positive ions was dictated by the relatively non-polar nature of the analyte, while sodium iodide was found to be an appropriate salt to be added in the mobile phase or in the sheath flow because of its excellent solubility in the respective solvents (THF or methanol).

As in our earlier SEC–ESI-MS experiment, the mass spectrum obtained by averaging the scans acquired during the elution of the sample (6.9 to 9.2 min) during μSEC–ESI-MS (Fig. 1) indicated that the median of the main "envelope" of the mass separated ions was between 1500 and 1700. The mass-to-charge ratio (m/z) of the peaks in this envelope followed the formula:

$$M[+Na^+] = 229 + 44n$$

Thus, ions in this series arose from the attachment of a single sodium cation to yield the molecular ions (n is the number of ethoxy units). There was also another distribution from m/z 600 to 1100 that can be attributed mainly to the doubly charged [M+

$2Na]^{2+}$ species, and low-intensity $[M+3Na]^{3+}$ ions were also observed. Accordingly, mass-to-charge ratios of the oligomer peaks in these series were as follows:

$$M[+2Na^+] = 126 + 22n$$

$$M[+3Na^+] = 91.67 + 14.67n$$

ESI mass spectra recorded during the elution of the analyte upon using the FT-ICR mass analyzer were similar to those recorded by the ion-trap instrument, except that the ESI source interfaced to the FT-ICR instrument produced less multiple charging. The flow-rate for μSEC–ESI-FT-ICR was ≈3 μl/min to reach optimal ESI conditions in this particular combination, while the stability of electrospray in the source of the ion-trap instrument improved significantly when the flow-rate of the mobile phase reached at least 4 μl/min. The difference in retention times in Figs. 1 and 2 is, therefore, due to the difference in flow-rates. The disparity in the shape of the TIC chromatograms may have originated in various factors. In addition to the difference in the propensity to produce multiple-charged ions by the ESI-FT-ICR instrument compared to the ion-trap analyzer, their upper m/z cut-off and the cycle time for mass analysis were also different. The FT-ICR acquisition was set to collect transients and

reconstruct mass spectra in 5-s cycles (the shortest time to trap sufficient number of ions, collect and process the transients for the instrument used in the study) in the m/z 300 to 4500 range, while the ion-trap analyzer had an upper m/z limit of 2000 (i.e., singly-charged ions of oligomers with $M_r >$ 2000 were not detected), but was able to scan at a maximum rate of 180 μs/u that resulted in a much more frequent acquisition of full-scan mass spectra than FT-ICR. Our aim was to demonstrate that μSEC is amenable to direct coupling with ESI mass spectrometry; therefore, alternative solvent delivery systems and mass analyzers were used. However, no rigorous study on comparing the performance of various modes of solvent delivery and mass analyzers has been done, because the respective ESI hardware was also markedly different.

During the μSEC separation, the sample components were separated by their size in solution with the largest components eluting first and the smaller components eluting successively later. We used ion (mass) chromatograms reconstructed from the SEC–ESI-MS analyses, as shown in Fig. 2, to compare the performance of the conventional and the microcolumn chromatography. In this comparison, the linear flow of the mobile phase was approximately equal. The present study has taken advantage of the development and commercial availability of a mixed pore-size 3-μm packing, which provides the state-of-the-art in SEC and introduces less problems in μSEC than an approach relying on coupling single pore-size columns. The number of plates (N) obtained for a late-eluting oligomer (monosodiated molecular ion at m/z 1330) of octylphenoxypoly(ethoxy)ethanol was 19 000/m for the conventional column, while the microcolumn had $N = 24\,000$/m – about 25% increase. The resolution factor, $D\sigma$, where D is the slope of the linear portion of the calibration curve and σ is the peak standard deviation [20], was 0.042 for the 300×8 mm I.D. column. Roughly a 15% increase in chromatographic resolution ($D\sigma = 0.036$) was obtained based on this resolution factor when μSEC was used instead of conventional SEC upon coupling to ESI mass spectrometry. This modest increase, compared to the 300% increase in the efficiency and a 50% decrease in $D\sigma$ reported upon the use of single pore-size μSEC columns and in-line UV detection [20], may be due to an extracolumn

band broadening caused by the relatively long transfer lines in order to connect the column effluent, after in-line UV detection, to the ESI source. The protocol adapted for packing microcolumns were optimized to single pore-size, $d_p = 5$ μm particles and fused-silica tubing [20], and future studies should address reoptimization of the packing procedure for the mixed-bead, $d_p = 3$ μm particles. Previous publications [17–20] did not report any problems on reproducibility of column packing. Reproducibility of our procedure has not been addressed rigorously in this study that concentrated on coupling μSEC with ESI-MS, but the few columns packed under the conditions described in Section 2 gave similar performances. The lifetime of these columns is affected by storage conditions; they must not be allowed to dry out in order to maintain performance [20].

In conclusion, we have demonstrated the feasibility of coupling μSEC with ESI-MS. This hyphenated method obviates the need for effluent splitting and, thus, offers benefits for oligomer and polymer characterization, including reduced solvent and sample consumption, as well as improved chromatographic resolution. Reduced sample consumption may be a specific advantage for polymer analysis involving multidimensional chromatography such as normal- or reversed-phase HLPC followed by SEC [22].

Acknowledgements

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