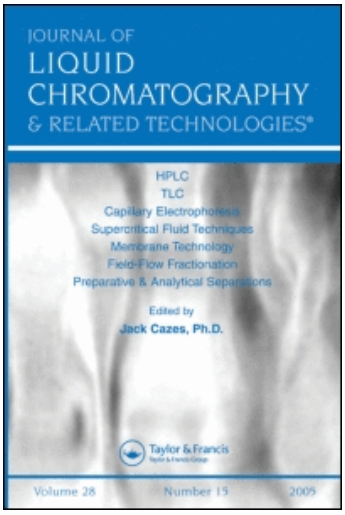

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ELECTROSPRAY MASS SPECTROMETRY AS ONLINE DETECTOR FOR LOW MOLECULAR WEIGHT POLYMER SEPARATIONS WITH FLOW FIELD-FLOW FRACTIONATION

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ABSTRACT

By coupling flow field-flow fractionation online to a high resolution mass spectrometer with electrospray ionisation, it was possible to separate low molecular weight polymers and to obtain mass chromatograms for specific polymers. Problems involved and their solutions in combining the mass spectrometer with field-flow fractionation are discussed. Separation efficiencies were tested for polystyrene sulphonates with different carrier solutions, and it was found that the ionic strength had to exceed about 20 mmol L^{-1} in order to achieve good separation. Salt clusters are formed in the electrospray interface at high ionic strengths, giving rise to a background in the mass spectra and it was found that the composition of the carrier solution and the tuning of the instrument were crucial to the signal to noise ratio. It was also found that, by adding a second electrolyte to the carrier solution, the extent of cluster formation was decreased.

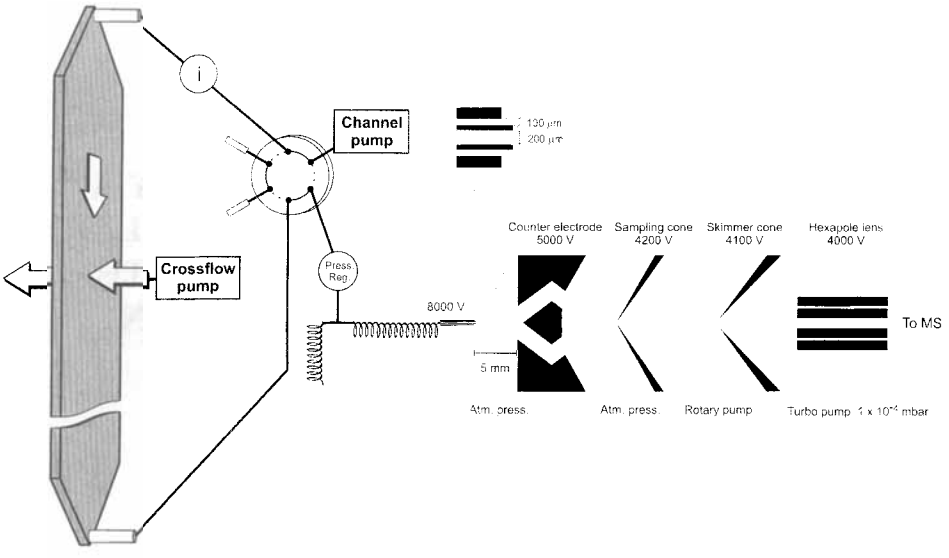


Figure 1. Experimental setup. Schematics of the FFFF channel, pumps, switching valve, flow splitting into 1/3 and the electrospray interface with counter electrode, sampling cone, skimmer cone and hexapole.

INTRODUCTION

Field-flow fractionation (FFF) has been shown to be suitable for the separation of polymers and for the determination of the size distribution of polymer systems.[1, 2, 3] Flow field-flow fractionation (FFFF) is the FFF technique with the widest dynamic range, from about 1000 Da, or a molecular diameter of 1nm, up to the steric inversion diameter of about 1 µm. The lower limit of FFFF is restricted by poor sample recovery due to loss of sample through the ultrafilter membrane.

Thermal FFF is suitable for high molecular weight polymer systems, especially hydrophobic systems in non-aqueous solutions, while FFFF is the more appropriate for low molecular weight hydrophilic polymers.

In general, FFF has minimal alteration to the sample, since it is a very gentle separation without any stationary phase that could cause interactions with the sample. The size distributions obtained consist of a polydispersity component of the sample plus a band broadening component and, since the field-flow fractionation techniques not have higher separation efficiencies than

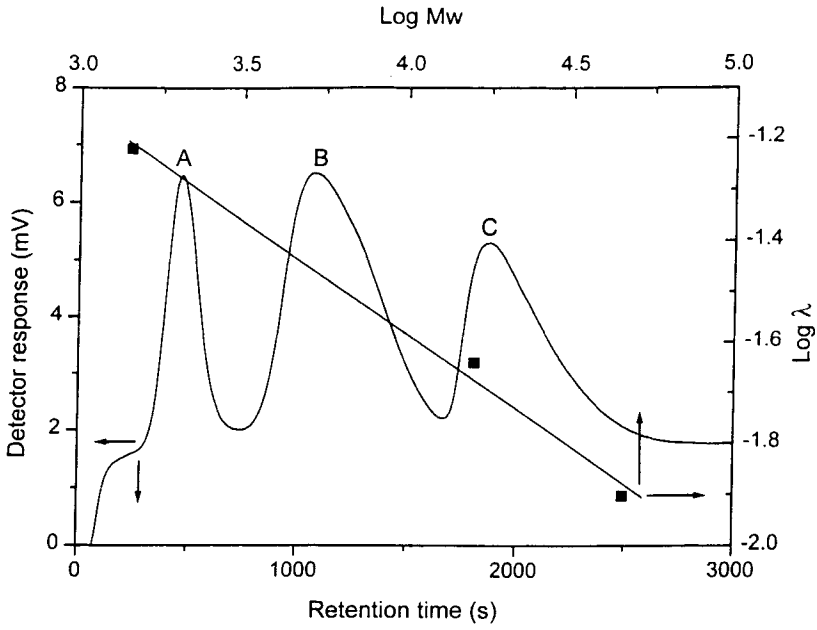
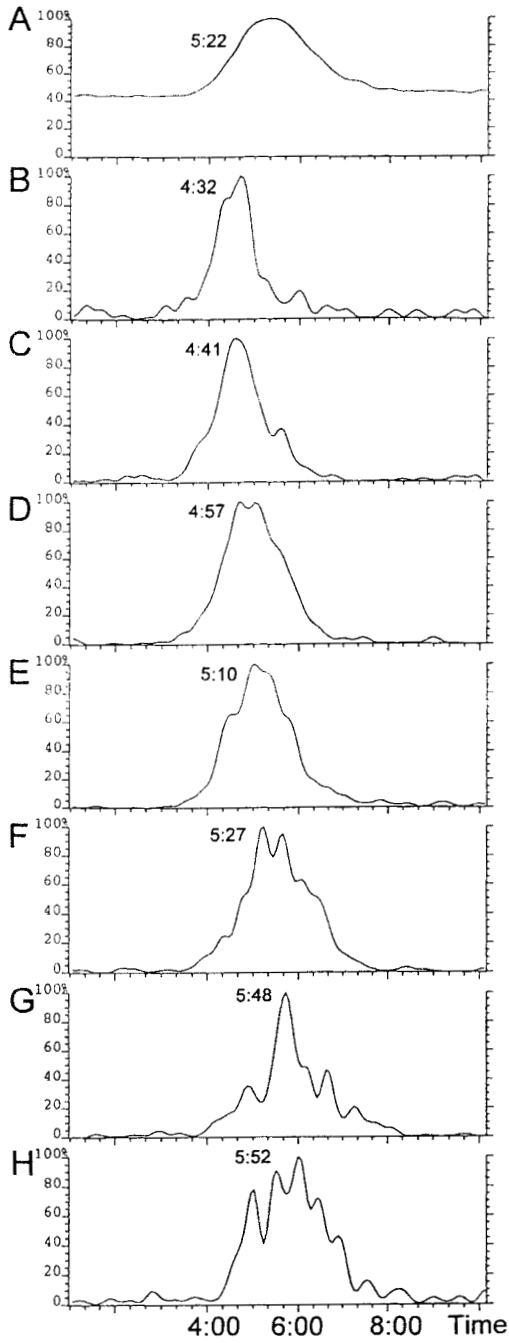


Figure 2. The figure shows a separation and the corresponding molecular weight calibration curve of three PSS standards with weight average molecular weight of 1430 (A), 16000 (B) and 46000 (C) Da. In the Mw calibration log λ is plotted versus log Mw where λ is calculated from $t_0/t_r = 6\lambda[\coth 1/2\lambda - 2\lambda]$. Carrier composition was tris 25 mmol L^{-1}, NaCl 20 mmol L^{-1} and HCl 12 mmol L^{-1}. Channel flow was 0.4 mL min^{-1}, crossflow 3.8 mL min^{-1}, injection loop 20 µL and concentration of sample was 150 mg L^{-1} of each; calculated number of plates, uncorrected for polydispersity, were 45, 31 and 131 for 1430, 16000 and 46000 Da respectively.

a few hundred theoretical plates, band broadening sometimes contributes significantly to the overall size distribution. The natural solution to this problem would be to take advantage of a more selective detector. When studying high molecular weight polymers, it has been shown that the multi angle laser light scattering detector (MALLS), combined with a concentration selective detector such as a refractive index detector, yields a mass selective detection system[4] which, consequently, gives information about polydispersity. However, the MALLS detector suffers from poor sensitivity for low molecular weight polymers, below about 10,000 Da.[5]



This was the reason to investigate the online combination of FFFF to a high resolution mass spectrometer with an electrospray (ES) interface, since the ES have proven to be suitable for low molecular weight polymers.[6, 7]

The basic principles of electrospray can be described as follows: small droplets from the exit of a capillary emerge in a dense electric field as an aerosol, assisted by the nebulizer gas, and evaporate in a warm counter-flow of nitrogen gas at atmospheric pressure, after which the analyte molecules are ionised either by exchange of protons (dissociation or protonation) or by adduction of charged groups, e.g. NH_4^+ or Na^+, added to the liquid phase.[8, 9]

MATERIALS AND METHODS

The electrospray experiments were carried out on a high resolution mass spectrometer (Zab-Spec, VG Analytical, Fisons instrument) equipped with the standard electrospray interface, including a hexapole placed before the acceleration path of the ions (Figure 1). The hexapole was scanned synchronously with the magnet which increases the transmission of ions considerably. The detector was the standard photomultiplier detector system.

The electric potentials in the ES interface, when run in the positive mode, were: spray needle +8000 V, counter electrode +5000 V, sampling cone +4200 V, skimmer cone +4100 V, hexapole and acceleration voltages +4000 V. Nitrogen was used as nebulizer gas purging out between the two capillaries and the bath gas, kept at 80°C entering in front of the counter electrode. The FFFF system was kept at ground potential and, therefore, a 2 m long and 0.12 ID mm PEEK capillary was inserted between the FFF channel and the needle. This sets the limit for maximum conductivity of the sample as corresponding to ~50-100 mmol L^{-1} of sodium chloride.

During these experiments, it was not necessary to clean the inlet system of the ES even though high salt contents were used. The separations were carried out in a commercially available FFFF system (F-1000, FFFractionation Inc.) equipped with modified polyethersulphone ultrafilter membrane, OMEGA,

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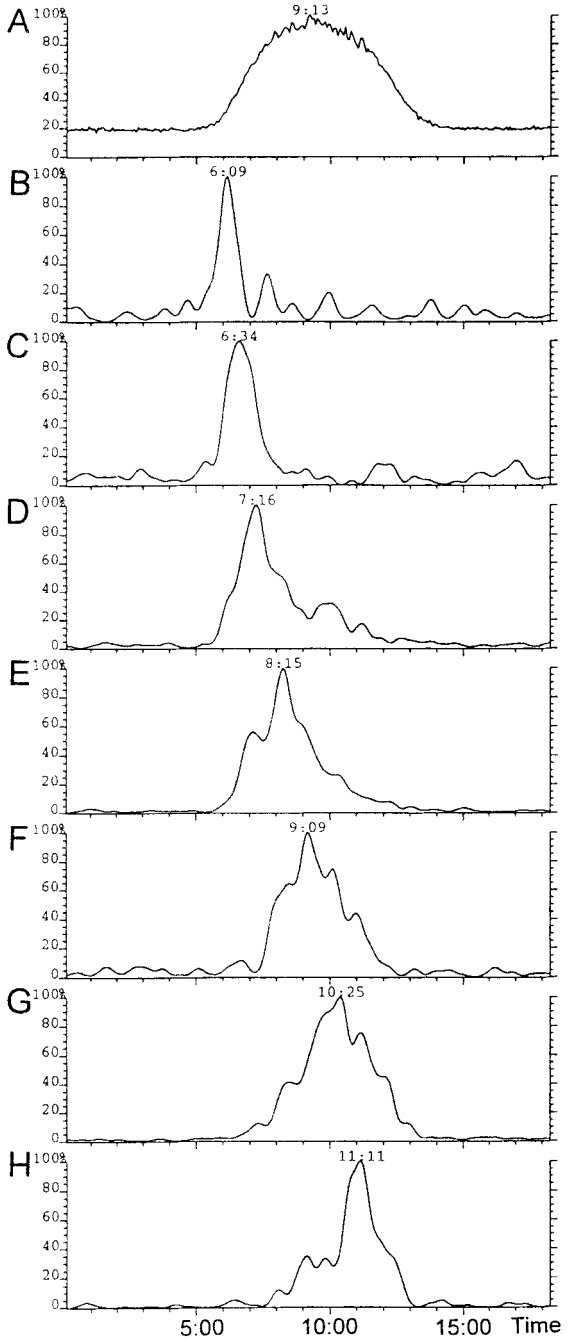
Figure 3 (left). Ion chromatogram and every third polymer in a separation of PEG 1500. Flows and injection volume were as in Figure 2. Total sample concentration was 200 g L^{-1}. Ion intensities in B, C, D corresponds to 1+ and 2+ charged complexes, E to 1+, 2+ and 3+ complexes and F and G to 2+ and 3+ complexes. Picture A shows Total Ion Current, B is mass 1204, C is 1334, D is 1422, E is 1553, F is 1729, G is 1906 and H is 1994.



(Filtron) with a nominal molecular weight cutoff at 1000 Da. This membrane has shown superior performance compared to regenerated cellulose membranes used for low molecular weight polymers, with respect to sample recovery.[10] The channel was cut out of a mylar spacer and the channel dimensions are 28 cm long tip to tip, 2 cm wide and 0.225 mm thick. The channel flow and crossflow were delivered by two HPLC-pumps (P-880, Jasco); channel flow rate was 0.4 mL min^{-1} and the crossflow rate 3.8 mL min^{-1}.

The samples were loaded onto the channel through a Rheodyne sample injection valve (20 μL) and the sample plug was relaxed at the channel inlet by the crossflow in a conventional stop-flow procedure by switching the computer controlled 6-way Valco valve (Figure 1). In order to minimise the formation of crystals and accumulation of solvents in the ES inlet system, the liquid flow was kept at about 140 μL min^{-1} by splitting the channel flow 1/3 using a T-union (Valco) and two pieces of 0.12 ID mm PEEK tubing.

The test polymers in the FFFF-ESMS system have been polyethylene glycol (PEG) standards. In order to save analysis time on the ESMS, poly(styrene)sulphonate (PSS) standards were used for separation optimisation due to higher UV absorptivity than the PEG standards. During these optimisations the PSS was detected using a UV-detector (Jasco UV-975) at 254 nm.

All reagents used, were of analytical grade, except ammonia, which was of supra pure grade. The carrier solution is defined by the pH buffer and ionic strength selected. The buffers tested were acetic acid (Merck)/sodium hydroxide (EKA Nobel) (pH 4.7), tris(hydroxymethyl)aminomethane (tris) (Merck) / hydrochloric acid (Merck)(50:50, pH 7.9), boric acid (Riedel-de Haën) / sodium hydroxide (pH 8.5), and ammonium/ammonia (Merck) (pH 9.3).

To increase the ionic strength, sodium chloride (Merck) or sodium nitrate (Merck) were used. The water used was Milli-Q water (Millipore) or Milli-Q water redistilled with sulphuric acid and potassium peroxydisulphate present during the distillation.

Figure 4 (left). Ion chromatogram and every third polymer in a separation of PEG 2000, 3000 and 4000. Flows and injection volume as in Figure 2. Total sample concentration 20 g L^{-1}. Ion intensities in B and C corresponds to 3+ complexes, D to 3+ and 4+, E and F to 4+ and G and H to 5+. Picture A shows Total Ion Current, B is mass 1778, C is 2218, D is 2658, E is 3098, F is 3758, G is 4418 and H is 4858.

Table 1

Test Results Determining a Suitable Carrier Solution for Both the FFFF Separation and the ES Interface

Carrier Solutions	FFFF	ES-MS
2 mmol L^{-1} tris and 1 mmol L^{-1} HCl	Poor separation	Minor $TrisH(TrisHCl)_n^+$ cluster formation
10 mmol L^{-1} Tris and 5 mmol L^{-1} HCl	Reasonable separation	Dominated by $TrisH(TrisHCl)_n^+$ clusters
10 mM NaCl	Reasonable separation	Dominated by $Na(NaCl)_n^+$ clusters
20 mmol L^{-1} HAc and 10 mmol L^{-1} NaOH	Good separation	Dominated by $Na(NaAc)_n^+$ clusters
10 mmol L^{-1} NH_3 and 5 mmol L^{-1} HNO3	——	Dominated by $NH_4(NH_4NO_3)_n^+$ clusters
20 mmol L^{-1} $B(OH)_3$ and 6 mmol L^{-1} NaOH	Good separation	Dominated by $Na(NaB(OH)_4)_n^+$ clusters
25 mmol L^{-1} tris, 20 mmol L^{-1} NaCl and 12 mmol L^{-1} HCl	Good separation (Fig. 2)	Minor cluster formation.

Polyethylene glycol (PEG) standards (PEG 1000, PEG 1500, PEG 2000, PEG 3000 and PEG 4000, where the number corresponds to the weight average molecular weight)(Fluka), and poly(styrene)sulphonate standards with weight average molecular weight of 1430, 16000 and 46000 Da (American Polymer Standards Corporation and Polymer Standards Service) were used. The malto-oligosaccharide std. was polydisperse maltodextrine, PZ9 (Reppe, Sweden).

RESULTS AND DISCUSSION

Electrospray in positive mode has been applied to all polymers used. For PSS, normally a negatively charged polymer, ES in negative mode were also tested. A major problem with ESMS at high ionic strength is the formation of matrix ion clusters or adducts[11] which give rise to a background in the mass spectrometer and, thus, further complicate the mass spectra. This can, to some extent, be avoided by minimising the amount of salt introduced to the electrospray; normally the ionic strength is kept below 1 mmol L^{-1}.

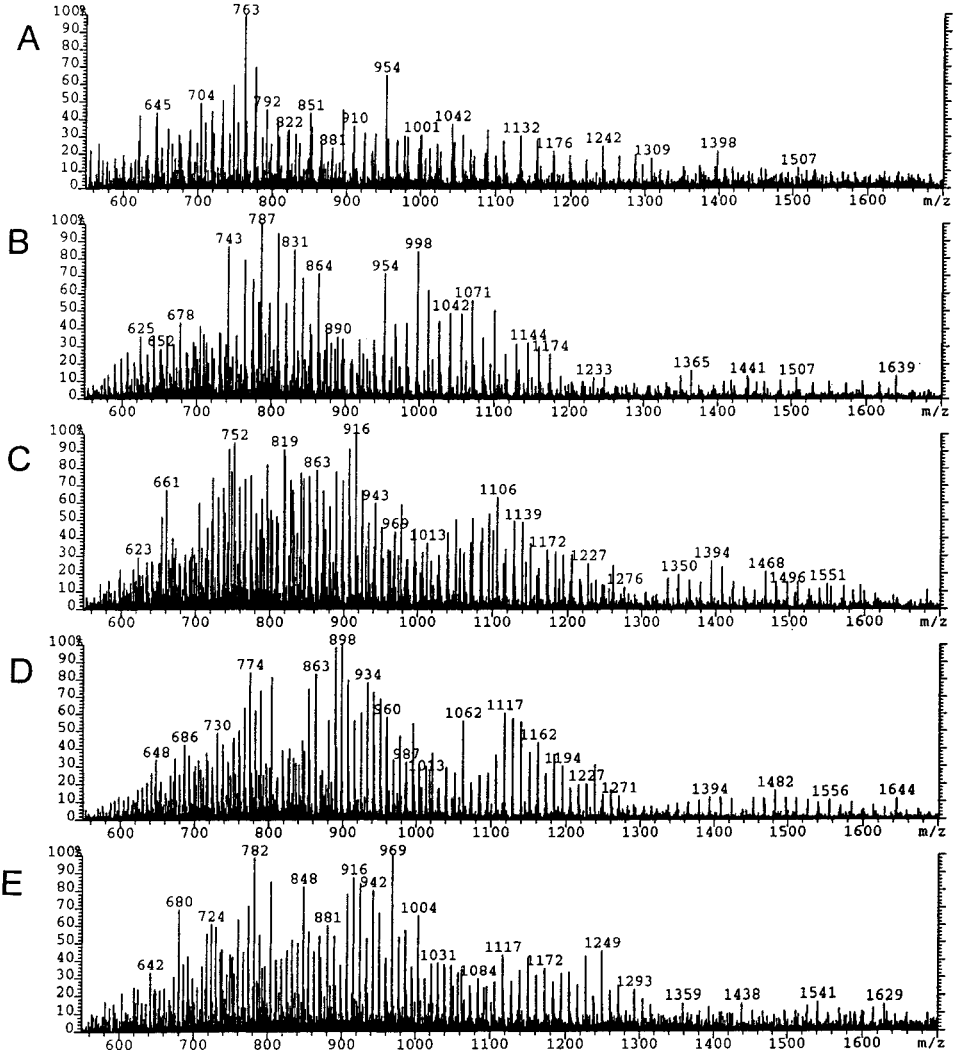


Figure 5. Mass spectra from selected parts of the separation in Figure 4. A corresponds to 5 min 55 s- 6 min 37 s. B to 7 min 27 s - 8 min 9 s, C to 8 min 59 s - 9 min 41 s, D to 10 min 31 s - 11 min 13 s and E to 12 min 3 s - 12 min 45 s.

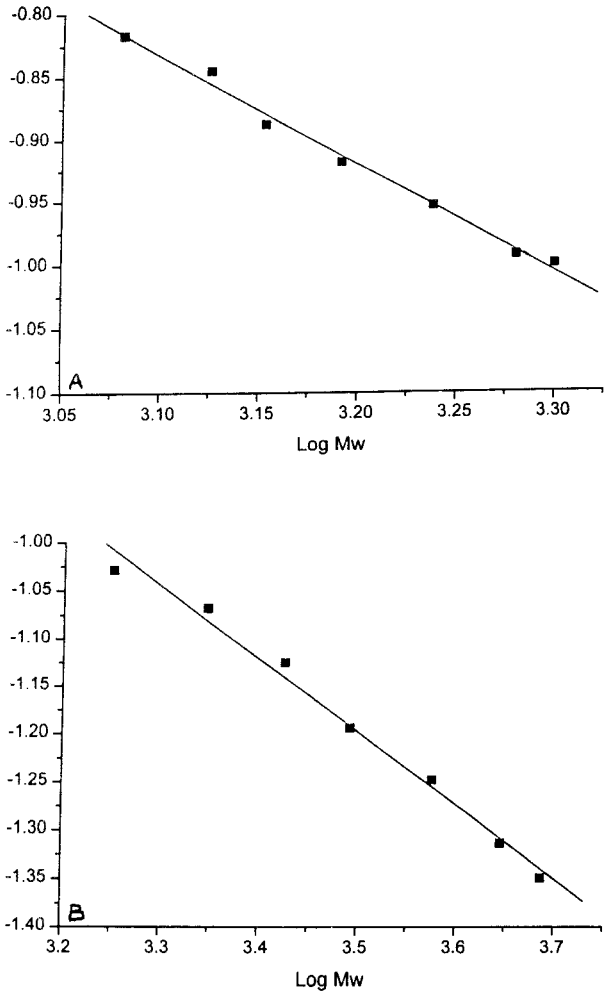


Figure 6. The plots show log λ versus log molecular weight, where λ is calculated as in Fig. 2. Figure 6A corresponds to the chromatogram in Figure 3 and Figure 6B corresponds to Figure 4.

Carrier liquids have been optimised to reconcile the demands of Flow FFF and ESMS with respect to ionic strength and ion composition. Effort was put into minimising the salt cluster background instead of just lowering the amount of salt present. It was found that cluster formation and, thus, the background,

were significantly reduced by tuning the skimmer cone voltage, to maximise the signal to noise ratio. It was also seen that the composition of the carrier liquid greatly influenced the formation of salt clusters.

Different buffering systems with pH from 4.7 to 9.3 were tested with respect to separation efficiency of PSS standards (Table 1). The pH did not have a significant effect on the separation efficiency, while ionic strength, on the other hand, has shown to be a very critical parameter. The apparent pH independence could be explained by the facts that both the PSS standards and the sulphonate groups on the OMEGA membrane have a constant negative charge in the pH range examined.

The separation of PSS standards collapsed when the ionic strength fell below a few millimoles per litre, and optimal separation is not achieved below approximately 20 mmol L^{-1}. An explanation for this behaviour can be that, for very low ionic strength, the negative charge on both the sample and the membrane exerts repulsive electrostatic forces which hinders the sample to come close enough to the accumulation wall but, when ionic strength increases, the electrostatic force is shielded and the sample clouds could establish at different distances from the wall. Another explanation for this ionic strength dependence could be conformational changes of the polymer for low ionic strength, which is stabilised in higher ionic strength.

Figure 2 shows a typical separation of PSS standards with corresponding molecular weight calibration data as logarithm of the retention parameter λ versus the logarithm of the molecular weight where λ is calculated from $t_0/t_r = 6\lambda[\coth 1/2\lambda - 2\lambda]$. Good FFFF separation was achieved with all of the higher ionic strength carrier solutions. On the other hand, ESMS worked well only for lower ionic strengths and for high ionic strength solution of tris, hydrochloric acid, and sodium chloride.

The latter mixture gave the lowest background, in combination with good separation efficiency. A probable explanation of this is that the lifetime of, e.g., a pure $Na(NaCl)_n^+$ cluster is long enough to travel through the MS (slightly less than 1 μs) while this is not the case for a mixed cluster. In order to further reduce the background, some hydrochloric acid was replaced with nitric acid and sulphuric acid in the above carrier solution, without any further improvement seen. The signal to noise ratio was studied by direct injection of PSS, PEG and malto-oligosaccharides dissolved in the different carriers. PSS gave low sensitivity both in negative and positive electrospray.

The malto-oligosaccharides were easily detected in ESMS[11,12], at the lower ionic strength, but turned out to exchange protons to sodium at sodium concentrations higher than 5mM. Because the intensities were shared by anumber of masses, the sensitivity decreases and the mass spectrum becomes complex. If the sodium concentration in the solution was decreased too much, clusters of tris started to become a problem. For this reason, the malto-oligosaccharides samples became difficult for FFFF-ESMS. PEG run on ESMS with tris/sodium chloride solution gave mainly sodium adducts with higher amounts of sodium attached to the larger PEG. The number of added sodium ions is highly dependent on the chain length, giving charges ranging from +1 to +6, and each molecule gave, normally, three peaks in the mass spectrum corresponding to three different charges (Figure 5).

When injecting PEG 1000 into the FFF channel, it was found that almost all of the sample passed through the membrane. The smallest PEG that could be separated and detected was around 1200 Da.

The membrane seems to have a somewhat higher molecular weight cutoff for PEG than for PSS for the same experimental setup. This could be due to the fact that PEG is neutral and does not experience the same electrostatic repulsion from the negative membrane as the negatively charged PSS.

Figure 3 shows the separation of PEG 1500, including seven of its components and the total ion current (TIC). The calculated number of theoretical plates, N, ranged between 60 and 215 with an average of 110. The FFFF peaks for different masses in the mass chromatogram are not baseline separated, but the centre points of the area for each mass are.

Figure 6A shows the logarithm of the retention parameter λ versus the logarithm of the molecular weight, which gives a linear fit ($r = 0.996$) showing good agreement between diffusion coefficient and molecular weight for the PEG chosen. It is, therefore, possible to obtain a calibration curve with a large number of points in just one run which could be useful in the study of the relationship between molecular weight and diffusion coefficient for different samples, in order to find relevant standards.

A separation of a mixture of PEG 2000, PEG 3000 and PEG 4000 is presented in Figure 4. In the mixture, all chain lengths from 40 to 115 (ca 1800-5100 Da) could be seen. There is no baseline separation of the chosen compounds but, when plotting the logarithm of the retention parameter λ versus the logarithm of the molecular weight, a linear fit with a good correlation ($r = 0.994$) is achieved (Figure 6B). Flow FFF simplifies the mass

spectra in terms of differently charged species (Figure 5). When comparing standards with samples of known molecular weight, it is possible to get a picture of three dimensional conformation or diffusion coefficients versus molecular weight. The calculated number of theoretical plates for the separation made with PEG 2000, PEG 3000 and PEG 4000 (Figure 4) ranged between 100 and 320 with an average of 176.

A comparison of the mass chromatograms on certain PEG components (Figure 3 and 4) with the TIC, which is the sum of all mass chromatograms, shows the benefit of the mass selective detection system. A comparative study as the slope of log retention ratio vs. log Mw for PSS and PEG (0.41 and 0.67 respectively) suggests a smaller effect of diffusion coefficient/molecular weight for PEG which likely is due to weaker van der Waal forces within the PSS than in the PEG or higher electrostatic repulsion within the polymer coils of PSS than for PEG.

To further reduce the background, which in this study, made the detection limits quite high, would be a major field of interest. As the sensitivity for many naturally uncharged compounds increases with sodium ion concentration, ESMS has the potential to be a sensitive detector if the carrier solution could be kept reasonably free from contaminants. A possible way to achieve this could be to recrystallise the buffer salts and purify the carrier solution by passage through a column, eg. C_{18}, before entering the channel.

CONCLUSIONS

We have presented a relatively simple method for using ES-MS as an online detector for flow FFF. Even though high electrolyte concentrations are used in the carrier solution, the ES system seems to be robust and can work for a longer time.

Because of the good correlation between log retention parameter and log molecular weight of the PEG components, the method can be useful for conformational or molecular weight distribution studies. To achieve better detection limits, more work with purifying the carrier solutions should be done.

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