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# Salt effect on size-exclusion chromatography of partially sulfonated alternating copolymers of maleic acid and styrene in a polar solvent<sup>☆</sup>

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## Abstract

The molecular mass distributions of poly[(maleic acid)-alt-styrene] (SMA) and poly[(maleic acid)-alt-(*p*-sulfostyrene, styrene)] (SSMA) were measured by size-exclusion chromatography (SEC) in *N,N*-dimethylformamide (DMF) and in DMF containing different amounts of LiBr. The degree of sulfonation of SSMA varies from 18.3 mol% (SSMA I), to 44.8 mol% (SSMA II) and to 83 mol% (SSMA III). Measurements were made at 40°C using a  $\mu$ Bondagel set of columns. SEC measurements were followed by viscosity measurements at 25°C in the same media and by light scattering measurements only for the SSMA I sample. The concentrations of LiBr added to DMF were 0.025, 0.075 and 0.10 *M*, at which concentrations of counter ions the viscosity effect of polyelectrolyte was suppressed in all samples. The addition of LiBr to DMF shifted the chromatograms towards longer elution volumes (lower molecular mass); however, this shift was not significant in the case of the SSMA III sample, although the concentration of LiBr was 0.1 *M*. With the highest concentration of LiBr there was a problem with solubility of the sample. The  $M_w$  values vary from  $24.1 \cdot 10^6$  (SSMA III in DMF) to  $5.7 \cdot 10^4$  (SSMA I in DMF–0.10 *M* LiBr). Light-scattering data showed that SEC measurements using the polystyrene calibration graph afford only relative values for the molecular mass distribution.

## 1. Introduction

The structure and consequently the solution properties of polyelectrolytes in a polar medium with and without added salt are radically different. Coulombic interactions between charges fixed along the macromolecule backbone or side

groups are responsible for the most unusual properties of polyelectrolytes, and these interactions are very sensitive to the presence of counter ions in solution. The presence of small electrolytes in polar media, which determines the solution ionic strength, screens out charges and the repulsive interactions between identical charges along the chain. The competition between repulsive forces along the fixed charges on the chain (or side group), and the screening effects brought about by the excess of counter ions (from the added salt) are manifested in a complex dependence of solution properties on

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these parameters (charge density and counter ion excess).

One of the most commonly employed solvents for studying the solution properties of polar polymers and polyelectrolytes is N,N-dimethylformamide (DMF) [1–4], and this solvent has found widespread application in the size-exclusion chromatographic (SEC) analysis of polyelectrolytes. The existence of polar groups in a polymer coil causes some specific problems in interpreting the results of SEC because, in addition to size exclusion, some additional mechanisms might be involved. As SEC separation is greatly dependent on solution properties, such as hydrodynamic volume or intrinsic viscosity, a thorough understanding of how the charge of the polymer and the polarity of the solvent influence these parameters is of utmost importance. According to the aforementioned, it is difficult to believe that the universal approach of Benoit et al. [5] is valid for solutions of polyelectrolytes, and comparison of molecular mass data obtained by methods, e.g., light scattering (LS) is necessary.

Many small electrolytes act as “non-solvents” when added to aqueous polar solutions of polyelectrolytes. Increasing amounts of these electrolytes tend to diminish the solvent quality of the media until finally, at some critical concentration of salt, phase separation occurs. The 1:1 electrolytes are generally the least effective of the simple salts for promoting phase separation in polyelectrolyte solutions. The tendency of low-molecular-mass electrolytes to salt out polyelectrolytes from polar media is probably a consequence of both an ionic atmosphere and site-binding phenomena [6].

In a second paper [7] we dealt with solutions of non-sulfonated poly[(maleic acid-alt-styrene)] (SMA) and sulfonated poly[(maleic acid)-alt-(p-sulfostyrene, styrene)] (SSMA) copolymers in DMF and DMF–0.05 M LiBr as media. The conclusion was that SMA was a weak polyelectrolyte and SSMA behaved as a strong polyelectrolyte. The concentration of added LiBr (0.05M) was not sufficient to suppress completely the additional separation mechanisms for SEC of polyelectrolytes.

In this work, we extended the concentration range of LiBr from 0.025 to 0.10 M, i.e., from low to moderate concentrations [8].

## 2. Experimental

### 2.1. Materials, intrinsic viscosity and high-performance SEC (HPSEC)

The preparation of samples, intrinsic viscosity measurements and HPSEC measurements have been described elsewhere [7].

The concentration range of copolymers for all viscosity measurements was 0.15–0.5 g/100 ml, but in the media containing 0.05 M LiBr the copolymer concentration ranged from 0.3 to 0.65 g/100 ml.

### 2.2. Light scattering

The average molecular mass,  $M_w$ , of copolymers was determined using a KMX-6 laser low-angle light-scattering photometer (LLALS) (Chromatix, Mountain Valley, CA, USA) [9] with a helium–neon laser as a light source ( $\lambda = 633$  nm) and a standard static 15-mm measuring cell. All measurements were made at room temperature and the measuring angle was 6–7° with a field stop of 0.2 mm. Copolymer solutions, usually of five different concentrations, ranging from 1 to 5 mg/ml, were filtered before use through 0.5- $\mu$ m membrane filters (Millipore, Bedford, MA, USA).

### 2.3. Differential refractometry

For the measurements of specific refractive index increment (SRII), a Brice-Phoenix differential refractometer was used. Measurements were performed at wavelengths of 436 and 546 nm at 25°C. The differential refractometer was calibrated with sodium chloride solutions of known concentration and refractive index [10]. Sample solutions, usually at five different concentrations ranging from 2 to 10 mg/ml, were filtered before use through 1- $\mu$ m Millipore filters.

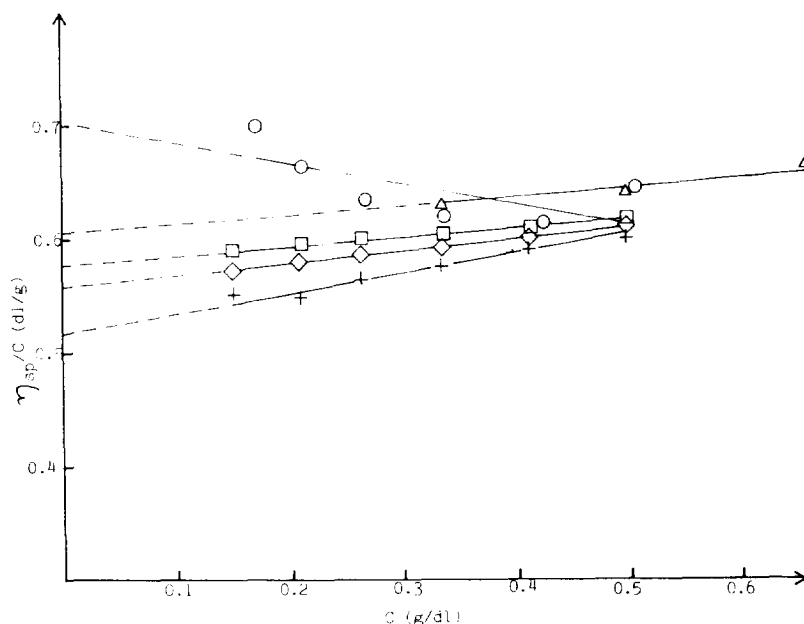


Fig. 1. Reduced viscosity vs. concentration of SMA copolymer in (○) DMF, (+) DMF–0.025 M LiBr, (△) DMF–0.05 M LiBr, (◇) DMF–0.075 M LiBr and (□) DMF–0.1 M LiBr.

### 3. Results and discussion

In a second study [7] we observed significant differences in intrinsic viscosity and molecular mass data for SMA and SSMA copolymers in DMF and DMF–0.05 M LiBr. Therefore, in this work we extended the measurements in the media with LiBr concentrations from 0.025 to 0.1 M.

Fig. 1 shows the results for the reduced vis-

cosity of SMA in DMF and DMF containing various amounts of LiBr. As can be seen, a concentration of 0.025 M LiBr suppressed the weak polyelectrolyte effect observed in salt-free DMF. In DMF of higher ionic strength (0.075 and 0.10 M LiBr concentrations) the reduced viscosity was higher than in DMF containing 0.025 M LiBr, which cannot be explained. Some discrepancies in the case of a 0.05 M LiBr concentration could be ascribed to small differ-

Table 1  
Dependence of intrinsic viscosity data on the degree of sulfonation and the concentration of LiBr added to DMF

Degree of sulfonation (mol-%)	LiBr added (M)				
	0	0.025	0.05	0.075	0.1
0 (pure SMA)	0.701	0.526	0.608	0.557	0.578
18	1.408	0.539	0.621	0.490	0.501
44.8	2.439	0.688	0.640	0.573	
83	3.246	0.888	0.812	0.682	0.584

ences in the concentration range of the copolymer in solution. The intrinsic viscosity calculated by first-order linear regression analysis is presented in Table 1 for all concentrations of added LiBr and for the salt-free DMF.

Fig. 2 shows the reduced viscosities for SSMA I (degree of sulfonation 18.3 mol-%) in salt-free

DMF and in DMF containing various concentrations of LiBr. A significant difference between salt-free DMF and DMF with the smallest amount of added LiBr is evident. The reduced viscosity of SSMA I in 0.025 M LiBr is close to that of SMA. Moreover, with a high concentration of LiBr (0.075 and 0.10 M), the reduced

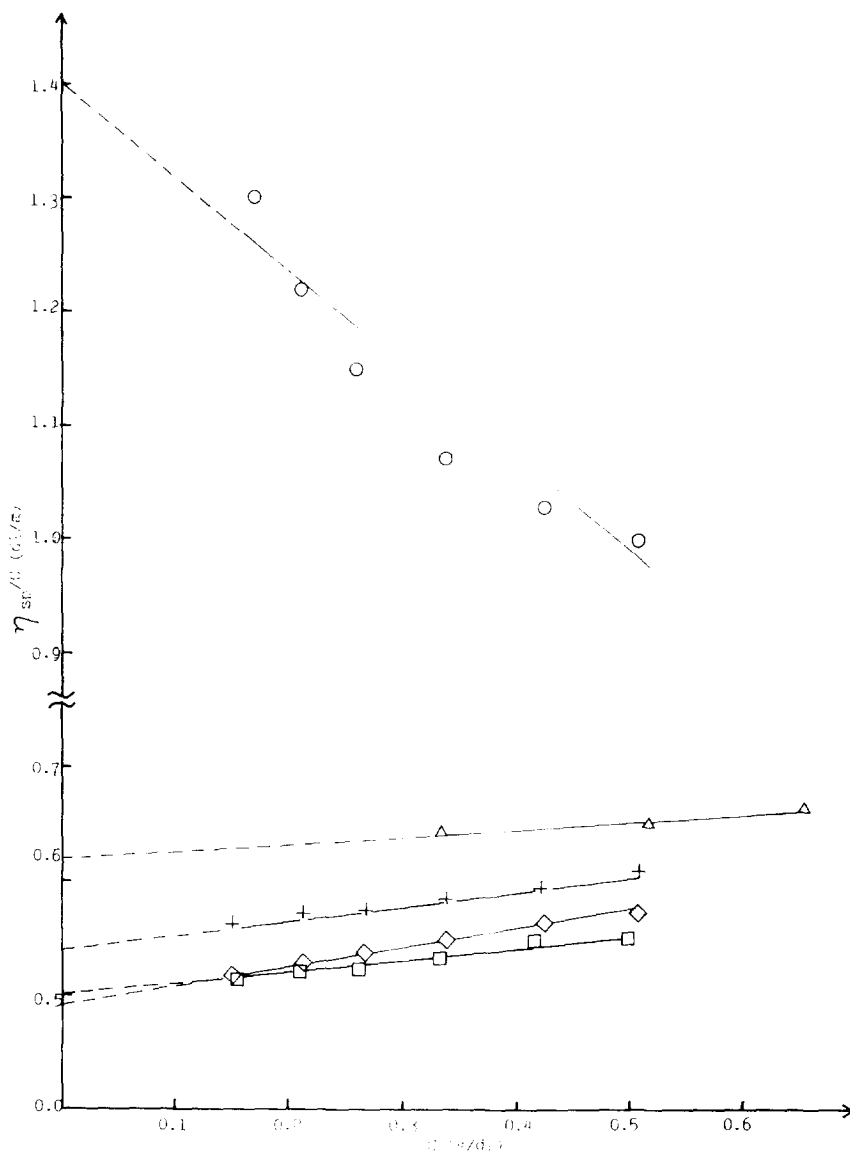


Fig. 2. Reduced viscosity vs. concentration of SSMA I copolymer in (○) DMF, (+) DMF-0.025 M LiBr, (△) DMF-0.05 M LiBr, (◇) DMF-0.075 M LiBr and (□) DMF-0.1 M LiBr.

viscosity is actually slightly lower than in the case of SMA. The calculated  $[\eta]$  values of SSMA I are presented in Table 1.

Fig. 3 illustrates the reduced viscosities of the SSMA II sample with a moderate degree of sulfonation (44.8 mol-%) in the same media. A significant difference is observed between the reduced viscosity in DMF and in LiBr-containing media. With the lowest concentration of LiBr, the reduced viscosity of SSMA II is slightly higher in comparison with that of SMA, but with a higher concentration of added LiBr (0.075 M), the reduced viscosities are much closer to each other. The calculated intrinsic viscosity (Table 1) confirms the aforementioned results.

Reduced viscosities of SSMA III (degree of sulfonation 83 mol-%) are presented in Fig. 4. Differences between the reduced viscosity in salt-free DMF and in DMF containing LiBr are

much more evident. Addition of 0.025 M LiBr to DMF significantly reduces the viscosity, but the value is still higher in comparison with SMA. Neither a concentration of 0.05 nor 0.075 M LiBr in DMF decreases the viscosity to that of SMA.

Only with the solution of DMF containing 0.1 M LiBr does the value of the reduced viscosity of SSMA III approach that of SMA. Corresponding  $[\eta]$  values are also shown in Table 1. Fig. 5 presents the size-exclusion chromatograms for three sulfonated copolymers (SSMA I, II, and III) in the measured media. The chromatograms of SSMA I and II are almost the same with DMF–0.025 M LiBr (Fig. 5a), but the chromatogram of SSMA III is still shifted toward lower elution volumes. Calculated values of  $M_w$  and  $M_n$  are given in Table 2.

The polystyrene calibration graph was used for

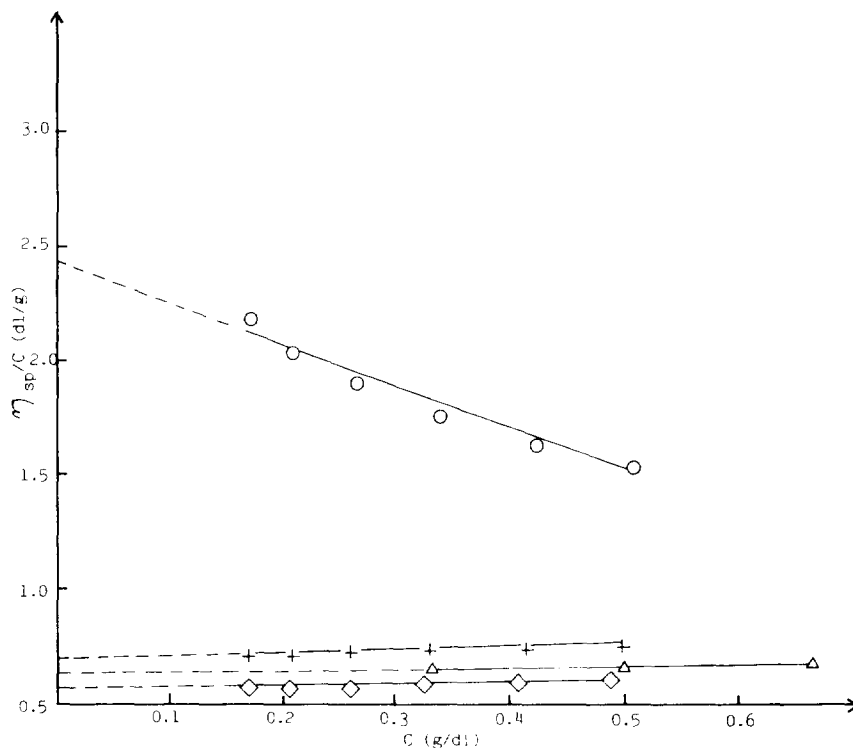


Fig. 3. Reduced viscosity vs. concentration of SSMA II copolymer in (○) DMF, (+) DMF–0.025 M LiBr, (△) DMF–0.05 M LiBr, and (◇) DMF–0.075 M LiBr.

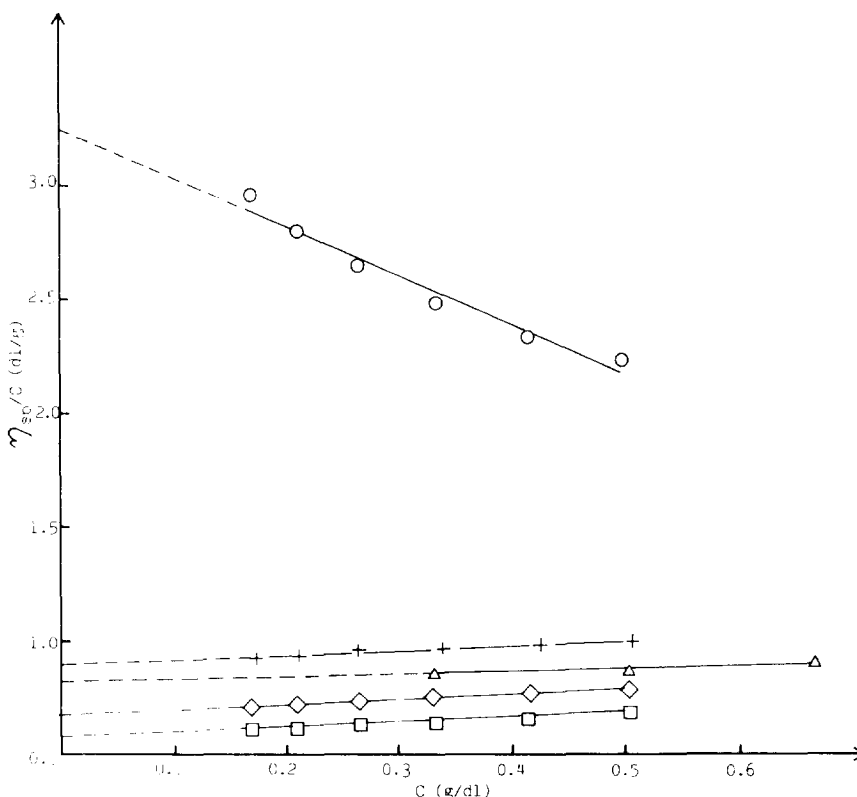


Fig. 4. Reduced viscosity vs. concentration of SSMA III copolymer in (○) DMF, (+) DMF–0.025 M LiBr, (Δ) DMF–0.05 M LiBr, (◇) DMF–0.075 M LiBr and (□) DMF–0.1 M LiBr.

calculation of molecular mass distribution data and  $M_w$  and  $M_n$  values. Fig. 5b shows chromatograms of copolymers with DMF–0.075 M LiBr eluent. SSMA I and II again show very close results and almost the same as for SMA with DMF–0.05 M LiBr eluent [7]. Earlier elution for SSMA III is still evident. The molecular mass data (Table 2) confirm the aforementioned. With DMF–0.10 M LiBr eluent (Fig. 5c), all the chromatograms are more similar to each other and at the same time to the chromatogram of SMA with, DMF–0.05 M LiBr [7]. The calculated molecular mass data for SSMA I and II are lower than those for SMA. Only the molecular mass for SSMA III is higher than that for SMA, but this difference is smaller than that in DMF with a lower content of LiBr. Comparing Fig. 5a, b and c, it is evident that the detector response

decreases with increasing amount of LiBr in DMF and with increasing degree of sulfonation.

During the preparation of solutions in DMF–0.10 M LiBr, a problem occurred in filtration through a 0.5- $\mu$ m filter, and the baseline stability during SEC analyses was worse in comparison with DMF solutions containing lower concentrations of LiBr. This was the reason why we did not try to perform measurements with a higher concentration of LiBr to check the behaviour of SSMA III. This might mean that the addition of higher amounts of LiBr diminishes the solvent power of DMF, and causes the precipitation of part of the solute in DMF–0.10 M LiBr or in solutions with a higher content of LiBr. To check the correct molecular mass for all samples we performed light-scattering measurements. Table 3 gives  $M_w$  data and refractive index increment

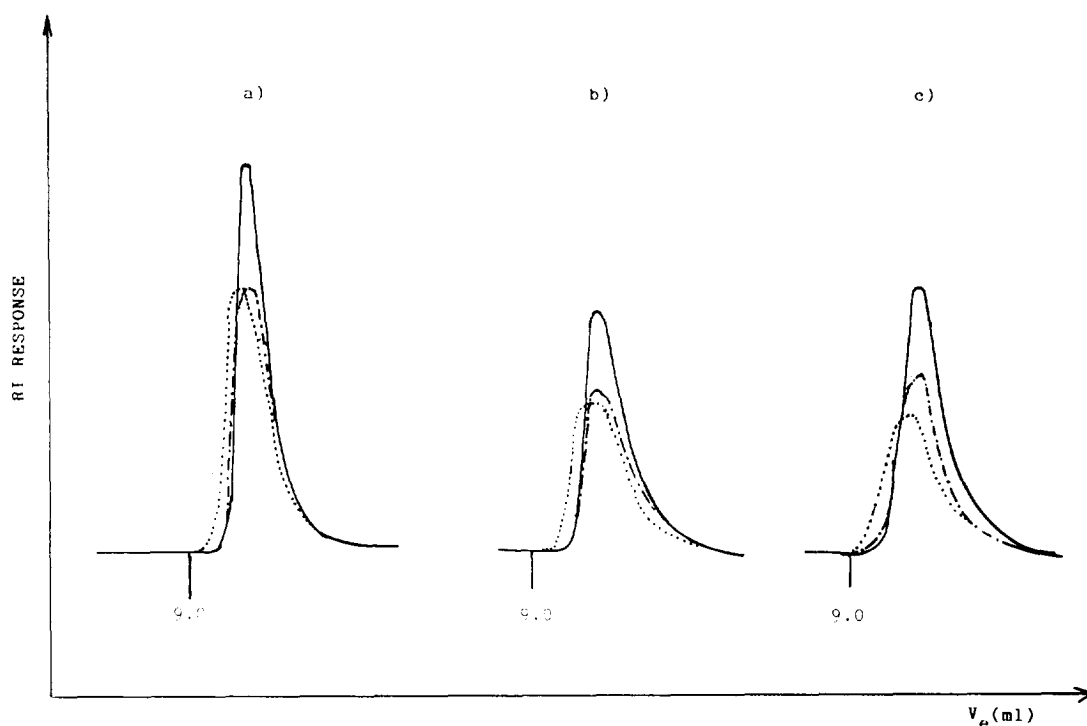


Fig. 5. HPSEC on  $\mu$ Bondagel column set (E-high and two E-linear) at 40°C and a flow-rate of 0.5 ml/min in (a) DMF–0.025 M LiBr (b) DMF–0.075 M LiBr and (c) DMF–0.1 M LiBr for (—) SSMA I, (---) SSMA II and (····) SSMA III.

data,  $dn/dc$ , for SSMA I in all four copolymer solutions of different ionic strength. For comparison,  $M_w$  and  $dn/dc$  for SMA in DMF were  $1.62 \cdot 10^5$  and 0.124 ml/g, respectively.

The measured  $M_w$  values are “apparent” because the refractive index increment,  $dn/dc$ , for a multi-component system is complex and

comprises all components in solution [11]. These effects may be more important for a higher concentration of salt ( $>0.5 M$ ). Because of the relatively modest salt concentration ( $<0.10 M$ ), the effects of measured parameters may be neglected and no measurements of  $dn/dc$  at a constant chemical potential were performed,

Table 2  
Dependence of  $M_w$  and  $M_n$ , determined by HPSEC, on the degree of sulfonation and concentration of LiBr added to DMF

Degree of sulfonation (mol-%)	LiBr added (M)									
	0		0.025		0.05		0.075		0.1	
	$M_w$	$M_n$	$M_w$	$M_n$	$M_w$	$M_n$	$M_w$	$M_n$	$M_w$	$M_n$
0 (pure SMA)	$4.06 \cdot 10^6$	$0.49 \cdot 10^6$			$7.33 \cdot 10^4$	$3.03 \cdot 10^4$				
18	$11.1 \cdot 10^6$	$1.21 \cdot 10^6$	$1.12 \cdot 10^5$	$5.54 \cdot 10^4$	$7.2 \cdot 10^4$	$3.67 \cdot 10^4$	$5.86 \cdot 10^4$	$3.25 \cdot 10^4$	$5.67 \cdot 10^4$	$3.03 \cdot 10^4$
44.8	$23.1 \cdot 10^6$	$1.38 \cdot 10^6$	$1.08 \cdot 10^5$	$5.47 \cdot 10^4$	$1.03 \cdot 10^5$	$5.16 \cdot 10^4$	$7.27 \cdot 10^4$	$3.42 \cdot 10^4$	$6.24 \cdot 10^4$	$3.25 \cdot 10^4$
83	$24.1 \cdot 10^6$	$1.25 \cdot 10^6$	$3.04 \cdot 10^5$	$7.81 \cdot 10^4$	$2.30 \cdot 10^5$	$4.72 \cdot 10^4$	$1.71 \cdot 10^4$	$4.72 \cdot 10^4$	$1.37 \cdot 10^4$	$4.36 \cdot 10^4$

Table 3  
 $M_w$  determined by LLALS for SSMA I in different media

Concentration of LiBr added ( $M$ )	$M_w$	$dn/dc$ (ml/g)
0.025	$1.82 \cdot 10^5$	0.122
0.05	$2.08 \cdot 10^5$	0.116
0.075	$2.28 \cdot 10^5$	0.115
0.1	$2.54 \cdot 10^5$	0.111

$$A_2 = (5.0-4.7) \cdot 10^{-4} \text{ mol ml/g}^2$$

even though we observed a small decrease in  $dn/dc$  values on increasing the LiBr concentration (Table 3). At the same time, there is a small increase in  $M_w$  with increased concentration of LiBr. The second virial coefficients decrease slightly with increasing concentration of LiBr ( $A_2 = 5.0 \cdot 10^{-4} \text{ mol ml/g}^2$  at 0.025  $M$  and  $4.75 \cdot 10^{-4} \text{ mol ml/g}^2$  at 0.10  $M$  LiBr), indicating again a decrease in solvent quality. Light-scattering data showed that the calculated  $M_w$  and  $M_n$  values from SEC measurements using the polystyrene calibration graph have only a relative meaning. This should be taken into account for the absolute molecular mass distribution.

#### 4. Conclusion

The results obtained by SEC measurements in solutions of different ionic strength have shown that in polar media SSMA samples behave as strong polyelectrolytes. Introduction of a sulfo group in the *para* position of the styrene unit of copolymers is responsible for a strong polyelectrolyte effect in comparison with a weak polyelectrolyte in the case of the non-sulfonated copolymer SMA. The polyelectrolyte effect depends on the degree of sulfonation. In the case of a copolymer with a low degree of sulfonation (SSMA I, 18.3 mol-%), a low concentration of LiBr is almost enough to screen out the charge and suppress the expansion of macromolecular coils caused by intramolecular repulsive forces. The effect of a higher degree of sulfonation

could be eliminated by increasing the concentration of LiBr (0.075 or 0.10  $M$ ). Only for the copolymer with the highest degree of sulfonation (SSMA III, 83 mol-%) is the concentration of 0.10  $M$  insufficient for the complete suppression of macromolecular expansion, but at this concentration the solvent quality decreases and copolymer becomes partially insoluble. SEC measurements in the mentioned eluent using the polystyrene calibration graph gave only relative values for molecular masses. Comparing the viscosity data and SEC data there is some doubt whether the separation process in SEC measurements is based only on size or if some additional processes are involved (ion inclusion and ion exclusion).

All the results observed have shown that SEC measurements are affected by the polarity and ion strength of the eluent and by the degree of sulfonation, i.e., charge density of copolymers.

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