

# Size Exclusion Chromatography of Polyelectrolytes in Dimethylformamide

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## Synopsis

A study of the size exclusion chromatographic behavior of styrene-methacrylic acid and epoxy-acrylic polymers has been made. These polymers exhibit strong "ion-exclusion" effects in DMF, similar to that found for water-soluble polyelectrolytes such as sodium polystyrene sulfonate. The addition of lithium bromide, at a concentration of 0.1M, overcomes ion exclusion on a set of silica-based, "deactivated" duPont SEC columns. Calibration of both the duPont columns and a set of styrene-divinyl benzene based columns ( $\mu$ -Styragel) is complicated by absorption of polystyrene standards in DMF and DMF/salt mobile phases. The absorption of monomeric solutes on the duPont columns in DMF/salt mobile phases is different from that of their corresponding polymers.

## INTRODUCTION

Development of so-called "water-dispersible" forms of polymers used in the coatings industry has been underway for some time now. Government regulations limiting emissions of volatile organic solvents from industrial users of coatings, and the steady price increases of these solvents continue to generate interest in such polymers. A wide range of polymers employed as solutions in the past have been modified to provide water dispersibility, including alkyd, acrylics, epoxy, polyester, and urethane polymers. Modification to achieve water dispersibility is usually through the incorporation of ionizable groups such as carboxylic acid or amino moieties. Neutralization of these groups with an appropriate counterion provides enough hydrophilic character to the polymer that self-stabilized colloidal dispersions or emulsions are formed in water or water-glycol ether mixtures.

Size exclusion chromatography (SEC) has been a valuable tool for the characterization of the molecular weight distributions of polymers of interest to the coatings industry since its development.<sup>1</sup> Recent advances in column technology and data reduction have made it possible to obtain the molecular weight distribution of uncharged polymers in organic solvents, particularly tetrahydrofuran, in 15-30 min. The application of modern SEC analysis to water-soluble or -dispersible resins is considerably more complex, however, and is the subject of this report.

The ionic strength of the solvent affects the hydrodynamic volume of polyelectrolytes in polar solvents, and thus the chromatographic behavior in SEC.<sup>2-4</sup> In addition to coil expansion effects in solvents of low ionic strengths, ion exclusion of polyions from the pores of a similarly charged SEC column can distort

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chromatograms. Direct interaction through absorption of polymeric solutes onto column packing surfaces can also lead to mixed mechanism separation, which are undesirable if calculation of average molecular weights is needed.

This study summarizes observations of and approaches needed to overcome polyelectrolyte effects in the SEC of acrylic-modified epoxy resins. These polymers are of the water-dispersible type having pendant carboxylate groups but little true solubility in water. They are only partially soluble in tetrahydrofuran, although the base epoxy resin and the carboxyl functional acrylic (in its acid form) are completely soluble. These polymers are soluble (whether ionized or not) in dimethylformamide (DMF), and show chromatographic behavior like aqueous solutions of polyelectrolytes. Domard et al. also studied DMF-soluble polyelectrolytes.<sup>5</sup>

## EXPERIMENTAL

Size exclusion chromatography was performed with a Waters Model 244 liquid chromatograph (Waters Associates, Milford, MA) equipped with a fixed wavelength ultraviolet detector and a differential refractometer. A Waters Data Module was used to determine retention times of standards and for SEC data reduction. Corrections to the average molecular weights calculated for instrumental band broadening were not applied.

Tetrahydrofuran was chromatography grade, unstabilized, from MCB Omnisolve (Cincinnati, OH). Dimethyl formamide also was Omnisolve grade. Solvents were used, without further purification, after filtration through a 0.45  $\mu\text{m}$  Fluoropore filter (Millipore Corp., Bedford, MA). Lithium bromide was from Aldrich Chemical (Milwaukee, WI).

Two types of SEC columns were investigated.

The primary goal of this study was to provide rapid size exclusion chromatograms for polymer processing studies. For this reason, rigid particle silica based SEC packings from duPont (Instruments Division, Wilmington, DE) were chosen. One each of a duPont PSM 1000s, PSM 60s, and SE4000, all 7.8 mm  $\times$  25 cm. prepacked columns, made up the column bank used for most of the experiments described herein. The 1000S and 60S columns are "bimodal" types, containing a mixture of pore sizes.<sup>6,7</sup>

These packings are described as deactivated by the supplier. This means that an attempt to minimize the number of surface silanol groups on the packing through reaction with proprietary organic modifier has been made. The 4000 column is a bare silica type which was used to increase resolution of very high molecular weight materials ( $>10^7$  exclusion limit relative to polystyrene standards). Flow rate of the mobile phase was 1.0 mL/min.

Some experiments were also performed with a series of  $\mu$ -Styragel columns (Waters Associates). One column each (7.8 mm  $\times$  30 cm) containing packings with the following pore sizes comprised this column set:  $10^6$ ,  $10^5$ ,  $10^4$ ,  $10^3$  Å. The flow rate with this column set was 1.5 mL/min.

Polystyrene standards of narrow dispersity were obtained from Polysciences, Inc. (Warrington, PA).

The water-dispersible polymers investigated were part of a series of model polymers prepared in a study of effects of processing on certain rheological parameters. The styrene-methacrylic acid copolymers were made using azoiso-

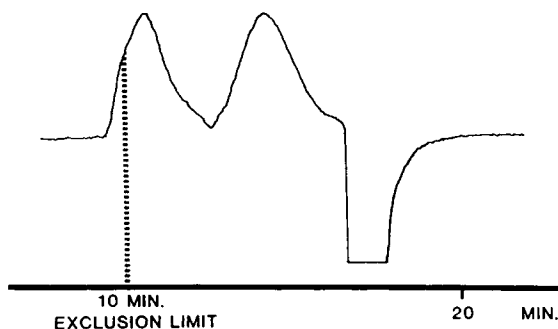


Fig.1. Chromatogram of epoxy-acrylic polymer obtained on the duPont column set. The mobile phase is neat dimethylformamide.

butyronitrile catalyst. Reaction of some fraction of the pendant carboxyl groups with the oxirane groups of bisphenol A epichlorohydrin epoxy resins of various molecular weights provided the acrylic-epoxy polymers studied.

## RESULTS AND DISCUSSION

Figure 1 illustrates the chromatogram of an epoxy-acrylic polymer obtained in DMF with the duPont column set. A significant fraction of this polymer is eluted at the exclusion limit of the column set. This behavior is typical of a polyelectrolyte in a solvent of low ionic strength. Electrostatic repulsion of a charged polyion from similarly charged column packing, the ion exclusion effect, and coil expansion are responsible for earlier elution and thus apparently higher molecular weight.<sup>4,8</sup>

Addition of an electrolyte to the mobile phase is necessary to prevent ion exclusion, whether the mobile phase is water or DMF.<sup>5</sup> In aqueous SEC there is a wide range of salts that can be used as mobile phase electrolytes: phosphates,<sup>3</sup> sodium chloride, sodium hydroxide,<sup>9</sup> and acetates<sup>10</sup> have been used. The use of DMF as the mobile phase restricts the choice of salt somewhat. Sodium nitrate and lithium bromide are common salts which have sufficient solubility in DMF for use as modifiers. Lithium bromide, at concentrations from 0.0065*M* to 0.5*M*, was used in this study as an electrolyte in DMF.

Figure 2 shows the effect of various lithium bromide concentrations on the chromatogram of the same epoxy-acrylic polymer. The excluded portion of the polymer begins to penetrate the pores of the packing with 0.0125*M* lithium bromide added. The retention time of the distribution maximum shifts as the salt concentration increases to 0.1*M*. Domard et al. found that, for analysis of acrylonitrile-sodium methallylsulfonate copolymers, a concentration of at least 0.5*M* sodium nitrate was necessary to prevent ion exclusion effects in DMF.<sup>5</sup> Bose et al. found the majority of the shift in retention time of sodium polystyrene sulfonate in water occurred between 0*N* and 0.1*N* NaOH.<sup>9</sup>

Compared to the deactivated silica-based duPont columns, the styrene-divinylbenzene-based  $\mu$ -Styragel packings should contain fewer potential ionic sites. It is thus interesting to note that a large shift in the chromatogram of the same epoxy-acrylic occurs when the mobile phase is changed from DMF to DMF/salt on this column set also. Thus, an apparently large decrease in the

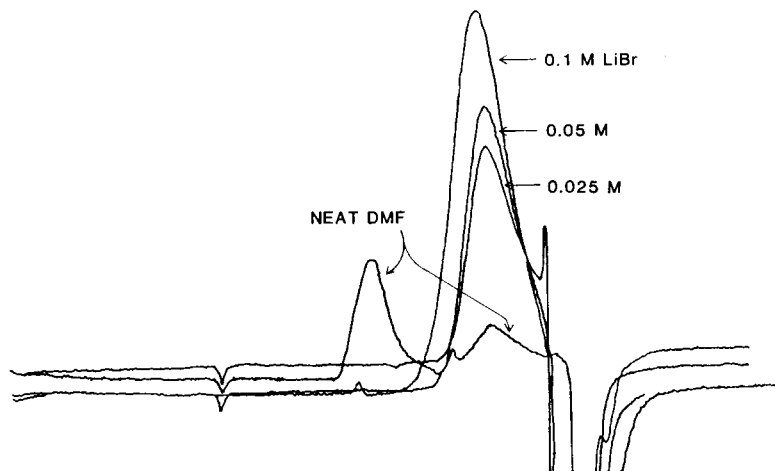


Fig. 2. The effect of lithium bromide concentration in DMF on chromatogram of epoxy-acrylic resin. duPont column set.

hydrodynamic volume occurs as the lithium bromide concentration increases, as well as a screening out of the ion exclusion effect (Fig. 3).

This large shift in retention volumes observed for these polymers may also be due to the destruction of intermolecular aggregates formed in DMF but split apart when a salt is added. Cha<sup>2</sup> investigated sulfonate functional polyelectrolytes and concluded that hydrodynamic volume changes were occurring, while Coppola et al.<sup>11</sup> considered the possibility of aggregate destruction in a similar polymer.

The chromatogram of the epoxy-acrylic polymer in DMF consists of apparently two populations. This interesting splitting of the distribution was found to have applications in the characterization of these complex polymers, as will be discussed below. The origin of such an effect could be what is termed the ion inclusion effect.

In a discussion of polyelectrolyte effects, Stenlund<sup>4</sup> reported that the presence of a high molecular weight solute, excluded from the pores of a packing, increases the retention volumes of lower molecular weight species, due to the electrostatic repulsion. Such a phenomenon can be exploited to obtain increased resolution of lower molecular weight solutes and also explains variations in chromatograms of polyelectrolytes caused by increases in injected sample mass.

Infrared spectra of the excluded fraction and another nonoverlapping fraction of lower apparent molecular weight both exhibited styrene, bisphenol and acid carboxyl absorptions. The relative acid content of the excluded fraction was much greater than that of the other fraction. Thus, a degree of heterogeneity of charge density is present in this polymer. This is not surprising since the epoxy-acrylics are products of reaction between two polymers, both of which exhibit distributions of molecular weights, and probably initial functionality. Domard<sup>5</sup> found a threefold change in apparent charge density across the molecular weight distribution of similar copolymers.

A copolymer of styrene and methacrylic acid was also analyzed as described above. Figure 4 illustrates the very large increase in retention volume as the lithium bromide concentrations increased. At relatively high salt concentrations,

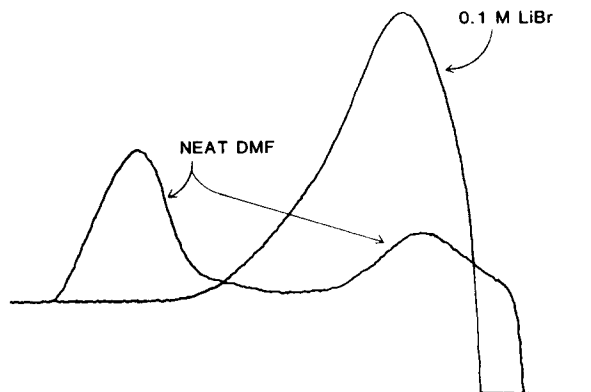


Fig. 3. Comparison of the chromatograms of epoxy-acrylic resin obtained in neat DMF and 0.1M lithium bromide in DMF.  $\mu$ -Styragel column set.

0.5M, some apparent adsorption onto the packing was observed. The optimum salt concentration for SEC appeared to be about 0.1M, similar to the case of the epoxy-acrylics.

A comparison of the chromatograms obtained in DMF and DMF with added lithium bromide of the epoxy base resin is shown in Figure 5. Since the epoxy polymer is nonionic, the salt concentration is found to have a negligible effect on hydrodynamic volume and hence elution volume. Similar behavior is exhibited by dextran standards in water.<sup>9</sup>

### CALIBRATION OF COLUMNS

Domard et al.<sup>5</sup> reports a "weak interaction" of polystyrene standards used for calibration of the column set with bare silica columns. This adsorption of polystyrene results in a shift of the calibration curve towards lower apparent molecular weights (larger retention columns). These shifts make interpretation of routine calibrations rather difficult since the adsorption of polystyrene has a different origin from the ionic effects already discussed.

In this study a significant amount of adsorption of the various polystyrene standards on the duPont columns was observed at all lithium bromide concentrations. As the salt concentration increased, the retention times of the polystyrenes increased. At 0.5M, lithium bromide elution of polystyrene standards of all molecular weights was apparently prevented. The effect of salt concentration on the calibration curve is shown in Figure 6.

This data suggests that the salt causes a partitioning of polystyrene between the mobile phase and the deactivated packing surface. Partitioning of this type would be expected to vary significantly between chemically different non-ionic solutes. This is, indeed, the basis for the separation of, for example, benzene and toluene in ordinary reverse phase liquid chromatography.

The practical result of the effect shown in Figure 6 is that if the calibration curve obtained from polystyrene standards in 0.1M salt is used to calculate average molecular weights for solutes which do not partition in the same manner as polystyrene, then erroneously high averages will be obtained. This will be discussed further below.

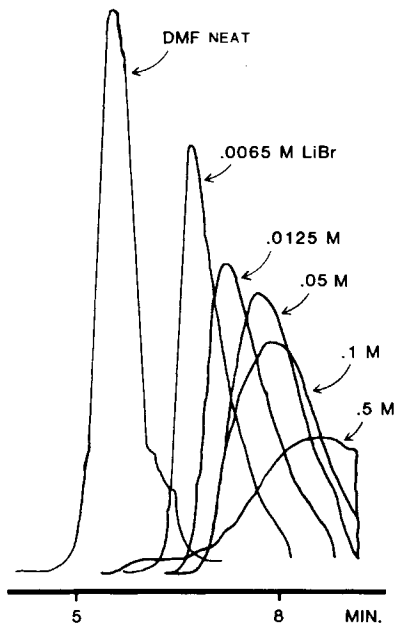


Fig. 4. The effect of lithium bromide concentration on chromatogram of styrene-methacrylic acid copolymer. duPont PSM 1000S and 60S columns. Flow rate = 1.0 mL/min.

Figure 7 illustrates the calibration curves obtained in neat DMF and THF for the same duPont column set. The two curves are similar over most of the molecular weight range, except near the high molecular weight end where the adsorption of polystyrene is evident. This adsorption is probably due to the 4000 column, which is a bare silica type. This column was added to the set to increase resolution in the  $10^5$ - $10^7$  MW range. It is not commercially available in a "deactivated" form.

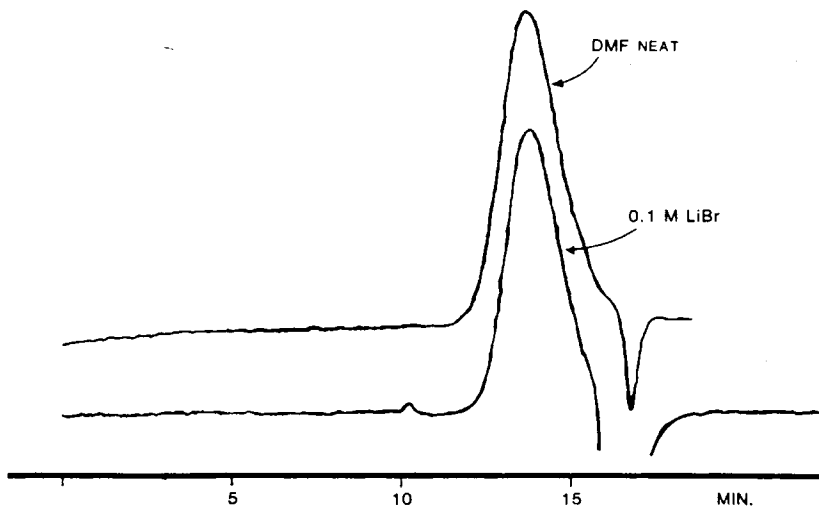


Fig. 5. Comparison of the chromatograms of the epoxy base resin in neat DMF and 0.1M lithium bromide in DMF. duPont column set.

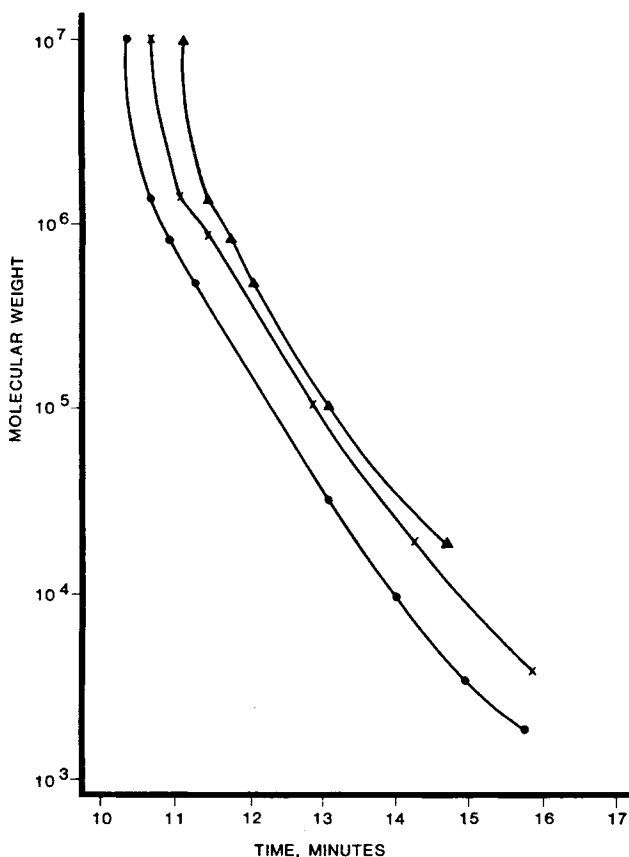


Fig. 6. Calibration curves obtained as a function of lithium bromide in DMF. duPont column set. (●) DMF; (x) 0.1M LiBr in DMF; (Δ) 0.25M LiBr in DMF.

A further investigation of the effect of high salt concentrations on the retention of small to moderate size molecules on the deactivated packing (duPont PSM 60S column) is summarized in Table I.

The decrease in the retention of methacrylic acid is apparently due to a partial reduction in hydrogen bonding with surface hydroxyls or silanols. The acid peaks exhibited significant tailing even at a 0.5M salt concentration. This behavior is precisely opposite to the ion exclusion of the polymers investigated with pendant carboxyl groups derived from methacrylic acid, which indicates that an important molecular weight effect is operating which requires further investigation.

Styrene and polystyrene show large differences in the magnitude of change in retention time with salt concentration. The polymer exhibits a large increase in retention with increasing salt levels while the monomer is only slightly affected. Thus, while adsorption of polystyrene on bare silica occurs at low ionic strength, what is apparently actual solvophobic<sup>12</sup> interaction occurs at high ionic strength on deactivated silica surfaces. It would be expected that such interaction would be a function also of the hydrophobic deactivating group's chain lengths and total loading.

Attempts to calibrate the  $\mu$ -Styragel column set with polystyrene at 0.1M

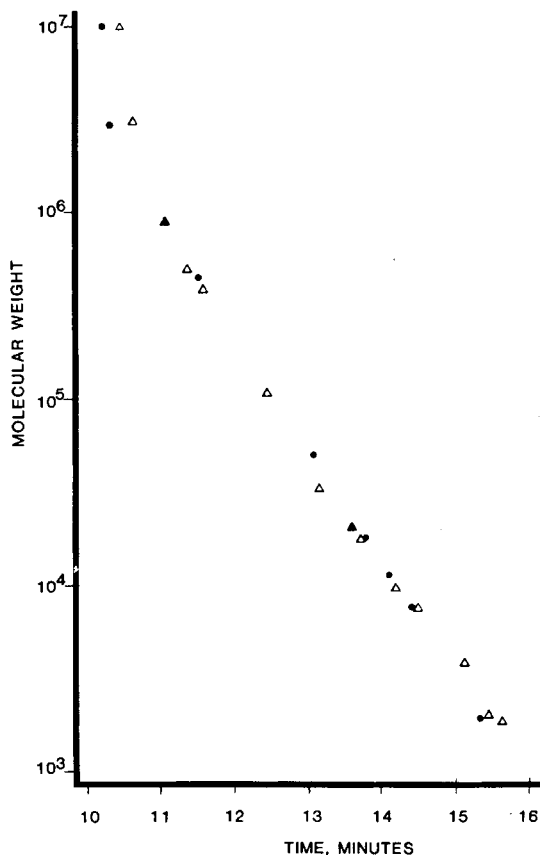


Fig. 7. Comparison of calibration curves of duPont column set obtained with tetrahydrofuran and dimethylformamide mobile phases. (●) Tetrahydrofuran; (Δ) dimethylformamide.

lithium bromide in DMF revealed that the standards were totally retained. The organic matrix of these column packings (crosslinked styrene-divinylbenzene) exposes a larger area of nonpolar surface for solvophobic interactions.

A comparison of calculated average molecular weights of several epoxy-acrylic polymers obtained from the different calibration curves for the duPont column set is given in Table II. The data illustrate the range of values calculated when the calibration is performed in 0.1M lithium bromide/DMF and in tetrahydrofuran. The latter solvent is recommended by the manufacturer and apparently does not cause adsorption of polystyrene.

TABLE I  
Retention Time (min) in DMF/Lithium Bromide

Solute	0.5M	0.25M	0.10M	0.0M
Methacrylic acid	7.03	7.73	Not eluted	Not eluted
Styrene	5.86	5.76	5.70	5.63
Polystyrene (MW = 2.04 × 10 <sup>4</sup> )	Not eluted	4.30	3.73	3.26
Diglycidyl ether of bisphenol A	5.53	5.53		5.23



TABLE II  
Average Molecular Weights of Epoxy-Acrylic Polymers in 0.1M Lithium Bromide Calibration  
Mobile Phase

Sample	THF			0.1M LiBr/DMF		
	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	$D$	$M_n \times 10^{-3}$	$M_x \times 10^{-3}$	$D$
EA-1	8.9	23	2.6	17	41	2.4
EA-2	8.6	25	2.9	17	33	1.9
EA-3	10.0	36	3.5	20	48	2.4
EA-4	9.8	31	3.2	20	45	2.3

Since the epoxy-acrylic polyelectrolytes are expected to change in hydrodynamic volume as a function of salt concentration, and also require salt to screen out ion exclusion effects, a practical approach to minimize variation in molecular weight averages is needed. Therefore the column set was calibrated with 14 polystyrene standards with THF as the mobile phase, while analysis of epoxy-acrylic polymers was conducted in 0.1M lithium bromide/DMF. This approach at least eliminates variation in the calibration curve caused by experimental error in the preparation of lithium bromide solutions in DMF. It emphasizes the fact that the measurement of the molecular weight distributions of the epoxy-acrylic polymers is a relative, not absolute measurement.

Bose et al.<sup>9</sup> found that the effect of sodium chloride on the elution of sodium polystyrene sulfonates was similar, but not identical, to that of sodium hydroxide in water. They explored the use of the universal calibration curve concept and the modification of it proposed by Coll and Prusinowski.<sup>13</sup> They found it impossible to collapse calibration curves of sodium polystyrene sulfonates obtained in the presence of sodium chloride or hydroxide onto a common line. They postulate that sodium chloride may be causing an interaction between the ionic solute and the column packing (Sephacrose CL-6B) or that some "supermolecular" structure exists in aqueous sodium chloride solutions of high ionic strengths.

This study has shown that both styrene-methacrylic acid and epoxy-acrylic polymers will exhibit interaction or retardation on the DuPont column set, at sufficiently high lithium bromide concentrations, in DMF. Although not extensively studied the shape of the chromatogram of typical epoxy-acrylic polymers on  $\mu$ -Styragel also suggested some interaction with this packing at only 0.1M salt.

The retardation of polystyrene on both the duPont and  $\mu$ -Styragel® columns as a function of salt concentration may be viewed as a "solvophobic" or "reverse-phase" interaction. Qualitatively, the lack of ion exclusion effects in the case of polystyrene may be the reason that these interactions occur at lower ionic strengths than with the polyelectrolytes studied.

## SUMMARY

A study of water-dispersible polyelectrolytes in DMF has found chromatographic behavior similar to that of water-soluble polymers. Ion exclusion effects can be overcome with the addition of lithium bromide to the mobile phase.

Calibration of the silica-based deactivated columns used with polystyrene standards is best accomplished using THF as a mobile phase. Calibration in

DMF/salt with polystyrene standards is unsuitable, due to interactions of the standards with the packing.

At high salt concentrations, some retardation or interaction of the polyelectrolytes also occurs on the duPont columns employed. In light of these interactions and those of lower molecular weight solutes, it is speculated that a solvophobic mechanism is operating. The SEC of polyelectrolytes in aqueous and solvent mobile phases necessitates compromise to achieve a minimum of "mixed mechanism" effects.

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### References

1. R. L. Bartosiewicz, *J. Polym. Sci., C*, **21**, 329 (1968).
2. C. Y. Cha, *J. Polym. Sci., B*, **7**, 343 (1969).
3. A. R. Cooper and D. P. Matzinger, *J. Appl. Polym. Sci.*, **23**, 419 (1979).
4. B. Stenlund, *Adv. Chromatogr.*, **14**, 37 (1976).
5. A. Domard, M. Rinaudo, and C. Rochas, *J. Polym. Sci., Polym. Phys. Ed.*, **17**, 673 (1979).
6. J. J. Kirkland, *J. Chromatogr.*, **125**, 231 (1976).
7. DuPont Instrument's Literature Sheet on PSM HPSEC Columns, Wilmington, Del.
8. B. Stenlund and K. G. Forss, *J. Polym. Sci. Symp.*, **42**, 951 (1973).
9. A. Bose, J. E. Rollings, J. M. Caruthers, M. R. Okos, and G. T. Tsao, *J. Appl. Polym. Sci.*, **27**, 795 (1982).
10. K. Fukano, K. Komiya, H. Sasaki, and T. Hashimoto, *J. Chromatogr.*, **166**, 47 (1978).
11. G. Coppola, P. Fabbri, P. Bice, and U. Bianchi, *J. Polym. Sci.*, **16**, 2829 (1972).
12. C. Horvath, W. Melander, and I. Molnair, *J. Chromatogr.*, **125**, 129 (1976).
13. H. Coll and P. Prusinowski, *J. Polym. Sci., Part B*, **5**, 1153 (1967).

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