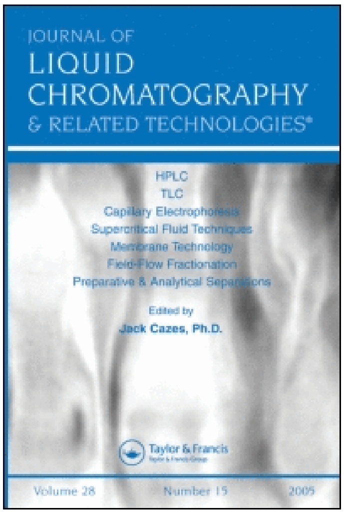

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OPTIMIZATION OF PEAK SEPARATION AND BROADENING IN AQUEOUS GEL PERMEATION CHROMATOGRAPHY (GPC) - POLY (SODIUM STYRENE SULFONATES)

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ABSTRACT

An experimental study of the gel permeation chromatography of a series of poly (sodium styrene sulfonates) in aqueous media has been undertaken. The objective of this study was to develop a set of operating conditions that would maximize peak separation and minimize peak broadening. Using CPG-10 porous glass packing, it has been found optimal to work at pH near 6 and ionic strength near 0.05. Under those conditions, ion exclusion is eliminated and the optimum balance between resolution and reproducibility is obtained. Addition of a few ppm neutral surfactant to the mobile phase prevents adsorption to the packing surface, and improves resolution.

INTRODUCTION

The objective of the present paper, as for previous papers in the series (1,2), was to develop an experimentally optimal system for aqueous GPC analysis of a particular class of polymer - in this case, a series of anionic poly(sodium styrene sulfonates). Phenomena that would interfere with a purely size exclusion mechanism in such analyses are adsorption to the surface of the packing material, and ion exclusion due to charge repulsion. The latter is expected to be a particular problem with polyelectrolytes. In order to approach a system in which peak separation and broadening are optimal, such phenomena must first of all be eliminated or minimized. At that point, it is possible to choose a column system with a combination of pore sizes that gives adequate peak separation without excessive peak broadening. As for previous work, we have chosen CPG-10 porous glass as a suitable packing material since it is available in a wide range of narrowly distributed pore dimensions and particle size. It is thus possible to choose a pore size combination to match the size range of the polymers of interest.

EXPERIMENTAL

The polymers investigated were a series of six sulfonated polystyrenes in the MW range 31,000 to 1.06 x 10^6 obtained from Pressure Chemical Company (Pittsburgh). These are prepared from polystyrenes of low polydispersity by sulfonation to a level of approximately one sulfonate group per styrene residue. Since sulfonic acids are strong acids, these materials would be expected to show polyanion behaviour over a wide range of pH. Molecular weight data supplied by Pressure Chemical Company are shown in Table I.

TABLE 1
Poly (sodium styrene sulfonate) Standards - M.W. Data of Supplier
(Pressure Chemical Co.)

Designation	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
Na PSS 31	31,000	~ 1.10
Na PSS 88	88,000	~ 1.10
Na PSS 195	195,000	~ 1.10
Na PSS 354	354,000	~ 1.10
Na PSS 690	690,000	~ 1.10
Na PSS 1060	1,060,000	~ 1.10

Chromatography was carried out using a Waters Associates Model ALC/GPC 300, with a differential refractometer. Polymer concentrations were in the range 0.01 to 0.1 wt % and mobile phase flowrates varied between 4 and 8 ml min^{-1}. Columns were dry-packed with CPG-10 glass packing (Electronucleonics, Fairfield, NJ). Experimental conditions such as flowrate, additive concentrations and pH are specified below where the results are presented. The neutral surfactant Tergitol NPX (Union Carbide), an alkylphenoxypolyoxyethylene, was used in some experiments in an attempt to minimize adsorption.

Intrinsic viscosities were measured at room temperature in various aqueous media over a range of pH and ionic strength using Cannon Ubbelohde dilution viscometers. Viscosity data were obtained to give information on the dimensions of polymer molecules in solution under differing conditions of pH, ionic strength, and concentration of various additives. Such information, is of assistance in the interpretation of GPC behaviour.

RESULTS AND DISCUSSION

Effect of pH and Ionic Strength on Polymer Dimensions in Aqueous Solution

A summary of intrinsic viscosity data in the pH range above 3.0 is shown in Fig. 1. For a given polymer, it is seen that [η] decreases sharply with increasing ionic strength. This would be expected on the basis of charge screening by the added electrolyte. For the lower MW polymers, the coil dimensions appear reasonably constant at ionic strength above 0.1, whereas [η] does not level off for higher MW polymers. These data suggest that SEC behaviour, regardless of any other effects, would be expected to depend on ionic strength at values less than about 0.3. Viscosity data showing the effect of ionic strength and presented in the form of the Mark-Houwink equation are shown in Fig. 2. The effect of charge is again clear from these data. Thus, while the slopes

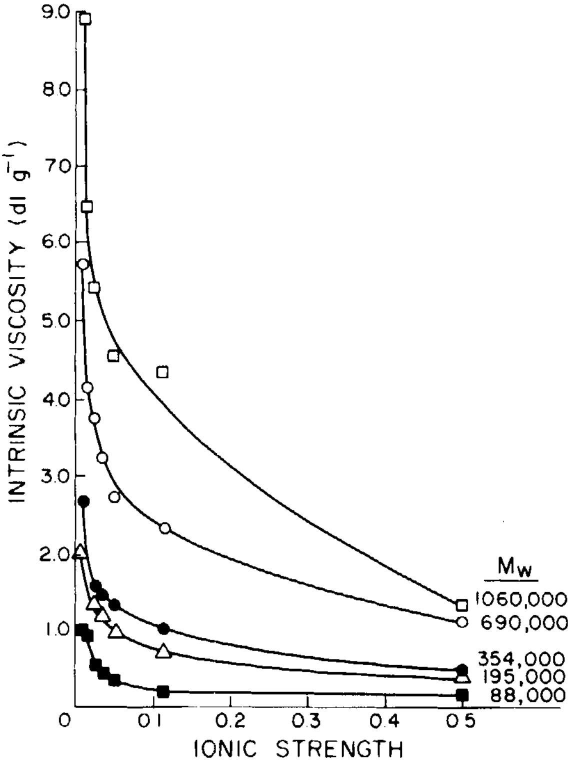

Figure 1 Effect of ionic strength on intrinsic viscosity of poly (sodium styrene sulfonates) (pH > 3, 25^{o}C).

of the plots are similar, the intercepts vary, increasing with decreasing ionic strength and increasing pH. These data again suggest that the polymer coil dimensions depend on solution conditions insofar as these influence the charge characteristics of the chains. The data also suggest that GPC should be conducted at moderate ionic strength for these polymers. Thus it is clear that at ionic strength greater than 0.1 the coils of the lower MW polymers are essentially "collapsed", a situation that would result in poor GPC resolution. At ionic strength less than 0.1 the coil size is larger but variable so that peak positions would

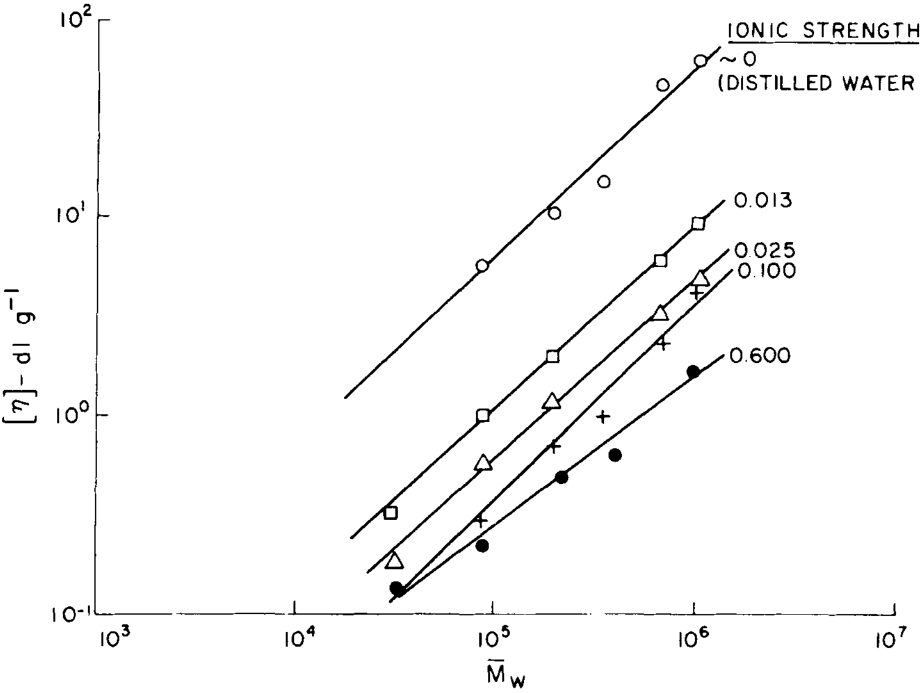

Figure 2 Intrinsic viscosity versus MW for poly (sodium styrene sulfonates), 25°C.

be highly sensitive to ionic strength and reproducibility might suffer. Clearly a balance between these two effects must be sought.

Effect of Ionic Strength on Elution Volumes

To determine the effect of various experimental variables (such as ionic strength and pH and presence or absence of surfactants) on chromatographic performance, molecular weight calibration curves were obtained under different conditions, using a single column. This column had dimensions of 4' x 3/8" and contained CPG-10 packing of 2000 Å pore size.

The effect of neutral salts is demonstrated in Fig. 3. In these experiments, the pH varied between 7.5 and 5.3, a range within which the ionization of sulfonic acid groups would be expected to be complete. Such pH variation should thus not affect the charge characteristics of the polymers. As can be seen from Fig. 3, all the polymers are essentially excluded from the pores in distilled water. Such exclusion could conceivably be a result of size in the case of the high MW polymers, but since even the

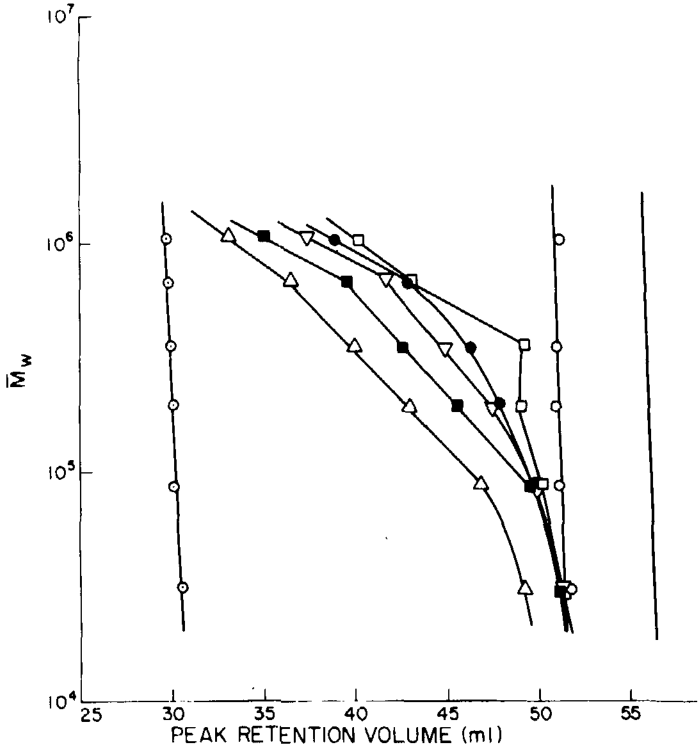

Figure 3 Effect of ionic strength on MW calibration curves of poly (sodium styrene sulfonates) on CPG-10. Column length 4 ft, pore size 2000 Å, flowrate 4.2 ml min^{-1}. Ionic strength: ʘ , zero; △, 0.01; ■ , 0.025; ▽, 0.05; ● , 0.10; □ , 0.30; ○, 0.5; ——, NaCl peak position.

lowest MW polymers are excluded, it is more likely that the effect derives from charge repulsion. The addition of small amounts of neutral salts (e.g. 0.01 M NaCl) allows pore permeation even for the high MW polymers and while such addition has been shown to decrease the polymer dimensions, it is again most probable that the salts are acting by a charge screening mechanism. At high ionic strength (0.5 M NaCl), all the polymers show total permeation of the column. Such behaviour cannot be explained by a size effect alone since, as the viscosity data show, the size of the polymers does not change significantly. It seems likely that these effects can be explained by the onset of adsorption at higher ionic strength. It can also be seen from Fig. 3 that the apparent total permeated volume suggested by the elution volumes of the polymers is less than that observed for NaCl. This effect was also noted for polyacrylamides and dextrans (1,2) and may reflect the existence of a bimodal pore size distribution in the column packing.

Effect of pH on SEC Elution Volumes

The pH of the mobile phase would be expected to have an effect on GPC behaviour through its influence on polymer and/or surface charge. Glass is generally believed to acquire a negative charge at intermediate and higher pH via dissociation of silanol groups. Hair and Hertl (3) have reported a pK value of the order of about 7. The sulfonic acid bearing polymers would be expected to be charged at pH above about 3. Thus, ion exclusion effects would be expected only at high pH, whereas at low pH, charge effects should be absent. The curves in Fig. 4 are in accord with these general precepts. At a pH of 1.6, there is almost total permeation of the packing by polymers of MW less than 200,000. Some adsorption is probably occurring under these conditions. Again, there appears to be a gap between the total volume available to polymers compared to small species such as NaCl. The particular pore size represented by this packing is clearly providing the best resolution for polymers in the MW range 10^5 to 10^6.

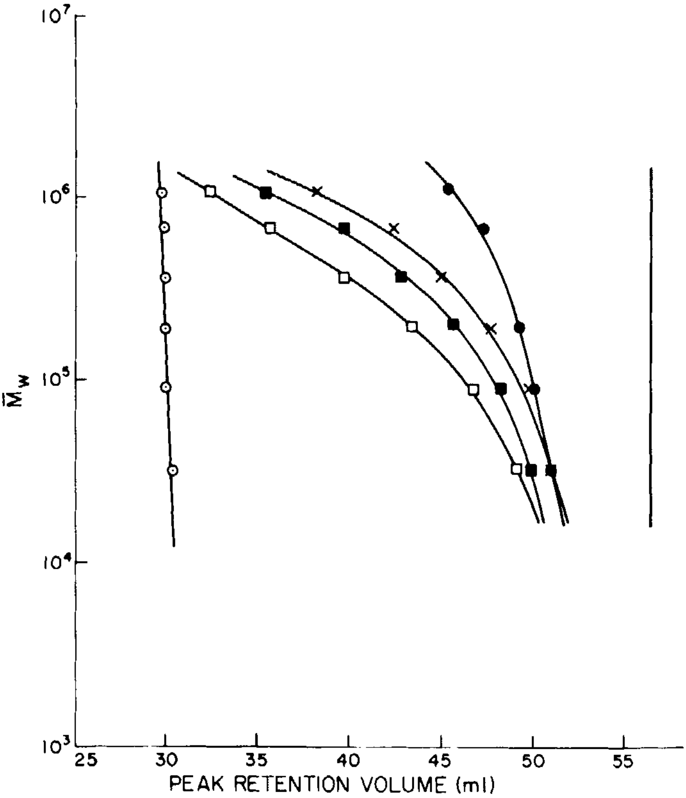

Figure 4 Effect of pH on MW calibration curves of poly (sodium styrene sulfonates) on CPG-10. Column length 4 ft, pore size, 2000 Å, flowrate 4.2 ml $\min^{-1}$. pH values: ⊙ , 7.0; □ , 5.19; ■, 2.28; x, 1.98, ●, 1.62; ——, NaCl peak position.

Effect of Neutral Surfactant on SEC Elution Volumes

Use of neutral surfactants in SEC has frequently been advocated as a means of minimizing adsorption (4), presumably by a pre-emptive adsorption of the surfactant itself. It was found in the present work that adsorption occurred at high ionic strength, especially on the smaller pore size packing. In line with previous investigations in this series it was also found that such

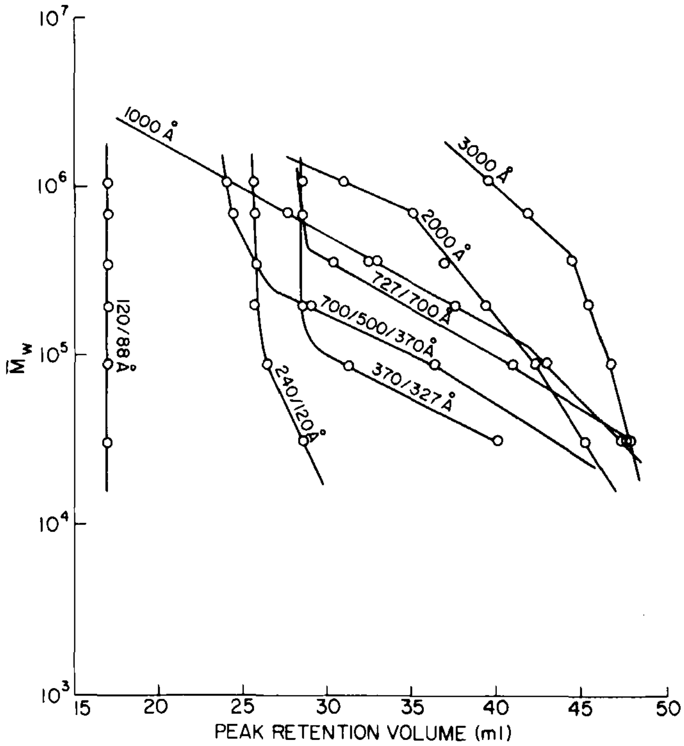

Figure 5 MW calibration curves of poly (sodium styrene sulfonates) for single columns of various pore sizes, pH 6.6, ionic strength 0.053, flowrate 4.2 ml min^{-1}, column lengths, 4 ft.

adsorption could be eliminated by addition of a few ppm of the nonionic surfactant Tergitol to the mobile phase (data not shown). It should be pointed out that even in the presence of Tergitol at high ionic strength (> 0.2) the polystyrene sulfonates were strongly adsorbed. It seems possible in this connection that the solubility of the polymers may be reduced in these media, perhaps due to a salting-out tendency.

In terms of the mobile phase, the optimal conditions appear to be at moderate ionic strength (~ 0.1) and pH in the neighbourhood of 6 in presence of ~ 40 ppm Tergitol. Under these conditions, both charge and adsorption effects are minimal.

Choice of Range of Pore Size (Column Combination)

The above discussion relating to mobile phase optimization was based on experiments with a single column packing with a pore size of 2000 Å. As was pointed out, good resolution of polymers above 10^5 MW is obtained but the lower MW materials are not well resolved. Clearly, if good resolution is to be obtained over a wider range of MW, it is necessary to use a multi-column system containing appropriate pore sizes.

Fig. 5 shows calibration curves using a number of single column systems covering a wide range of pore sizes. The mobile phase was selected based on the results presented above and had the following composition: ionic strength 0.055, 0.04 g/l Tergitol, pH 6.6. This mobile phase, in addition to eliminating charge and adsorption, was also found to resolve the lowest MW polymer (31,000) well from the added salt, Na_2SO_4 (which is also present in the as-received polymers).

Based on these data, several column combinations were studied in order to provide an optimal SEC system for these polymers. The MW averages and polydispersities calculated by integration of the experimental chromatograms using the appropriate calibration curve were used as the criteria of optimal conditions. Table 2 shows a selection of data for several column combinations. In terms of minimum polydispersity over the whole range of MW's, the system employing a 28.7 foot column containing pore sizes 2000, 1000, 729, 700, 500, 370 and 327 Å, operating at pH 6.6 and ionic strength 0.053 gave the best results (system #1). The calibration curve for this system is shown in Fig. 6. At higher ionic strength the resolution (data not shown) was considerably reduced.

The results of this study may be compared to those of Spatorico and Beyer (5) who also investigated polystyrene sulfonates as standards for aqueous SEC. These workers prepared their own standards by sulfonation of narrow polystyrene fractions

TABLE 2

Measured MW Averages and Polydispersities of NaPSS Standards using Selected GPC Systems

System #	1			2			3			4		
Calibration Curve	$M(v) = 0.339\times10^{9} \exp(-0.151v)$			$M(v) = 0.636\times10^{9} \exp(-0.17v)$			$M(v) = 0.347\times10^{10} \exp(0.283v)$			$M(v) = 0.131\times10^{10} \exp(-0.227v)$		
Column Length (ft)	28.7			28.7			21.4			20.8		
pH	6.6			6.8			3.4			3.25		
Ionic Strength	0.053			0.031			0.025			0.013		
	$\bar{M}_w$ $\times10^{-3}$	$\bar{M}_n$ $\times10^{-3}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$ $\times10^{-3}$	$\bar{M}_n$ $\times10^{-3}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$ $\times10^{-3}$	$\bar{M}_n$ $\times10^{-3}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$ $\times10^{-3}$	$\bar{M}_n$ $\times10^{-3}$	$\bar{M}_w/\bar{M}_n$
	32.60	27.90	1.17	33.40	28.90	1.16	31.80	26.90	1.18	52.10	47.00	1.11
	81.50	73.30	1.13	81.30	70.80	1.15	89.00	73.20	1.22	97.40	86.80	1.12
	194.00	171.00	1.14	204.00	178.00	1.14	231.00	195.00	1.19	199.00	172.00	1.16
	366.00	327.00	1.12	392.00	342.00	1.14	383.00	317.00	1.21	360.00	299.00	1.21
	606.00	470.00	1.29	605.00	453.00	1.34	602.00	447.00	1.35	582.00	389.00	1.50
	965.00	812.00	1.19	889.00	698.00	1.27	819.00	561.00	1.46	904.00	572.00	1.58

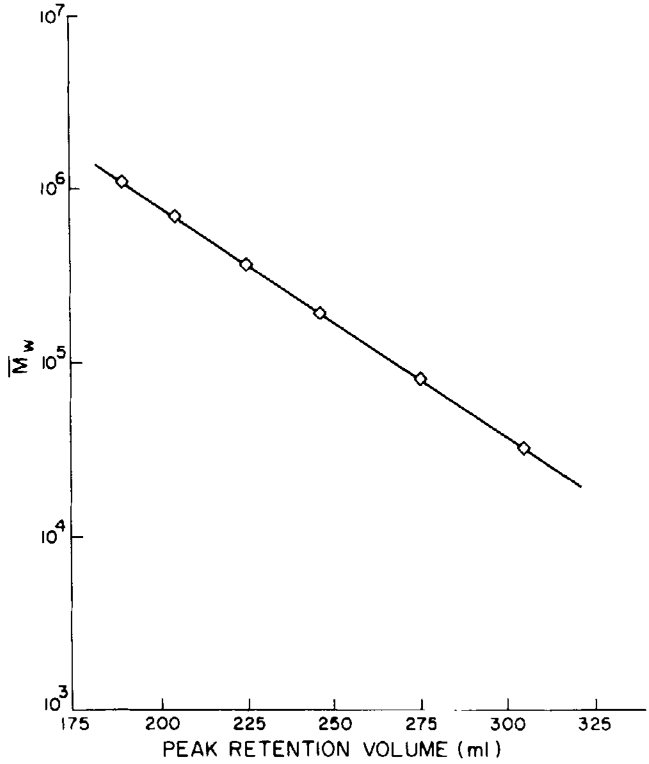

Figure 6 MW calibration curve for poly (sodium styrene sulfonates) for optimally chosen mobile phase and pore size combination. Column length 28.7 ft; pore diameters: 2000, 1000, 729, 700, 500, 370 and 327 Å; pH 6.6; ionic strength 0.053; flowrate 4.2 ml min^{-1}.

in the MW range 8,000 to 800,000. They obtained resolution comparable to that reported here using CPG-10 packing (pore sizes 1250 to 75 Å) with a mobile phase of 0.2 M Na_2SO_4 (ionic strength = 0.6). The pH, although not specified was presumably in the neutral range. A comparison of the two studies can be made in terms of the most obvious differences between them, namely the level of ionic strength and the analysis time, both of which are greater in the work of Spatorico and Beyer.

As indicated previously our intrinsic viscosity results suggest that a compromise is required between loss of resolution at high ionic strength and poor reproducibility at lower ionic strength (but still high enough to eliminate ion exclusion). More specifically our experience suggests that the mobile phase used by Spatorico and Beyer, with an ionic strength of 0.6, would give less than optimal resolution since the polymer coils appear to be effectively collapsed. It is possible that this has been compensated by inclusion of very small pore sizes in the system (5). In this connection it should be noted (Fig. 6) that using our mobile phase conditions of moderate ionic strength the polymers were completely excluded from 120/88 Å pores and were not well resolved at pore sizes less than 300Å.

With regard to analysis time this too is presumably influenced by the use of very small pores. The column lengths in the two studies are about the same while the column diameters are respectively 0.375" (this study) and 0.17" (5), leading to a system volume ratio of about 5. Since the respective flowrates were 4.2 (this study) and 0.5 ml min^{-1} (5) the analysis time is less in the present study by a factor of about 2.

No attempt has been made in the present series of studies (1,2) to test the applicability of the universal calibration concept. Indeed we have found that each water soluble polymer system requires a somewhat different mobile phase for optimal resolution so that the universal calibration approach simply is not appropriate. Studies touching on this point are those of Spatorico and Beyer (5) and Rollings et al (6). Spatorico and Beyer found that for four different polymer types (including NaPSS) a common universal-type calibration curve was obtained in 0.2 M Na_2SO_4. Furthermore, the curve was independent of Na_2SO_4 concentration in the range 0.2 to 0.8 M. The recent work of Rollings et al (6) showed that a common universal-type calibration curve could not be obtained for any of a variety of mobile phases. However when an excluded volume parameter was included in the hydrodynamic volume term a common curve was obtained. In agreement with Spatorico and Beyer these authors obtained a common curve only at high ionic strength (0.1 M or greater, NaCl). As has already been pointed out, resolution can be improved for each individual polymer system by careful choice of conditions. Thus although the universal calibration may or may not be applicable for a given set of conditions, it would seem preferable where possible to operate under "customised" optimal conditions for the polymer system under consideration.

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