

Polyelectrolytes as Secondary Calibration Standards for Aqueous Size-Exclusion Chromatography

A. BOSE,* J. E. ROLLINGS,† J. M. CARUTHERS,‡ M. R. OKOS, and G. T. TSAO, *School of Chemical Engineering, Department of Agriculture Engineering, and Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, Indiana 47907*

Synopsis

The effect of the ionic strength of the solvent on the size-exclusion chromatography of narrow molecular weight dextrans and monodisperse sodium polystyrene sulfonates has been investigated. In both NaOH and NaCl solutions the elution volume of the sodium polystyrene sulfonates increased as the ionic strength was increased, while the elution volume of the dextrans decreased slightly as the ionic strength was increased. The elution data for various ionic strength solvents could not be described by the traditional universal calibration procedure of plotting the product of the intrinsic viscosity and the molecular weight vs. the elution volume. By using a modified calibration procedure which includes excluded volume effects, the elution data can be described for NaOH solution of moderate and high ionic strength. This modified calibration technique was unable to describe the elution data for very low ionic strength NaOH solutions and for the NaCl solutions. Possible explanations of the inability of straightforward size exclusion chromatographic calibration techniques to quantitatively describe all the observed elution behavior are discussed.

INTRODUCTION

The molecular weight and the molecular weight distribution of both synthetic and biological polymers can be determined by size-exclusion chromatography (SEC). The development of SEC for organically soluble synthetic polymers has been rapid; however, the characterization of water-soluble polymers by SEC is not completely understood.^{1,2} The development of aqueous SEC has not been as rapid as the development of organic SEC because of (i) a lack of readily available monodisperse water-soluble polymer standards and (ii) difficulties inherent in the description of the dilute solution conformation statistics of polyelectrolytes. Monodisperse sodium polystyrene sulfonates (NaPSS) as well as dextrans with relatively narrow molecular weight distributions have recently become commercially available. It is possible to use these readily available polymers as secondary standards for the calibration of aqueous SEC systems.³ However, if the NaPSS and dextrans are to be used as calibration standards for the study of other water-soluble polymers, a detailed understanding of the effects of the ionic strength of the solvent in aqueous SEC is required. An experimental study on the effects of the ionic strength of the solvent on the aqueous SEC separation of polyelectrolytes will be reported in this paper.

The principal objective of SEC is to determine the molecular weight and/or

* Present address: Battelle, Columbus Labs, Columbus, OH 43201

† Present address: Department of Chemical Engineering, Worcester Polytechnic Institute, Worcester, MA 01609.

‡ To whom correspondence should be addressed.

molecular weight distribution of the macromolecules. However, the separation process in SEC depends primarily on the size of the polymer. In order to effectively interpret SEC elution data, the relationship between the size of the macromolecule in solution and the molecular weight must be available. Flory has proposed that the molecular weight M is related to the hydrodynamic volume $\langle r^2 \rangle^{3/2}$ as follows^{4,5}:

$$[\eta] = \Phi_0 \langle r^2 \rangle^{3/2} / M = \Phi_0 \alpha^3 \langle r_0^2 \rangle^{3/2} / M \quad (1)$$

where $[\eta]$ is the intrinsic viscosity, $\langle r^2 \rangle$ is the mean-square end-to-end distance, $\langle r_0^2 \rangle$ is the unperturbed mean square end-to-end distance, α is the expansion factor, and Φ_0 is a constant equal to 3.6×10^{21} . Grubisic and co-workers have used the relationship given in eq. (1) as the basis for the calibration of SEC.⁶ The product $M[\eta]$ is proportional to the hydrodynamic volume; therefore, a plot of $\log M[\eta]$ vs. the SEC elution volume should yield a common curve for a given chromatographic column irrespective of the chemical structure of the polymer. The calibration curve can be constructed from SEC and intrinsic viscosity data of polymer standards with known molecular weights, typically monodisperse polystyrenes. Since the intrinsic viscosity can be independently measured for any polymer, the molecular weight of an unknown polymer can be determined from the elution volume and the SEC calibration curve. The result of this procedure is the so-called universal calibration curve, and it has enjoyed considerable success in the molecular weight determination of organic polymers in nonpolar solvents.

In the derivation of eq. (1) excluded volume effects were not considered in the calculation of the front factor Φ . More complete theoretical treatments of the intrinsic viscosity that include the contributions of excluded volume have been summarized by Yamakawa.⁷ Representative of these theories is the relationship developed by Ptitsyn and Eizner⁸:

$$[\eta] = \Phi \langle r^2 \rangle^{3/2} / M = \Phi_0 f(\epsilon) \langle r^2 \rangle^{3/2} / M \quad (2)$$

where $f(\epsilon) = 1 - 2.63\epsilon + 2.86\epsilon^2$, $\epsilon = (2a - 1)/3$, and a is the Mark-Houwink exponent. Coll and Prusinowski have proposed a SEC calibration procedure based on Ptitsyn and Eizner's theoretical development, where $\log \{M[\eta]/f(\epsilon)\}$ is plotted against the elution volume.⁹ Several researchers have analyzed SEC data by both the procedure proposed by Grubisic and the Coll-Prusinowski method; however, they were unable to determine any difference between the two procedures.^{10,11} If the Mark-Houwink exponents are similar for the various polymer-solvent systems to be examined by SEC, eqs. (1) and (2) are identical up to a multiplicative constant so that the two proposed calibration techniques will be indistinguishable.

The size of an uncharged, isolated macromolecule in solution as specified by the mean square end-to-end distance depends upon the molecular weight, the interaction between the polymer and the solvent, and intramolecular polymer-polymer interactions. The conformation statistics of neutral polymers in nonpolar solvents are reasonably well understood⁴; however, this is not the case for polyions in polar solvents.¹² For polyelectrolytes the molecular conformation depends upon the amount and type of charged species, the ionic strength of the solvent, and the molecular weight. Thus, the separation of polyelectrolytes by SEC will depend upon the ionic strength of the solvent. The effect of ionic

strength in aqueous SEC was studied by Cha¹³ and later by Cooper and Matzinger¹⁴; however, these studies did not describe quantitatively the effect of ionic strength on the elution behavior in SEC. In addition, interactions between the polymer and the chromatographic support, and also between the support and the eluent, can influence the SEC of polyions.¹⁴⁻¹⁸

Electrostatic interactions present in polyelectrolytes are not explicitly accounted for in eqs. (1) or (2). The expansion of polyelectrolytes in solvents of low ionic strength has been treated theoretically as an excluded volume effect due to electrostatic interactions.^{12,19-22} If the effects of excluded volume on the intrinsic viscosity in polyelectrolytes are the same as in neutral polymers, the SEC calibration procedure proposed by Coll and Prusinowski⁹ should be equally applicable to polyelectrolytes. In this study we will examine the implications of this hypothesis.

EXPERIMENTAL

Two series of well-characterized polymers were used in these studies. The sodium polystyrene sulfonate (NaPSS) standards were obtained from Pressure Chemical Co. (Pittsburgh, Pa.) and the dextran standards, from Pharmacia Fine Chemical (Piscataway, N.J.). The polyelectrolytes are nearly monodisperse, whereas the dextrans have much broader molecular weight distributions. The properties of both polymer series as quoted by the manufacturers are summarized in Table I.

Aqueous solutions of NaOH and NaCl were prepared. The alkaline solutions were standardized by the potassium acid phthalate method, and the salt solutions were standardized gravimetrically. Both solutions were degassed prior to use. The polymer solutions were prepared at the highest concentration in volumetric flasks and dilutions were made by volume. The solutions were filtered through 0.8 μm membranes.

The dilute solution viscosity of the polymer standards in NaOH and NaCl of various concentrations were determined with a Cannon-Fenske viscometer. The measurements were performed in a viscosity bath maintained at $25 \pm 0.1^\circ\text{C}$. Flow times were determined to 0.5 s and the flow time of pure solvent was always greater than 200 s; hence kinetic energy corrections were unnecessary. The re-

TABLE I
Properties of the Standards

Polymer	\bar{M}_w	\bar{M}_w/\bar{M}_n
NaPSS-700	708,000	1.195
NaPSS-400	400,000	<1.100
NaPSS-200	200,000	<1.100
NaPSS-87	87,000	<1.100
NaPSS-65	64,900	<1.100
NaPSS-18	17,500	<1.100
NaPSS-5	4,800	1.143
T-500	466,000	2.03
T-65	64,400	1.57
T-40	41,000	1.46
T-10	9,400	1.71

ported intrinsic viscosity of a sample is the arithmetic average of the intercepts of the η_{sp} vs. c and $\ln \eta_r/c$ vs. c plots. The two extrapolated values of the intrinsic viscosities agreed to within 0.5% in all cases.

A detailed description of the SEC apparatus used in this study is reported elsewhere.²³ The chromatographic support was unfractionated sepharose CL-6B (Pharmacia Fine Chemicals). The flow rate of the eluent through the column was slightly greater than the value obtained for gravity flow. The temperatures of both the SEC column and the refractive index detector were maintained at $25 \pm 1.0^\circ\text{C}$. A sample size of $50 \mu\text{L}$ was used throughout these experiments.

The elution volume data was normalized in the usual fashion by using the K_{AV} parameter:

$$K_{AV} = \frac{V_e - V_0}{V_t - V_0} \quad (3)$$

where V_e is the peak retention volume of the sample, V_0 is the void volume of the column, and V_t is the total interstitial volume of the column. V_0 was determined from retention volume of NaPSS 700 which was totally excluded, and V_t was determined from the retention volume of glucose.

RESULTS

Intrinsic Viscosity of NaPSS and Dextrans

The molecular weight dependence of the intrinsic viscosity for NaPSS and dextrans in NaOH solutions of varying ionic strength and for NaPSS in various NaCl solutions are shown respectively in Figures 1–3. The intrinsic viscosity data can be described by the Mark–Houwink relationship

$$[\eta] = KM^a \quad (4)$$

where K and a are empirical constants and M is the weight average molecular weight of the polymer. The Mark–Houwink constants for all the systems studied in this investigation are given in Table II. At low ionic strengths the Mark–Houwink exponent a increases significantly with decreasing ionic strength for the NaPSS in both NaOH and NaCl solutions; however, at higher ionic strength NaOH solutions the effect is reversed. The exponent a is essentially independent of ionic strength for the dextrans.

The effect of ionic strength on the intrinsic viscosity at a given molecular weight is qualitatively different for NaPSS as compared to the dextrans. Representative intrinsic viscosity data is shown in Figure 4. The intrinsic viscosity of NaPSS increases with decreasing ionic strength, and, as the ionic strength decreases below $0.1N$, the intrinsic viscosity increases dramatically. Alternatively, the intrinsic viscosity of the dextrans is relatively insensitive to the ionic strength except at very low ionic strengths, where a slight decrease in the intrinsic viscosity is observed. Similar behavior was observed for dextrans and NaPSS of different molecular weights and for the dextrans and NaPSS in NaCl solutions.

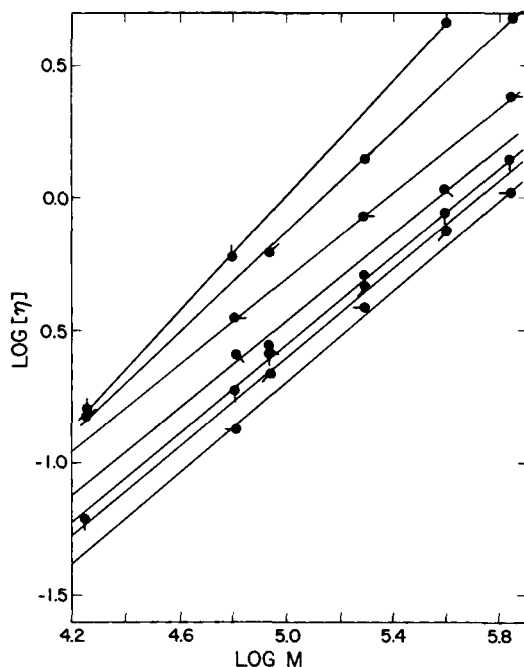


Fig. 1. Molecular weight dependence of intrinsic viscosity for NaPSS at 25°C; NaOH concentrations of 0.005*N*, 0.0185*N*, 0.097*N*, 0.287*N*, 0.501*N*, 0.671*N*, and 0.877*N* indicated by pips starting upwards and moving clockwise at 45° angles, respectively.

Size Exclusion Chromatography

The elution profiles of the NaPSS and dextrans were obtained for the NaOH and NaCl solutions enumerated in Table II. SEC elution profiles are shown in Figure 5 for NaPSS with a molecular weight of 87,000 g/mol. Similar SEC data for a dextran of molecular weight 65,000 g/mol are presented in Figure 6. For both dextrans and NaPSS the shape of the elution profile is insensitive to the ionic strength of the solvent. For the NaPSS data in Figure 5 the peak of the elution profile shifts to higher elution volumes as the ionic strength of the solvent is increased. Since an increasing elution volume corresponds to a decreasing molecular size, the NaPSS data indicate that the conformation of the macromolecules expand as the ionic strength of the solvent is decreased. The effect of the ionic strength of the solvent on the SEC elution profiles for dextrans as shown in Figure 6 is qualitatively different than the NaPSS data. The elution volume for the dextrans decreases as the ionic strength is increased, in contrast to the NaPSS data, and the magnitude of this shift is considerably less than the shift observed with NaPSS.

The molecular weight dependence of the elution volume for NaPSS and dextrans in a 0.097 *N* NaOH solution is shown in Figure 7. For a given molecular weight, the elution volume of the dextrans is greater than that of the NaPSS. This data indicates that the NaPSS are in an expanded molecular conformation as compared to the dextrans.

The SEC data for dextrans and NaPSS in NaOH solutions of varying ionic strength are plotted according to the calibration procedure of Grubisic et al.⁶

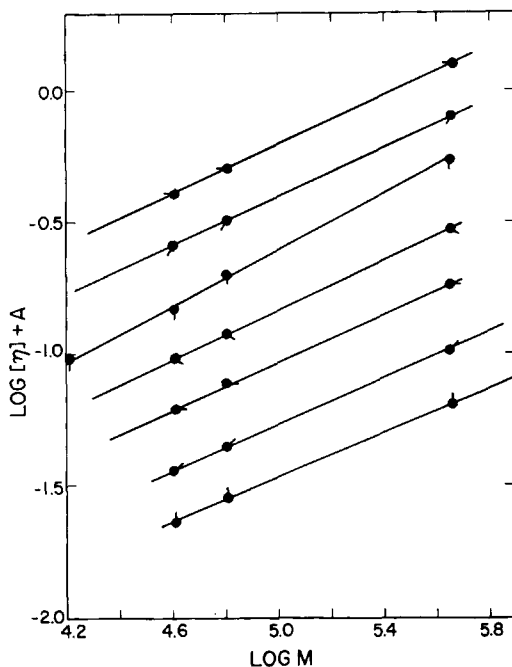


Fig. 2. Molecular weight dependence of intrinsic viscosity for dextrans at 25°C; NaOH concentrations are the same as indicated in Figure 1. The values of the constant A are: -0.9 , -0.7 , -0.5 , -0.3 , -0.1 , and $+0.3$, and increase with the ionic strength.

in Figure 8 and according to the Coll-Prusinowski procedure⁹ in Figure 9. Examining the data in Figure 8, we observe that the calibration curve is different for the dextrans as compared to the NaPSS, and both the dextran and NaPSS calibration curves depend upon the ionic strength of the solvent. Thus, the relationship between molecular size and the molecular weight as described in eq. (1) is not appropriate for these polyelectrolytes. For a given value of $[\eta]M$ the dextrans exhibit a larger elution volume than the NaPSS, irrespective of the ionic strength of the solvent. The dextran's SEC data is relatively insensitive to the ionic strength of the solvent with a slight shift of the calibration curve to smaller elution volumes with increasing ionic strength. In contrast, the NaPSS data are quite sensitive to the ionic strength of the solvent, and larger elution volumes are observed with increasing ionic strength in contrast to the behavior observed for the dextrans. The Coll-Prusinowski calibration procedure as shown in Figure 9 is significantly better than the SEC calibration procedure used in Figure 8. In Figure 9 we observe a single curve for all the dextran data and for the NaPSS data except for the very low ionic strength solutions. The SEC data for NaPSS in 0.005 and 0.0185*N* NaOH is shifted towards smaller elution volumes, indicating the molecular conformation is expanded in these low ionic strength solvents.

The SEC elution behavior of NaPSS in various ionic strength solutions of NaCl was also investigated. The molecular weight dependence of the elution volume is shown in Figure 10. For a given molecular weight the elution volume increases with increasing ionic strength, and the amount of this increase in the elution volume is greater for the low molecular weight species. A plot of $[\eta]M$ vs. the

TABLE II
Mark-Houwink Constants for Dextran and Polystyrene Sulfonates

Ionic strength	Polystyrene sulfonate			Dextran		
	NaOH		NaCl	NaOH		
	$K \times 10^5$ (dL/g)	a	$K \times 10^5$ (dL/g)	$K \times 10^3$ (dL/g)	a	
0.005N	0.38	1.079	0.57	1.80	0.438	
0.0185N	1.46	0.935	1.06	1.85	0.431	
0.097N	4.58	0.805	2.09	1.58	0.454	
0.287N	2.81	0.815	2.85	1.24	0.474	
0.501N	1.98	0.828	4.16	1.32	0.478	
0.671N	1.74	0.830		1.47	0.465	
0.877N	1.10	0.851		1.51	0.464	

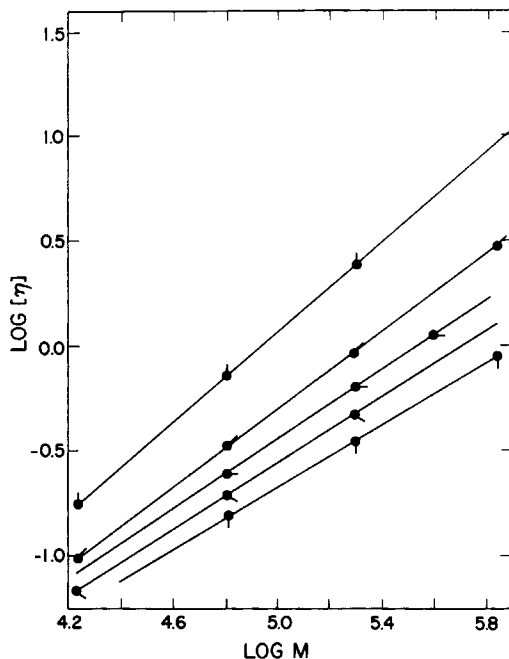


Fig. 3. Molecular weight dependence of intrinsic viscosity for NaPSS at 25°C; NaCl concentrations of 0.005*N*, 0.05*N*, 0.2*N*, 0.5*N*, and 1.0*N* indicated by pips starting upwards and moving clockwise at 45° angles respectively.

elution volume is given in Figure 11 for the NaCl solutions of NaPSS. As with the NaOH solutions of NaPSS, a single calibration curve is not obtained. As shown in Figure 8, at high ionic strengths the NaPSS SEC data for NaOH solutions appear to be approaching a common calibration curve; however, the NaCl solutions do not exhibit the same high ionic strength limit. The Coll-Prusinowski procedure was applied to the elution data for the NaCl solutions and is shown in Figure 12. Although the Coll-Prusinowski calibration procedure was successful in describing the SEC data for NaPSS in moderate and high ionic strength solutions of NaOH, the elution behavior for NaCl cannot be described by this method. The Coll-Prusinowski procedure does reduce the differences between various NaCl solutions as compared to the discrepancies seen in the Grubisic calibration method, but significant variations still exist. The NaPSS elution data does not even appear to be approaching a common calibration curve as the NaCl ionic strength is increased. Thus, over the same range of ionic strengths the SEC behavior of NaPSS in NaOH solutions is qualitatively different from the behavior observed in NaCl solutions.

DISCUSSION

We have observed that the separation of polyelectrolytes by SEC and the intrinsic viscosity of these polyelectrolytes are sensitive to both the ionic strength of the solvent and the exact composition of the added electrolyte. The primary separation mechanism in SEC is the size of the polymer in solution, and the intrinsic viscosity of a macromolecule also depends upon the molecular size. If the macromolecules contains moieties in which a counterion can dissociate from

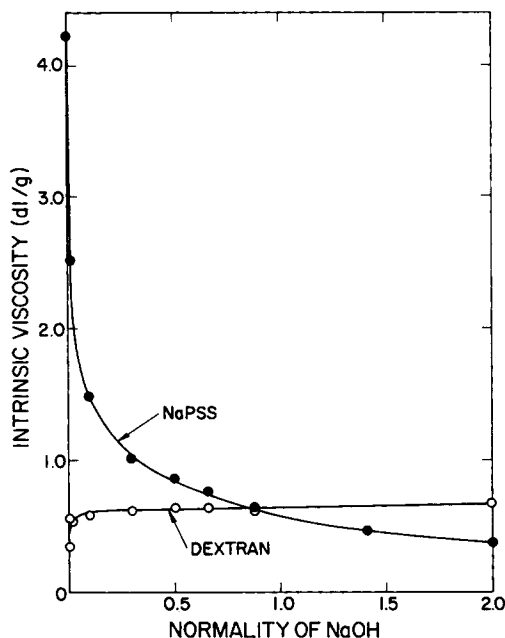


Fig. 4. Variation with NaOH concentration of intrinsic viscosity for NaPSS-400 and dextran T-500.

the charged polymer, the conformation of the polyelectrolyte will be sensitive to the ionic strength of the solvent.⁷ Alternatively, if the macromolecule does not contain counterions which can dissociate, the effect of ionic strength on the chain conformation will be minimal. The SEC and intrinsic viscosity data for NaPSS and dextrans which we have reported are consistent with this qualitative picture of charged polymers. The NaPSS contain counterions which can dissociate and the experimentally observed viscosity and SEC behavior were quite sensitive to the ionic strength of the solution. The intrinsic viscosity and SEC behavior of the dextrans, which do not have an ionizable counterion, were relatively insensitive to the ionic strength of the solvent.

The most significant observation of this study is the inadequacy of conventional SEC calibration procedures for polyelectrolytes. As shown in Figures 8 and 11, the traditional calibration procedure of Grubisic et al.⁶ is not appropriate for polyelectrolytes. Significant deviations between the dextran and NaPSS SEC data were observed with NaOH solutions even when the ionic strength of the solvent was the same for both polymer solutions. When excluded volume effects were included during calculation of the intrinsic viscosity factor Φ , the corresponding SEC calibration procedure was noticeably improved for moderate and high ionic strength NaOH solutions. However, as shown in Figure 9, the NaPSS SEC data in low ionic strengths NaOH solutions did not collapse to a common calibration curve using the Coll-Prusinowski procedure. The Coll-Prusinowski SEC calibration procedure⁹ uses the intrinsic viscosity factor Φ predicted by Ptitsyn and Eizner.⁸ Kurata and Yamakawa¹⁹ have predicted a slightly different form of the factor Φ ; however, when the Kurata-Yamakawa form of Φ is used, the NaPSS SEC data for 0.005*N* and 0.0185*N* NaOH solutions still do not collapse to a common calibration curve. Intrinsic viscosity and light

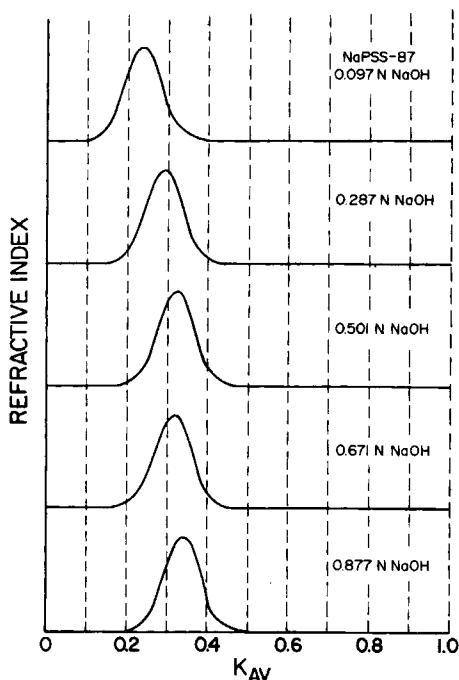


Fig. 5. Chromatograms of NaPSS-87 in various NaOH solutions.

scattering measurements have been used by Takahashi et al. to experimentally determine the dependence of Φ on the chain expansion α for NaPSS in NaCl solutions of varying ionic strength.²⁰ Although the experimental determination Φ by Takahashi et al. differed from the theoretical predictions of Ptitsyn-Eizner⁸ and Kurata-Yamakawa,¹⁹ the SEC data for NaPSS in the two lowest ionic strength NaOH solutions could not be reduced to a common calibration curve, even when the experimentally determined values of Φ were used.

Since excluded volume effects have been unable to account for the SEC data in NaOH solutions of low ionic strength, other phenomena must be important in the separation mechanism. The separation of macromolecules in SEC is usually described in terms of the volume of the macromolecule in solution, i.e., $\langle r^2 \rangle^{3/2}$; however, the primary separation mechanism is more closely related to the projection of the macromolecular conformation on a plane. A macromolecule can diffuse into a pore of the SEC column if the projection of the macromolecule on the entrance to the pore does not exceed the size of the pore's entrance. If the macromolecules are spherically symmetric, an SEC calibration technique based on separation by the volume of the polymer in solution will be indistinguishable from a procedure based on the projection of that volume on the entrance to the pore. However, if the molecular conformation is not spherically symmetric, significant differences can result. Kurata has predicted that the conformation of a polyelectrolyte in solution will be in the shape of a prolate spheroid instead of being spherically symmetric.^{21,22} Thus, the inability to collapse the SEC data for NaPSS in NaOH solutions at the two lowest ionic strengths may be related to the nonspherical nature of the macromolecular conformation.

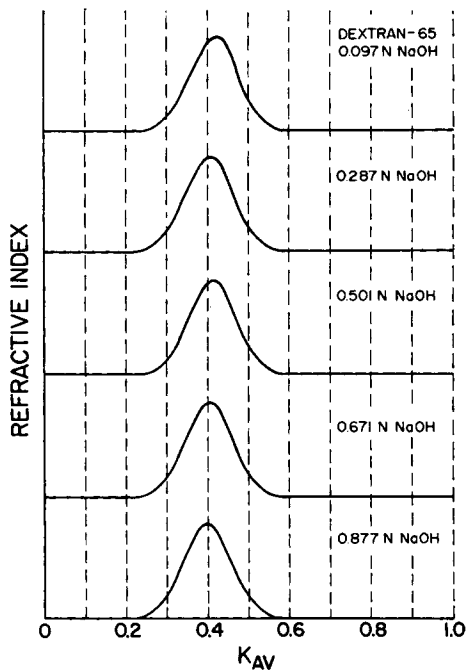


Fig. 6. Chromatograms of dextran T-65 in various NaOH solutions.

The area A projected on a plane by a prolate spheroid with a major and minor axis of lengths a and b , respectively, is given by

$$A = \pi b(a^2 \sin^2 \theta + b^2 \cos^2 \theta)^{1/2} \tag{5}$$

where θ is the angle between the major axis of the spheroid and the normal to

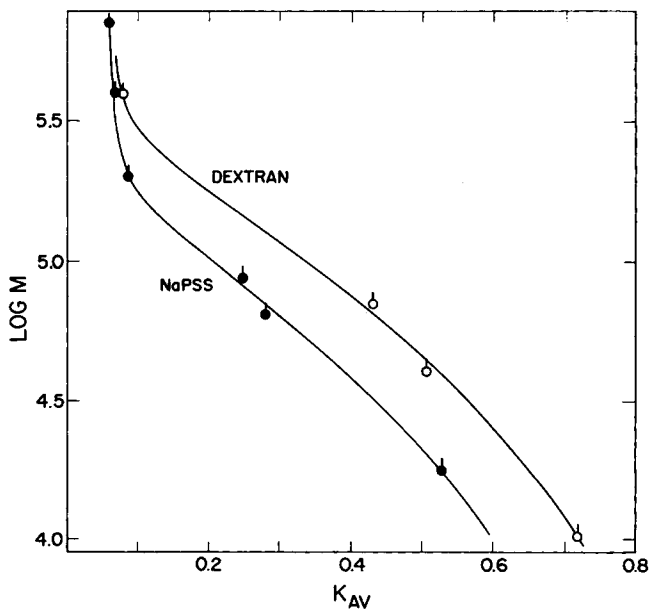


Fig. 7. Relationship between $\log M$ and K_{AV} for NaPSS and dextrans in 0.097N NaOH.

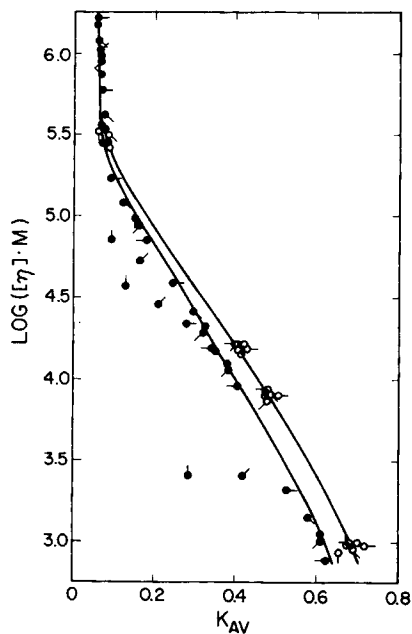


Fig. 8. $\log [\eta] \cdot M$ vs. K_{AV} for NaPSS and dextrans; NaCl concentrations are the same as indicated in Figure 1.

the surface. If we assume that all orientations of the spheroid are equally probable, the average area \bar{A} projected by the prolate spheroid on a plane is determined to be

$$\bar{A} = \frac{\pi b^2}{2} \left(1 + \frac{e^2}{\sqrt{e^2 - 1}} \sin^{-1} \sqrt{1 - 1/e^2} \right) \quad (6)$$

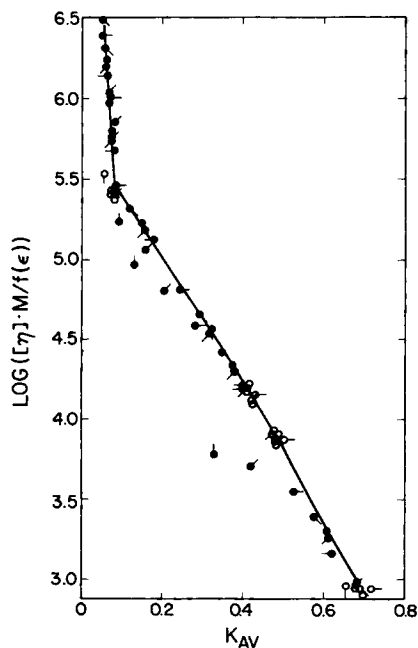


Fig. 9. Coll-Prusinowski calibration curve for NaPSS and dextrans; NaOH concentrations are the same as indicated in Figure 1. $\epsilon = (2a - 1)/3$; $f(\epsilon) = 1 - 2.63\epsilon + 2.86\epsilon^2$.

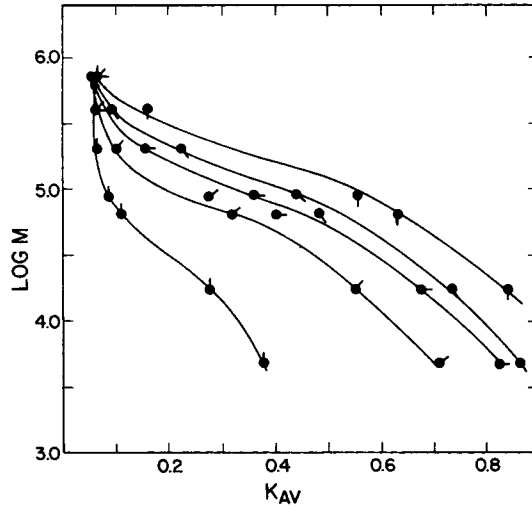


Fig. 10. Relationship between $\log M$ and K_{AV} for NaPSS: NaCl concentrations are the same as indicated in Figure 3.

where $e = a/b$. The area projected on a plane by a sphere that has the same volume as the prolate spheroid is $\pi b^2 e^{2/3}$. The ratio of the average area projected on a plane by a prolate spheroid to the area projected by a sphere of equivalent volume is given by

$$F(e) = \frac{1}{2} e^{-2/3} \left(1 + \frac{e^2}{\sqrt{e^2 - 1}} \sin^{-1} \sqrt{1 - 1/e^2} \right) \quad (7)$$

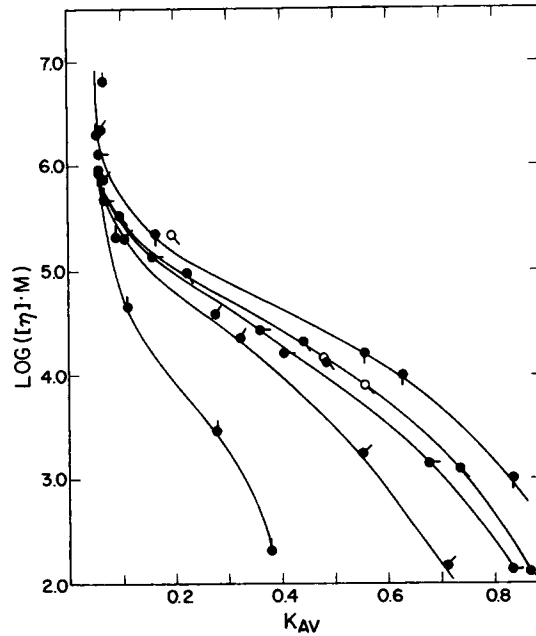


Fig. 11. $\log [\eta] \cdot M$ vs. K_{AV} for NaPSS; NaCl concentrations are the same as indicated in Figure 3.

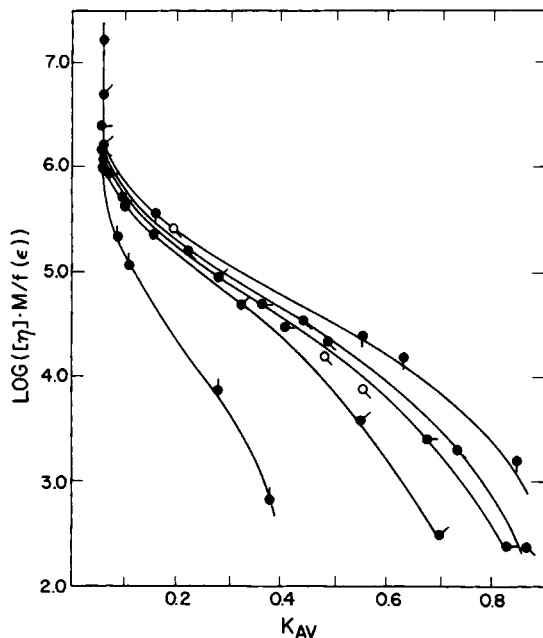


Fig. 12. Coll-Prusinski calibration curves for NaPSS; NaCl concentrations are the same as indicated in Figure 3.

The calibration curves for SEC should be constructed by plotting $F(e)\langle r^2 \rangle^{3/2}$ vs. the experimentally determined elution volume. If the Ptitsyn-Eizner theory⁸ is used to express $\langle r^2 \rangle$ in terms of the intrinsic viscosity and the molecular weight, SEC calibration curves can be constructed by plotting $[\eta]MF(e)/f(\epsilon)$ vs. the elution volume. Thus, if e can be theoretically predicted, the molecular weight can be determined from intrinsic viscosity and SEC data.

Although it should be possible to determine the length-to-diameter ratio e by light scattering, we do not have such an estimate at the present time. However, it is instructive to determine the ratio e that would be required to collapse the SEC elution data into a common curve. The SEC data indicates qualitatively that the ratio e increases with decreasing molecular weight and with decreasing ionic strength. Both these observations are consistent with our understanding of the conformation statistics of polyelectrolytes.¹² Examining the data in Figure 9, for NaPSS in 0.0185*N* NaOH at $K_{AV} = 0.25$ the function $F(\epsilon)$ would need to be 1.45, corresponding to an e of 6. For the same ionic strength NaOH solution the length-to-diameter ratio would need to be 7.5 at $K_{AV} = 0.35$. These length-to-diameter ratios are quite reasonable; however, values of e greater than 100 are required to collapse the 0.005*N* NaOH SEC elution data into the common curve. Although it is not inconceivable, it is unlikely that polyelectrolytes have such large length-to-diameter ratios. Our model assumes that the polyelectrolytes are excluded from the pores of the SEC column because of their shape, but once the molecule enters a pore the diffusion of the macromolecule in the pore is unaffected by the length-to-diameter ratio. This is probably a reasonable assumption, when the molecule is not in a highly extended conformation; however, for large length-to-diameter ratios the diffusion behavior in the pore is probably quite different. In addition, at very low ionic strengths other separation

mechanisms such as ion exclusion can become important. In order to critically assess the effects of anisotropy on SEC, independent measurements of the length-to-diameter ratio will be required.

In the preceding analysis we have assumed that both excluded volume and anisotropic effects contribute to the SEC behavior of polyelectrolytes. It is instructive to consider if anisotropic effects alone are sufficient to explain the observed SEC data. Let us assume that the discrepancy between the NaPSS elution volumes at high ionic strengths presented in Figure 9 and the dextran elution volumes is solely due to anisotropy. Furthermore, if we assume that the conformation of the dextrans are spherically symmetric, an e of ~ 10 would be required in order to obtain a common calibration curve. It is highly unlikely that NaPSS's in 0.9*N* NaOH are in such a highly extended conformation. Therefore, both the effects of excluded volume and the anisotropy of the molecular conformation need to be considered in analyzing SEC data of polyelectrolytes.

The differences between the SEC elution data for NaPSS in NaOH as compared to NaPSS in NaCl solutions is somewhat perplexing. The Coll-Prusiniowski calibration procedure, which was successful in describing the SEC data for NaPSS in moderate and high ionic strength solutions of NaOH, does not collapse the SEC data for NaPSS in various ionic strength solutions of NaCl to a common curve. Although the Coll-Prusiniowski procedure does reduce the differences between the data at different ionic strengths, substantial differences still exist, even at very high ionic strengths. Since the specific nature of the polyelectrolyte-counterion binding should not be important at these high ionic strengths, the only reasonable explanations of the observed elution data are that (i) either the NaCl causes an interaction between the polyelectrolyte and the sepharose support or (ii) some supermolecular structure exists in high ionic strength NaCl solutions. Further investigation of these phenomena are necessary if aqueous size exclusion chromatography is to be used effectively.

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