the polymer blend containing PCEA was lower than EtCz–PDNBM. By the calculation according to Onsager's theory, it was found that the number of excited ion pairs was lower and the probability of the dissociation of ion pairs was higher for the polymer blend than those for EtCz–PDNBM system.

Registry No. PCEA·PDNBM, 118631-47-3; PDNBM, 82008-07-9; PDNBM·NEt_3, 118631-48-4; EtDNB·NEt_3, 118631-46-2; PDNBM·*N*-ethylcarbazole, 118631-49-5.

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Agarose Gel Electrophoresis of High Molecular Weight, Synthetic Polyelectrolytes

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ABSTRACT: The electric-field-induced motion of poly(styrenesulfonate) in agarose gels has been studied with two objectives, to probe the field-biased dynamics of polyelectrolyte chains in a porous matrix and to assess the quality of the molecular size fractionations that evolve as a result of these dynamics. For molecular weights from 7×10^3 to 15×10^6 the electrophoretic mobility of poly(styrenesulfonate) has been measured as a function of gel concentration, electric field strength, and ionic strength. High-resolution separations based on chain length have been observed for nearly all conditions, with the useful analytical range extending beyond the highest molecular weight examined. Mobilities qualitatively follow the experimental trends observed with DNA except in the ionic-strength dependence; a mobility maximum has been observed at intermediate ionic strengths for poly(styrenesulfonate) of low molecular weight, while a chain-length-independent mobility has been found at higher molecular weight when the ionic strength is low.

Introduction

Despite experimental simplicity and high resolution, gel electrophoresis has generally been neglected as a tool for measuring the molecular weight distributions of synthetic polymers. In sharp contrast, electrophoretic methods have become so ubiquitous in biopolymer research that complex protein and DNA mixtures are analyzed routinely.[1] We have been motivated by the success of such biopolymer applications to examine electrophoresis of highly charged, synthetic polymers. In this initial contribution attention will focus on electrophoretic migration of poly(styrenesulfonate) (PSS) in horizontal agarose gels. Synthetic polymers are heterogeneous in both chemical structure and size; how this heterogeneity affects electrophoretic behavior constitutes the biggest uncertainty in use of electrophoresis for characterizing synthetic polymer materials.

Chen and Morawetz[2] recently described electrophoresis of synthetic polymers in chemically cross-linked polyacrylamide gels, media conventionally employed to study proteins and small DNA fragments. For DNA chains less than 300 base pairs in length, polyacrylamide gel electrophoresis permits complete separation of mixtures when chain lengths differ by as little as one base pair. Chen and Morawetz limited their attention to PSS and poly(acrylic acid) samples with molecular weights in the range 2×10^4–1×10^5; we are primarily interested in molecular weights above this range. Separation in polyacrylamide gels will not be discussed further as the migration of a free polymer within these dense gels is likely to be somewhat different than in agarose gels.

Agarose gels are normally used for separations of molecules too large to penetrate the tighter pore structure of polyacrylamide gels. The largest proteins and higher molecular weight DNA chains fall in this category. Agarose gels are formed by physical cross-linking upon solution cooling, and the interconnecting strands are fiberlike molecular aggregates rather than individual chains.[3] The mean pore size depends on the concentration of agarose, with 100 nm being a characteristic length for a 1% gel.[3,4] Agarose is a polysaccharide possessing only a residual density of covalently bound ionizable groups.

Although size exclusion chromatography (SEC) is a valuable analytical tool for low and medium molecular weight polyelectrolytes, fractions of ultrahigh molecular weight ($M > 1.0 \times 10^6$) have generally resisted molecular weight analysis by SEC.[5,6] For this reason, synthetic polymers in this high molecular weight category have often been insufficiently characterized. Agarose gel electrophoresis could offer considerable advantages over difficult methods such as band sedimentation, which have been the only alternatives when SEC fails. The advantages of gel electrophoresis would include reduced cost, shorter run times, and increased ability to separate similarly sized fractions.

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Table I
Poly(styrenesulfonate) Standards[a]

PSS sample	parent PS M_w	parent PS M_w/M_n	N	PSS nominal M	PSS M_w/M_n
1	4000	<1.06	38	7100	≤1.10
2	9200	<1.06	88	16000	≤1.10
3	20400	<1.06	195	36000	≤1.10
4	32600	<1.06	313	58000	≤1.10
5	53700	1.05	515	95000	≤1.10
6	98700	1.07	947	175000	≤1.10
7	200000	<1.06	1,920	354000	≤1.10
8	392000	1.12	3,760	693000	≤1.10
9	600000	<1.06	5,760	1060000	≤1.10
10	1030000	1.10	9,900	1800000	1.02
11	1860000	1.10	17,900	3300000	1.05
12	2950000	1.07	28,300	5200000	1.07
13	4400000	1.06	42,200	7800000	1.14
14	6770000	1.14	65,000	12000000	1.19
15	8500000	1.20	81,600	15000000	1.24

[a] Values for PSS 1–9 are as reported by the supplier, Pressure Chemical. The PS parents for PSS 10–11 were purchased from Polysciences, parents for PSS 12–14 from Polymer Laboratories, and parent for PSS 14 from Toya Soda. Polydispersities for PSS 10–15 were measured by gel electrophoresis. Nominal PSS molecular weights are for the fully ionized, 100% sulfonated form.

The separation in gel electrophoresis occurs as charged polymers migrate through a fixed matrix of constraints (i.e., the gel) under the influence of an electric field. Several models for molecular motion in this setting have recently been advanced,[7-13] and these models will be tested against the data collected in the current study. Results from DNA electrophoresis have already been employed for this purpose,[4,14,15] but the experimental perspective with synthetic polymers is somewhat different and perhaps broader. Electrophoresis studies of star molecules, for example, are possible only with synthetic polymer systems. Comparisons of the dynamics of star molecules with those of their linear analogues should provide new insights into the appropriateness of "reptation" theories for the motion of entangled chains. This contribution will discuss only the behavior of highly charged, linear polymers; further contributions will address questions of molecular topology and heterogeneous chemical structure in the polymer chain.

We have performed most experiments with PSS samples prepared from narrow-distribution, anionically polymerized polystyrene (PS). PSS exhibits well-understood polyelectrolyte behavior[16,17] and has frequently served as a model compound in studies of the solution properties of charged polymers. In the first portion of this study, the narrow-distribution PSS samples have been employed as molecular weight standards to construct chain length/mobility calibration curves. Chain length and chain-length distributions have then been obtained from corresponding measurements of mobility distribution. In the second phase of this investigation, we have examined the mobility of PSS as a function of gel concentration, electric field strength, ionic strength of buffer, and molecular weight. Many of these experiments provide data that can be compared directly to predictions of the newer theoretical models for electrophoresis,[7-13] as well as the classical models, which are somewhat older.[18-20]

Experimental Section

Polymers. Nine PSS samples with narrow molecular weight distributions were purchased from Pressure Chemical Co. Six other PSS samples were prepared in our own labs from PS standards obtained from Polysciences, Polymer Laboratories, and Toyo Soda. Table I lists the properties of all the PSS samples and their unsulfonated parents.

Sulfonation of Polystyrene. PS samples were sulfonated by the method of Vink,[21] with two small modifications. First, lower PS concentrations (<0.1 g in 75 mL of cyclohexane) were employed to keep the polymer concentration below the critical overlap concentration. It was observed that such low concentrations were necessary to prevent formation of intermolecular cross-links during sulfonation. Second, to ensure that sufficient P_2O_5 was present to catalyze the sulfonation of PS, the mass of added P_2O_5 was increased to 1.5 times that called for in Vink's procedure. The water-soluble product of the sulfonation was dialyzed extensively against 0.0017 M NaCl and stored in a refrigerator.

The concentrations of the PSS solutions were determined by absorbance at 227 nm by using an absorbance/concentration calibration curve. The concentration standard for this curve was established gravimetrically, with polymer mass in the standard solution being measured after vacuum drying at 95 °C for 24 h. Concentrations of the final, dialyzed PSS solutions were typically in the range of 1000 ppm. From preliminary electrophoresis runs it became apparent that these PSS solutions degraded if left at room temperature for more than a day or two. Refrigerated samples were observed to degrade if kept for more than two or three weeks. Results tainted by possible PSS degradation will not be reported.

Degree of Sulfonation. Carbon-13 NMR was performed on a Varian XL-300 at 75 MHz to determine the degree of sulfonation. A peak at 144.0 ppm was assigned to sulfonate-substituted para carbons, while a peak at 152.3 ppm was assigned to phenyl carbons linked to the chain backbone. The ratio of the areas of these peaks provided the degree of sulfonation. Peak assignments were made by using the samples purchased from Pressure Chemical; these polymers displayed the same NMR peaks as the high molecular weight samples prepared according to Vink's procedure. A typical sample of the in-house materials, PSS 10, was 73.5% sulfonated. The sulfonations of the Pressure Chemical samples were higher, in the range of 80–95%.

The sulfonation levels of many of the PSS samples we prepared were not accessible as the mass of polymer in the final, dilute solution was insufficient for NMR measurements. The critical overlap concentration decreases with molecular weight so the problem was most acute for the highest molecular weight products. Samples subject to quantitative NMR were first recovered by lyophilization and then redissolved at 1 wt % in deuterated water. NMR run times were on the order of 12 h. As will be discussed later, we believe accurate knowledge of sulfonation levels to be unnecessary for understanding the electrophoretic behavior of PSS. Given the possible variation in sulfonation levels, however, the degree of polymerization N rather than molecular weight will be employed as a measure of chain length. The average degree of polymerization for the sulfonated polymer is assumed to be identical with that of its parent PS.

Equipment. Gels were cast in a horizontal electrophoresis submarine cell from Bio-Rad, and the electric field was provided by an Ephortec 500-V power supply from Haake Buchler. The power supply was operated in its constant voltage mode. Densitometry of stained PSS bands was performed with an ISCO Model 1312 gel scanner operating at its slowest scanning rate. To minimize the buildup of electrolytic products near the electrodes and to reduce temperature gradients, buffer was recirculated between the two buffer reservoirs with a Cole-Parmer Model 7520-00 pump. The conductivity of the buffer never changed over the duration of a run by more than 10% from its original value.

Preparation of Agarose Gels. The selected mass of SeaKem GTG agarose from FMC was dissolved in 200 mL of a Na_2HPO_4 buffer by heating the solvent and suspended powder to 95 °C. After slow cooling to 60 °C, the agarose solution was cast into a 15 cm × 15 cm gel tray. Sample wells were formed from a 15-well comb with 5.5 mm × 0.75 mm teeth. The gel was allowed to solidify at room temperature for an hour, and the comb was then carefully removed. Both the Na_2HPO_4 concentration and the mass of agarose added to the buffer were varied.

Electrophoresis. The gel tray was placed in the submarine cell, and 5.0-μL sample volumes (50 ppm PSS) were carefully pipeted into the wells. The buffer reservoirs were then filled with the buffer until the solution level was even with, but not covering, the top of the gel. The selected voltage gradient was imposed across the gel for 15 min before the power was turned off and the

gel covered with recirculating buffer. Electrophoresis was then resumed at the initial electric field strength for the remainder of the run. All experiments were performed at room temperature, 21.0 ± 1.0 °C.

The electric field was measured by inserting platinum electrodes in the gel at known spacing; the observed electric field matched well with the applied field (calculated from the dimensions of the gel) except when the applied field was small. In this case some discrepancy was noted, probably due to the complicated electric fields induced near the immersed platinum wires used to apply the potential drop. The electric field was always uniform throughout the gel.

Detection. PSS bands were visualized after staining in an aqueous solution of methylene blue (0.01 wt %) that had been adjusted to pH 4.0 by addition of glacial acetic acid. The gel was immersed in the dye solution for about 15 min and then removed and destained in distilled water for at least several hours; the best contrast was obtained after destaining overnight. Methylene blue is a cationic dye, so its binding to the negatively charged polymer is presumably driven by electrostatic interaction. The dye appears irreversibly bound to polymer, so the destaining procedure simply removes unbound dye from the agarose gel.

Polymer concentrations in the stained gel were determined by transmission scans at 580 nm. To obtain a stable and reproducible base line, the gels were covered with a thin, transparent glass plate. This scanning process enabled measurement of polymer concentration as a function of position for concentrations in the gel below 5 ppm. For these low polymer concentrations the optical density was linear in the local PSS concentration.

Size Exclusion Chromatography. A mobile phase containing 0.01 M sodium dodecyl sulfate and 0.1 M NaCl was pumped at 1.0 mL/min through three successive TSK PW columns, G6000, G5000, and G3000 (Varian). After elution from the columns, the injected polymer was detected at 227 nm by a Kratos Spectroflow 783 absorbance detector.

Results and Discussion

Molecular Weight Calibration. A calibration of an electrophoresis gel can be constructed, as in SEC, by measurements on molecular weight standards. Due to the lack of a comprehensive theory for gel electrophoresis, however, there is no assurance that a unique mapping of position onto chain length (or molecular weight) actually exists. Noolandi et al.[22] have predicted and observed that two different molecular weight DNA chains can migrate, in some circumstances, to identical positions in the gel. Problems of this sort can easily be detected experimentally if a full set of molecular weight standards is available, as in this study. For all conditions except one, which will be described later, we have found that position of a polymer fraction in the gel, as defined by the position of the peak in the densitometry scan, correlates with chain length for a homologous set of polymers.

The position of a polyelectrolyte in the gel can be expressed in terms of its mobility μ:

$$\mu = \frac{x}{Et} \tag{1}$$

Position is denoted x, while E is the electric field and t is the run time. In our experiments, run times are typically on the order of 5 h, x is of the order 15 cm, and E is of the order 2 V/cm. Mobility nearly always decreases with chain length, so the highest molecular weight fractions are found nearest the sample well at the completion of a run. A plot of the logarithm of PSS chain length versus mobility is displayed in Figure 1 for a set of typical run conditions.

The density of ionizable groups along the chain backbone might seem an important parameter in correlating the mobility of poly(styrenesulfonate) and similar, potentially heterogeneously charged polymers. If so, a plot such as Figure 1 would be useful only for uniformly charged samples. This project was initiated after recognizing that "counterion condensation"[23-25] might play a significant role in electrophoresis. In its simplest form, counterion condensation predicts that the effective linear charge density β, made dimensionless with the Bjerrum length l_b, is controlled by a parameter ξ:

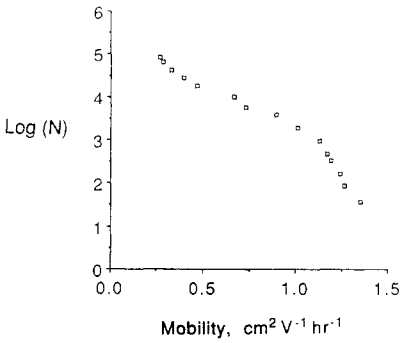

Figure 1. Calibration curve derived from narrow-distribution PSS samples. N is the degree of polymerization. The highest value of N corresponds to a molecular weight of 15×10^6 (0.6% agarose; 1.3 V/cm; 7.3 h; I = 0.03 M).

$$\xi = l_b/l_c \tag{2}$$

where l_c is the average spacing of charges along the chain. The Bjerrum length is calculated from the formula

$$l_b = e^2/\epsilon kT \tag{3}$$

in which e is the electron charge, ϵ is the bulk dielectric constant of the solvent, k is the Boltzmann constant, and T is the temperature.

In the linearized theory, first proposed by Manning[23,24]

$$\beta = \xi, \quad \xi < 1$$
$$\beta = 1, \quad \xi \geq 1 \tag{4}$$

For a highly sulfonated poly(styrene) chain in water (neutral pH), ξ is about 2.5–3.0, so the dimensionless effective linear charge density is independent of the actual degree of sulfonation. For less highly charged polymers ($\xi < 1$), variations in charge substitution will lead to variations in mobility. In accord with this prediction, we have observed an approximately linear dependence of mobility on the degree of carboxyl substitution for three lightly hydrolyzed polyacrylamides of identical molecular weight; experimental details of this work will not be reported here.

More complete theories for the electrostatic properties of charged polymers are available;[25] all these theories predict essentially the same plateau of β at high linear charge density as found with the linear theory of Manning. Experimental support for the principle of counterion condensation has been obtained in many studies, with perhaps the most convincing demonstration being the data measured by Whitlock using isotachophoresis.[26] Whitlock's contribution shows that electrophoresis experiments will be sensitive to the effective charge density β rather than the charge density calculated from polymer covalent structure. This result implies that the condensed ions are highly associated with the chain, acting hydrodynamically as if a part of the chain. Such behavior also follows from the theoretical analysis of Zimm[27] for the spatial disposition of the condensed ions.

The mobility data of Figure 1 have been successfully correlated with chain length despite measurable variations in the degree of sulfonation, a result in complete accord with the predictions of counterion condensation. Figure 1 can thus be regarded as a calibration curve from which the chain-length distribution of an unknown sample, run simultaneously with the polymer standards, can be determined. This side-by-side migration of unknowns and

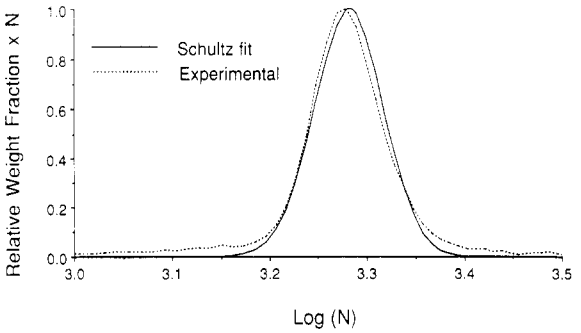

Figure 2. Molecular weight distribution of the narrow-distribution PSS standard PSS 7. Polydispersity of experimental curve = 1.02; Schulz fit polydispersity = 1.007. (The number-average chain length was held constant at the value in Table I during the fit.)

standards is necessary as preparation of two identical gels has proven difficult; the apparatus permits electrophoresis of up to 15 standards and/or unknowns at the same time.

Molecular Weight Distributions. Three chain-length distributions inferred by comparison of densitometry scans to calibration curves of the type shown in Figure 1 will be discussed, two involving fairly narrow distribution samples and the third involving a sample with a much broader distribution. To facilitate conversion of the measured concentration distribuitons in the gel to chain-length distributions, the mobility data for the standards are first fit to a cubic polynomial. Base-line corrections for each lane are applied by subtracting a densitometry scan of a corresponding sample-free section of the gel, and the well position for each lane is located by scanning across the entire gel with the densitometer; the entry face of the well shows up as a large "glitch" in such scans.

The chain length distribution of one of the standards purchased from Pressure Chemical, PSS 7 ($M_w \sim 354000$), is illustrated in Figure 2. The solid line indicates a best fit of the measurements to a Schulz distribution.[28] The degree of polymerization provided by the supplier is 1920, while that obtained by gel electrophoresis is 1908. This is not an absolute value, of course, since the measurement is relative to standards from the same source. The high resolution of gel electrophoresis is illustrated by the measured polydispersity of 1.02, compared to the "less than 1.10" polydispersity provided by the supplier. The polydispersity of the Zimm–Schulz fit is 1.007, well above the theoretical limit 1.0005 calculated from the formula

$$M_w/M_n = 1 + (N - 1)/N^2 \quad (5)$$

for the Poisson distribution associated with a perfect anionic polymerization.[28]

The accuracy of the measurement of polydispersity is probably limited by the finite ratio of the well thickness to the total distance migrated by the sample. In contrast to SEC, no band broadening (spatial dispersion) can arise in gel electrophoresis from convective transport; band broadening results from diffusion alone.[1] For higher molecular weight PSS samples diffusion over the time of an experiment is relatively unimportant as the associated diffusion coefficients are small. For the smallest PSS samples, however, some diffusion of the bands during electrophoresis and the staining procedures can be observed. Smeared and/or distorted bands are also produced when the concentration of PSS in the gel is too high or the electric field is too large. Small errors in calculating the polydispersity can also be traced to the difficulty in defining the starting and ending point of a stained polymer band in the gel.

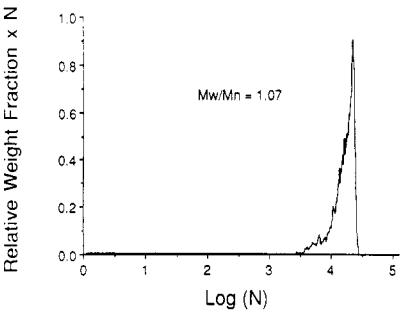

Figure 3. Molecular weight distribution of PSS 12.

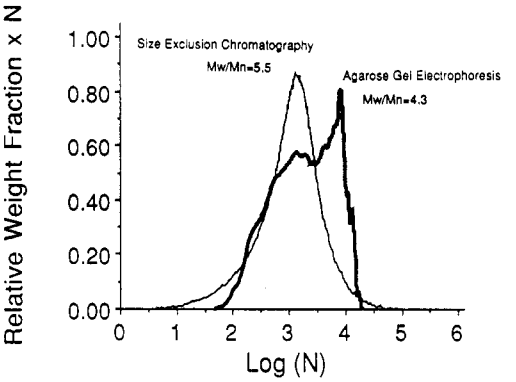

Figure 4. Comparison of the molecular weight distributions obtained by SEC and by agarose gel electrophoresis for a broad-distribution PSS sample.

A feature of gel electrophoresis that could be important to its application as an analytical technique is the relatively low slope of the chain length/mobility curve in its central region. In comparison to the corresponding region in SEC calibration curves, the low magnitude of this slope in gel electrophoresis reflects greater separation between similarly sized polymer fractions. On the other hand, the length of this optimal operating region is smaller in gel electrophoresis than in a well-configured set of SEC columns. The location of the linear portion of the calibration curve can be adjusted by varying gel concentration. (Effects of gel concentration will be more fully discussed in the next section.) The ease of this adjustment can be compared to the relative difficulty of selecting and installing SEC columns with an appropriate pore size for a given sample. Using techniques similar to the ones discussed in this article and the lowest concentration agarose gels that can be prepared (~0.1%), Fangman[29] has been able to separate DNA fractions with molecular weights up to 500×10^6.

The chain-length distribution of a second narrow-distribution sample, one prepared in our own labs, is shown in Figure 3. The nominal molecular weight of this sample is 5.2×10^6, and its polydispersity is 1.07. Similar polydispersities are measured for the other samples prepared in our labs, with nominal molecular weights ranging up to about 15×10^6. This series constitutes the first set of well-characterized synthetic polyelectrolyte standards with molecular weights greater than 1.2×10^6. Standards of this type should be useful in a variety of research problems such as the flow of solutions in porous media, flocculation of colloids, and electric birefringence. Previously, the only narrow-weight-fraction polyelectrolyte standards available in this size range were relatively fragile DNA fragments.

Finally, Figure 4 shows the application of gel electrophoresis to a sample with a broad chain-length distribution. The supplier (Polysciences) provides only a nominal molecular weight of 5×10^5 for this material, and the sample actually examined had previously been subjected

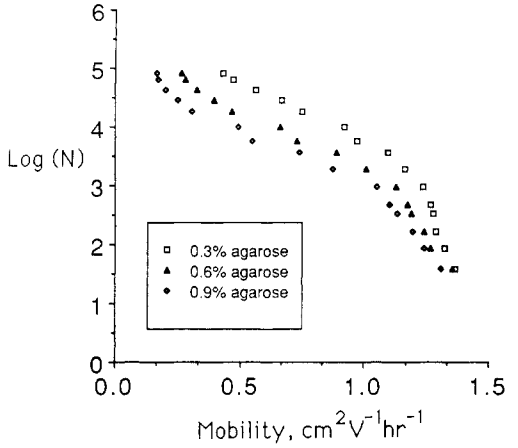

Figure 5. Mobility as a function of the degree of polymerization for three agarose concentrations (1.3 V/cm; 7 h; I = 0.03 M).

to an elongational flow experiment which may have caused some chain degradation. The same sample has been examined with SEC, and the result is shown in the figure. Both techniques provide similar polydispersities and average chain lengths, although there are some features at high molecular weight that are observed only in the gel electrophoresis data. We believe this discrepancy arises from the greater resolution of our gel electrophoresis analysis and that these high molecular weight features truly represent the chain-length distribution of the material.

Comparisons to Theory. There are four easily manipulated variables in gel electrophoresis: gel concentration, chain length, electric field strength, and ionic strength. We have systematically varied all four, comparing our results to the trends predicted by recent reptation-based theories for gel electrophoresis. Most comparisons of these theories to our experiments will be qualitative, as the pore structures of agarose gels are probably not well enough understood to accurately characterize in terms of the parameters appearing in models.

Gel Concentration. Experiments have been conducted at three gel concentrations: 0.3%, 0.6%, and 0.9%. Below this range agarose gels are so weak that reproducibility in gel preparation is difficult. At gel concentrations much above 0.9%, on the other hand, the migration of PSS samples is slow, producing impratically long run times. Figure 5 shows the effect of gel concentration on the relationship between chain length and mobility. As mentioned earlier, the linear range on these semilog plots is somewhat dependent on gel concentration, extending to higher molecular weight for lower concentration gels.

A more illuminating presentation of these data is shown in Figure 6, which displays mobility as a function of agarose concentration at constant chain length. Mobility always increases with decreasing gel concentration, and if the concentration is sufficiently low, this mobility increase is linear.[30] For higher molecular weight polyelectrolytes the linear regime begins at lower gel concentrations, explaining the nonlinearity in some of the curves in Figure 6. These trends can be understood in terms of the increase in average pore size, which is associated with a decrease in agarose concentration. Below a threshold gel concentration, which depends on the molecular weight, the flexible polyelectrolyte chain is no longer entangled in the gel matrix, and electrophoretic transport is more akin to that in free solution than in a dense gel. Above the onset of entanglement, electrophoretic transport occurs by a distinctly different mechanism, one tentatively identified as reptation.

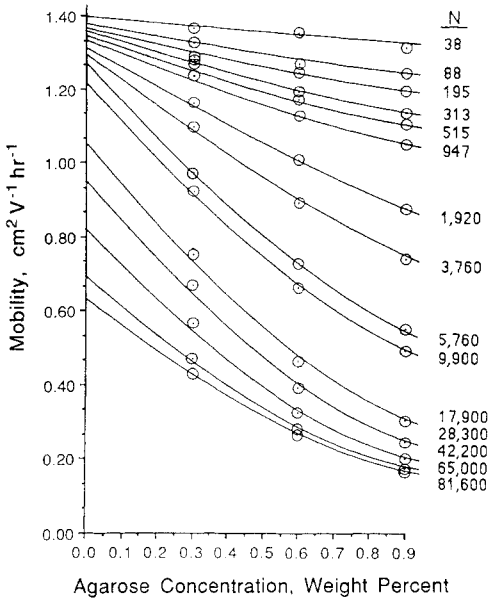

Figure 6. Mobility as a function of agarose concentration for the data of Figure 5. Degree of polymerization = N. The solid lines are solely for clarity of presentation.

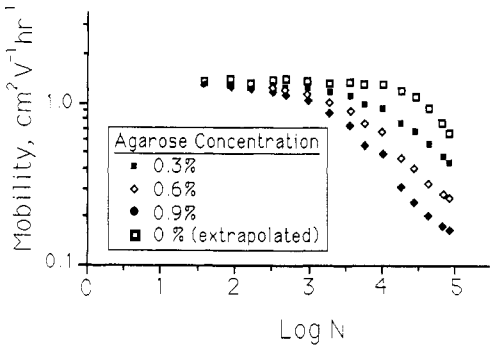

Figure 7. Mobility as a function of chain length for different agarose concentrations. Top curve is an extrapolation to zero agarose concentration using the data of Figure 6 (1.3 V/cm; I = 0.03 M).

Assuming that the data in agarose gels can be extrapolated smoothly to the zero gel concentration limit, we should be able to infer the free solution electrophoretic mobility of PSS as a function of chain length. Such extrapolated data are presented in Figure 7. The result is rather surprising—the apparent free solution mobility is chain-length independent over almost 2 magnitudes of chain length. At higher molecular weights the observed mobilities decrease, but these are the data extracted from nonlinear extrapolation of mobility with respect to gel concentration so this trend can be disregarded as a free solution result.

Chain-length-independent mobilities in free solution have previously been reported for other polymers,[31-33] and a semiquantitative theory for this behavior based on a porous sphere model was advanced by Hermans and Fujita over 30 years ago.[20] A chain-length-independent mobility appears naively consistent with the Rouse model, with both the electrical and hydrodynamic forces on the chain proportional to N. Zimm dynamics are obviously more likely and, in this case, predict that the mobility scales as $N^{0.5}$ (ignoring excluded volume). Hermans and Fujita proposed that the effects due to electrical forces on dissociated counterions could lead to molecular-weight-independent mobilities at high ionic strength. The ionic strength, 0.03 M, for the experiments illustrated in Figure 7 may be regarded as intermediate. A flexible coil model for po-

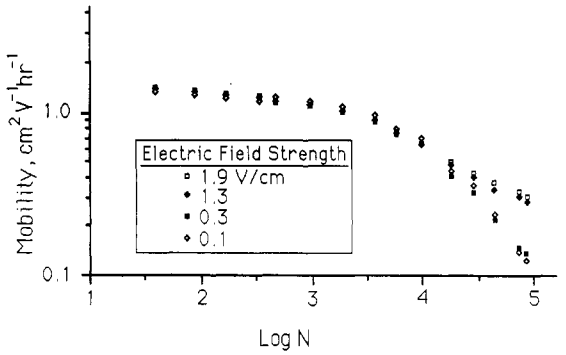

Figure 8. Effect of electric field on mobility (0.6% agarose; 4–36 h; I = 0.03 M).

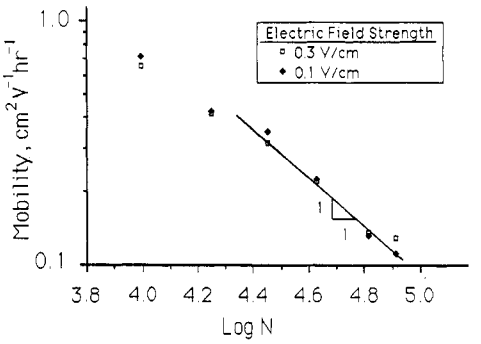

Figure 9. Mobility as a function of N for the six highest molecular weight PSS samples in Figure 8. The solid line has the slope −1, predicted by reptation theory for low electric-field strengths.

lyelectrolyte mobility in free solution is apparently unavailable.

Chain Length. In the strongly entangled limit encountered at high chain length, motion of polyelectrolyte chains in gel electrophoresis can be analyzed by using "biased" reptation models.[7-13] In the limit of low electric field strength, at which the polymer conformation is assumed to remain approximately Gaussian in the gel even with the field turned on, the Einstein relationship provides a link between the mobility and the center of mass diffusion coefficient D:

$$\mu = \frac{DQ}{kT} \tag{6}$$

where Q is the effective total charge on the chain expressed in terms of the charge on an electron. From reptation D scales as N^{-2}, and Q scales as N, so we obtain

$$\mu \sim N^{-1} \tag{7}$$

Plots of log mobility versus log N are presented in Figure 8 for four different electric field strengths. At the lowest two applied fields, 0.1 and 0.3 V/cm, the mobilities are, within experimental error, independent of field strength for the higher molecular weight samples. These data therefore represent the regime represented by eq 7. An expanded view of these data is presented in Figure 9, with a line of slope −1 fitted through the highest four molecular weights. The consistency between the predictions of the reptation model and experiment is clear. With some caution a high-field asymptotic behavior for the high molecular weight data of Figure 8 can be inferred; the slope of the 1.3 and 1.9 V/cm curves in this regime is close to −1/2. Further discussion of electric field effects is presented in the next section.

Electric Field Strength. Equation 7 is only proper in the zero-field-strength limit, for which the polymer coil is relaxed. At higher field strengths the coil becomes distorted by electrical forces; this distortion leads to mobilities that depend on the magnitude of the applied field. Lumpkin et al.[9] and Slater and Noolandi[11] have employed modified reptation models that incorporate deformation of the chain in the gel by a small electric field. The result is the addition of a field-dependent term to eq 7:

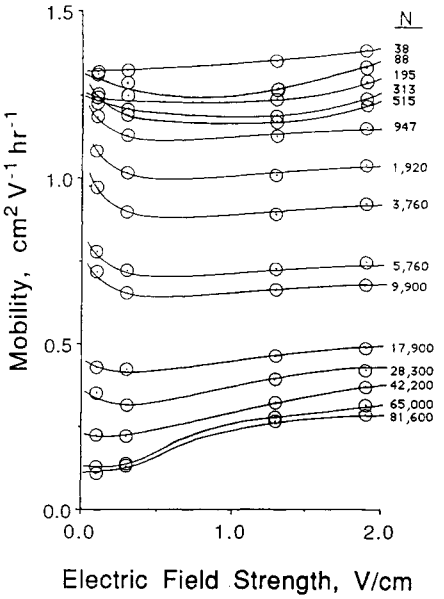

Figure 10. Mobility as a function of electric-field strength. Solid lines are for clarity of presentation (0.6% agarose; 4–36 h; I = 0.03 M).

$$\mu = \frac{DQ}{kT}\left[1 + \frac{NE'^2}{3}\right] \tag{8}$$

where E' is the dimensionless electric field strength given by

$$E' = \frac{aEQ}{2kT} \tag{9}$$

The mesh size of the gel, denoted a, has been assumed to be proportional to the segment length of the equivalent Gaussian chain representation of the polyelectrolyte in the gel. Alternately, the factor D/kT in eq 8 can be replaced by $(3N^2\zeta)^{-1}$, where ζ is the friction coefficient of a segment in the fictitious tube confining the chain.

From eq 8 and 9 we expect the mobility of a 5×10^6 molecular weight PSS in a 1% agarose gel to become field-strength dependent at electric fields greater than approximately 0.25 V/cm. This prediction is roughly in accord with our experimental findings for the high molecular weight PSS samples, as displayed in the bottom five curves in Figure 10 (which is a replotting of the data in Figure 8). The transition to field-strength-dependent behavior in these curves qualitatively follows the form of eq 8, although the necessity of comparing mobilities from several separately prepared gels induces significant uncertainty into this comparison.

Complicated, field-strength-dependent data have been observed for the smaller PSS samples in the same gels, as shown in the top curves of Figure 10. At the lowest field strength the mobility of the N = 515 sample is greater than the mobility of the N = 313 sample. A unique mapping of position onto mobility therefore does not exist in this chain-length range and at the specified field strength. The inversion of mobility for these two samples has been verified by repeated experiments at identical conditions. We have no explanation for the field-strength-dependent behavior of the lower molecular weight PSS samples.

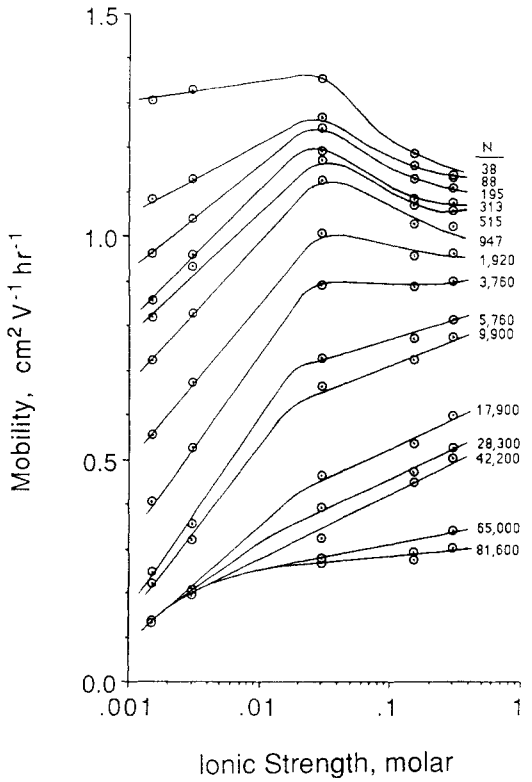

Figure 11. Mobility as a function of ionic strength. Solid lines are for clarity (0.6% agarose, 1.3 V/cm; 6 h).

Noolandi et al.[22] observed similar mobility inversion in DNA, which they ascribed to mechanisms related to "trapping" of chains in looplike states; this explanation predicts more significant trapping effects for higher molecular weight chains. In our experiments the inversion of mobility has been seen only with comparatively low molecular weight polymers.

We also note that the apparent high-electric-field plateau displayed in Figure 10 for the higher molecular weight samples reflects a mobility that is lower by at least a factor of 2 than the free solution mobility inferred in Figure 7. Slater and Noolandi[11] suggest that these mobilities should coincide.

Ionic Strength. None of the theories cited thus far can predict the ionic strength dependence of the mobility of polyelectrolytes in an entangled gel. The Hermans and Fujita theory was derived only for free solution, and the ionic-strength-mediated expansion of charged polymer coils was completely ignored. The reptation models implicitly assume that electrical forces act only on covalently bound and/or associated ions; counterion condensation predicts that the number of these ions is essentially independent of ionic strength, leading to a prediction from the reptation models of an ionic-strength-independent mobility. The reptation models also assume that the polymer conformation is Gaussian, with segment size equal to the mesh spacing of the gel network; this picture does not easily allow incorporation of polyelectrolyte expansion either by charge repulsion or by the excluded volume of the gel.

Experimentally, the mobility of PSS as a function of ionic strength is rather complex. Results at constant gel concentration and electric field strength are shown in Figure 11. For lower molecular weights a maximum in the mobility is observed with respect to ionic strength, while for higher molecular weights, the mobilities converge onto a universal curve at lower ionic strengths. This latter effect is strikingly illustrated in Figure 12, which presents a semilog plot of chain length verus mobility. At low ionic

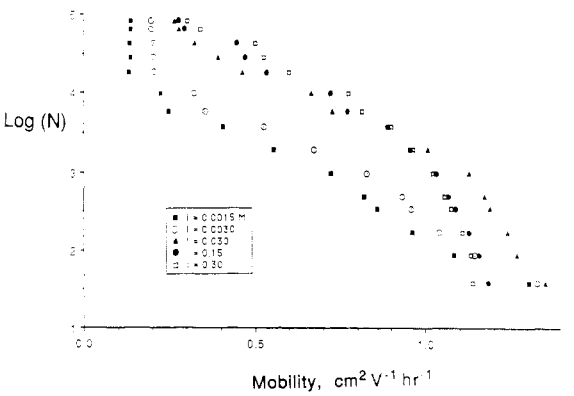

Figure 12. Mobility as a function of chain length for different ionic strengths. Note that mobility reaches a limiting value for the lowest ionic strength in the higher molecular weight range (0.6% agarose, 1.3 V/cm; 6 h).

strengths no separation with respect to chain length occurs. The transition to this mobility plateau is rather abrupt.

Explanations for all these effects are lacking, but their impact on analytical applications of electrophoresis are obvious. One might tentatively suppose that the interplay between the coil conformation as a function of ionic strength and the electrophoretic forces on comigrating counterions is important. These data will hopefully provide motivation for further theoretical analysis.

Comparison to Agarose Gel Electrophoresis of DNA. Stellwagen,[4] Hervet and Bean,[15] and Edmondson and Gray[14] have all provided much of the same type of data for electrophoresis of linear DNA as that just discussed for PSS. The trends with gel concentration, chain length, and electric field strength are quite similar to those we have presented. Significantly, the inverse chain-length dependence of mobility at high molecular weight and the molecular-weight-independent mobility in free solution have been well-documented. The ionic strength dependence of DNA electrophoresis has not been thoroughly examined. Hervet and Bean[15] and Ross and Scruggs[34] have observed a monotonic decrease in mobility with ionic strength, but the range of molecular weights and ionic strengths may not have been sufficiently broad to resolve the features seen here with PSS. In previous studies of the free solution electrophoresis of PSS and poly(vinyl sulfate),[35,36] evidence for a maximum in the mobility–ionic strength relationship was noted but not explained.

Conclusions

Agarose gel electrophoresis provides a new and perhaps unique tool for studying highly charged, high molecular weight polyelectrolytes. Use of the technique for polymer characterization is straightforward for highly sulfonated polystyrene and probably for other highly charged polymers as well. Efforts to extend these results to less highly charged, carboxyl-containing polymers are planned inasmuch as carboxyl-containing polymers constitute the largest class of polyelectrolytes. For these materials the complications of counterion condensation may be severe since the linear charge density of the chain may fall into an intermediate regime. This problem is often evaded in protein electrophoresis by overwhelming the native charge of the chain with the charges carried by an associating, anionic surfactant like sodium dodecyl sulfate.

Although reptation provides a reasonable picture of electrophoretic motion of long-chain polyelectrolytes in the highly entangled regime, theories for motion of shorter chains would also be helpful but are not available. The biggest puzzle is the ionic-strength dependence of mobility;

a proper accounting of counterion effects and polyelectrolyte coil expansion might help explain the observed behaviors. In this regard it may be useful to compare the conformation and mobility of the identical samples both in free solution and in a gel medium.

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Registry No. Agarose, 9012-36-6.

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Force between Surfaces That Confine a Polymer Solution: Derivation from Self-Consistent Field Theories

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ABSTRACT: Self-consistent field (SCF) theory for an incompressible polymer solution confined between parallel surfaces predicts the existence of a nonuniform hydrostatic pressure field to maintain the density constraint. The hydrostatic field is a local isotropic stress given by the excess chemical potential of the solvent relative to the adjacent bulk solution plus effects of nonlocal interactions between solvent and polymer segments. Based on the mechanical work to displace the surfaces, mean-field analysis of the polymer solution as a continuum also leads to the definition of a local deviatoric (shear) stress, which is derived from the equivalence of interfacial tension and the excess free energy of the gap solution (per unit area) established by SCF theory. The deviatoric stress arises from restricted equilibrium effects and nonlocal interactions. The sum of isotropic and deviatoric stress components defines the local axial stress aligned along the coordinate normal to the surfaces. The incremental work to displace surfaces with constant cross-sectional area yields the force per unit area on each surface, which reduces to the axial stress evaluated at the *midpoint* of the gap. For complete equilibrium without nonlocal effects, the force per unit area is simply given by the excess chemical potential of the solvent (relative osmotic pressure) at the center of the gap. The work per unit area (interaction potential) to assemble the surfaces from large separation to stable contact is the integral of the force per unit area over the full range of displacement. It is shown that changes in the interaction potential differ from changes in the excess free energy per unit area of the gap by the cumulated value of the change in stress field with respect to displacement (analogous to the $V\,dP$ term associated with the change in a Gibbs potential). This leads to a longer range interaction between surfaces than that predicted by the distance dependence of the excess free energy. Experimental data for interaction potentials between phospholipid bilayer surfaces in concentrated aqueous solutions of large nonadsorbent polymers correlate well with predictions of the mean-field analysis.

I. Introduction

Thirty years ago, Mackor and van der Waals analyzed the excess free energy of a solution of dimeric molecules confined between rigid surfaces.[1] Since then, self-consistent field (SCF) theories have been developed to predict excess free energy of polymer solutions confined between rigid surfaces. Both lattice mean-field models[2] and continuous space approximations[3,4] have been used to derive

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