Diffusion of Labeled Polyelectrolyte Probes in Unlabeled Polyelectrolyte Matrix Solutions

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Introduction. The diffusion of polymers in complex fluids is central to many industrial and biological processes. Fundamental understanding of the transport of polyelectrolytes remains limited due to difficulties in theory, simulation, and experiment. Unlike a neutral polymer in solution, a polyelectrolyte chain stays in close communion with a cloud of counterions that exactly balances its charge, maintaining electrical neutrality. Coulombic interactions dominate the physics of polyelectrolytes at low ionic strength, giving rise to a wealth of interesting behavior. One unusual feature is the "slow mode" or "extraordinary transition" that first became apparent in a dynamic light scattering (DLS) investigation[1] of poly-L-lysine, but which has since been observed in other systems, including sodium polystyrenesulfonate (NaPSS), which is the subject of the present work. For an entry to this controversial topic, see refs 2−4. Recently, the reversibility of the transition to slow mode behavior for a 100% sulfonated and never-dried sample of NaPSS was demonstrated, without involvement of filters, through the gentle process of in-situ dialysis DLS.[5] This shows conclusively that the putative aggregates require neither contact with hydrophobic materials nor the presence of hydrophobic patches, which can arise from incomplete sulfonation.[6,7] Such aggregates cannot be dismissed as poorly dissolved bits of matter.

Searching for features that might help define the nature of the temporal aggregates, Sedlak investigated solutions containing bimodal mixtures of polyelectrolytes having different molecular weights.[8,9] Three decay modes were found: a fast process representing the collective motion of polymer segments strongly interacting with rapidly diffusing ions in the osmotically stiff solution, a medium mode, and the slow mode. It is not easy to interpret these results, as is often the case when DLS is applied to solutions in which the scatterers interact strongly. NMR-based diffusion methods have been applied to low-salt solutions of a single polyelectrolyte.[10−13] They may seem to be ideal for the study of bimodal polyelectrolyte mixtures, but in practice the NMR signal from the dilute probe diffuser would be small compared to the strong background from the concentrated matrix. Worse, the ability to separate the probe and matrix signals of chemically identical polymers by rate of diffusion could change with the concentration of matrix.

Fluorescence photobleaching recovery (FPR) offers a practical alternative for the study of polyelectrolyte solutions.[14−17] This method, also called FRAP for fluorescence recovery after photobleaching, selectively detects a fluorescently labeled component by monitoring the recovery rate of optical gradients created in the sample after "erasure" of some fluorophores in a defined region or pattern. No change in the overall polymer concentration is induced by photobleaching, and polymers with trace levels of dye erased are very similar to those with their dye moieties still intact. As a result, and in contrast to DLS, which senses cooperative or mutual diffusion, FPR is relatively insensitive to thermodynamic interactions among the various components. The FPR diffusion coefficient closely approximates the self-diffusion coefficient of the fluorescently labeled species. In turn, this can be associated with the self-diffusion coefficient of unlabeled probe molecules if the dyes do not induce any conformational changes, which is the case for NaPSS.[15]

In this study, FPR is used to investigate the translational diffusion of labeled NaPSS in aqueous solutions containing a matrix of unlabeled NaPSS. Aided by a recently developed dialysis cell for FPR, the effects of matrix concentration, the molecular weight of LNaPSS, and added salt are examined. The study is limited to just one matrix molecular weight, which is appropriate for testing a hypothesis relating to the slow mode that will be described in the next section. Implications well beyond the slow mode will become apparent, too, even though variation of matrix molecular weight and other trappings of a full-scale probe/matrix/solvent investigation are beyond the scope of this work.

Background. It has been learned[5,18] that the FPR decay mode in low-salt polyelectrolyte solutions lies in between the DLS fast and slow modes but should not be regarded as the average of the two because of the fundamental differences in what the methods detect. The observations suggest that the aggregates must maintain integrity for at least 0.05 s, and that the residence time of a single chain in a temporal aggregate may be shorter than the FPR time scale (∼20 s). Narrowing this imprecise estimate is a challenging task for the future; meanwhile, further testing of the basic premise that molecules enter and leave the temporal aggregates many times on the FPR time scale seems appropriate. The temporal aggregates are thought to have a size that does not depend strongly on molar mass.[19] If sufficient amounts of them exist, and if labeled probe polyelectrolytes were to enter into stable (longer than the FPR recovery time, which exceeds 20 s in this work) associations with them, then the associated diffusion should depend only weakly on probe molecular weight. Conversely, the inverse relation of diffusion to molecular weight should remain or be exaggerated if probe molecules do not enter the temporal aggregates in significant numbers for significant times.

The problem at hand would be important even were it not for its role in understanding the slow mode. For reasons ranging from polydispersion to biocomplexity, polyelectrolytes are often found mixed with others. Yet it is single polyelectrolyte/solvent systems that have received most of the attention.[20−22] Although the selection of polymer molecular weights in this study is not extensive, reflecting its origin in slow mode phenomenology, it is possible to obtain a preliminary assessment of the behavior of diffusion in bimodal polyelec-

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Table 1. Properties of NaPSS Samples

sample	source	M_w	M_w/M_n	data source
NaPSS-6500	Polysciences, Inc.	6 500	<1.1	vendor
NaPSS-70000	SP^2 Inc.	70 000	<1.4	ref 15
NaPSS-77000	Polysciences, Inc.	77 400	<1.1	vendor
NaPSS-100000	Polysciences, Inc.	100 000	<1.1	vendor
LNaPSS-167000	fractionated from LNaPSS-70000	167 000	1.2	ref 5
NaPSS-170000	Polysciences, Inc.	178 000	<1.1	vendor
NaPSS-350000	Polysciences, Inc.	350 000	<1.1	vendor
NaPSS-680000	Polysciences, Inc.	680 000	<1.1	vendor
NaPSS-990000	Polysciences, Inc.	990 000	<1.1	vendor

trolyte systems. One may expect that the self-diffusion coefficient of labeled NaPSS probe (D) rises with added salt (c_s) and falls with matrix concentration (c_{matrix}), but details are lacking.

The following relations for *unimodal* polyelectrolyte solutions at low salt provide convenient landmarks, even though scaling laws for semidilute polyelectrolyte solutions are valid[23] only at high degrees of polymerization, N:

$$D \sim c^0 N^{-1} \quad \text{low salt, semidilute unentangled} \quad (1)$$

$$D \sim c^{-1/2} N^{-2} \quad \text{low salt, entangled} \quad (2)$$

where c is the concentration of polymer. At high salt, this becomes

$$D \sim c^{-1/2} c_s^{1/2} N^{-1} \quad \text{high salt, semidilute unentangled} \quad (3)$$

$$D \sim c^{-7/4 \text{ or } -7/5} c_s^{5/4} N^{-2} \quad \text{high salt, entangled} \quad (4)$$

The triple screening calculation of Muthukumar[20] results in a concentration exponent of −7/5 in the entangled, high-salt regime, while the scaling argument of Dobrynin, Colby, and Rubinstein[21] gives −7/4.

Experimental Section. a. Materials. The sources, molecular weights (M_w), and polydispersity (M_w/M_n) appear in Table 1. NaPSS with M_w of 70 000 is designated as NaPSS-70000; the labeled counterpart is referred to as LNaPSS-70000. Other molecular weights follow the same naming scheme. Labeling was accomplished through chlorination of narrowly distributed NaPSS in $POCl_3$ followed by the attachment of fluoresceinamine isomer 1. Additional details appear in the Supporting Information.

b. Instrumentation. Details of the FPR measurements were reported previously.[24] The important features are the use of periodic boundary conditions imposed by photobleaching in a striped pattern and the shallow bleach depth enabled by a modulation detection scheme. This practice also ensures that each diffuser in a multicomponent mixture (e.g., free polymer vs polymer bound in a stable aggregate) is assigned a single, separate exponential decay term. For sealed-cell FPR experiments, samples were loaded into a rectangular glass cell (Vitrocom) having a path length of 100 μm and flame-sealed. Dialysis FPR uses a cell[5] that sandwiches the sample between a microscope cover glass and semipermeable membrane capable of retaining the polymer while exchanging salts with an external bath.

c. Data Analysis. Except for LNaPSS-167000, which was fractionated from LNaPSS-70000, all other LNaPSS samples were prepared from narrowly distributed NaPSS standards; thus, the FPR contrast signal (ac amplitude) from the modulation detector should be a monomodal, exponential decay:

$$C(t) = \exp(-K^2 D t) + \text{baseline} \quad (5)$$

where the spatial frequency of the grating is $K = 2\pi/L$, with L representing the periodic distance in the pattern and D the apparent self-diffusion coefficient.

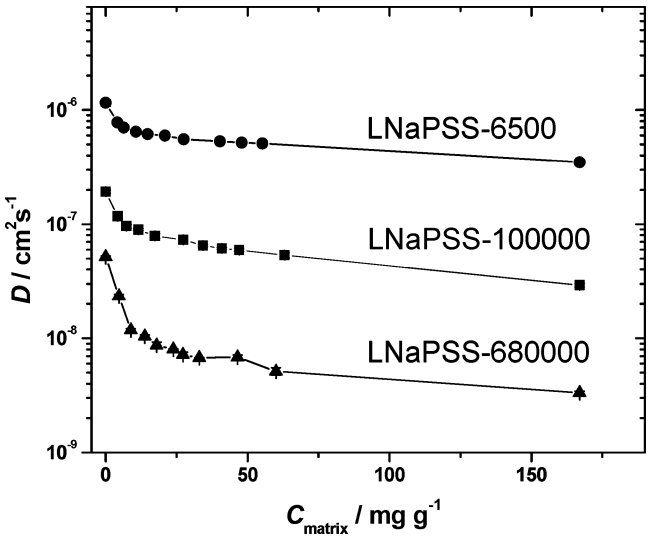

Figure 1. Dependence of self-diffusion coefficient of LNaPSS with indicated molecular weights in a matrix of NaPSS-990000 at low added salt. The concentration of LNaPSS was 0.68 mg/mL.

Results and Discussion. a. Nature of the FPR Signals. Systems without added salt showed two decay modes, the dominant one and a weaker, faster process comparable to the diffusion of free dye. The fast mode results from hydrolysis, as demonstrated by an increase in its significance after samples were heated to more than 100 °C to accelerate degradation. As the present work focuses on the dynamics of LNaPSS in a matrix, only the dominant decay process was used.

b. Diffusion of Labeled NaPSS in Matrix at Low Added Salt. The diffusion of LNaPSS in a polymeric matrix was investigated using sealed-cell FPR. LNaPSS-6500, LNaPSS-100000, and LNaPSS-680000 served as probes, while unlabeled NaPSS-990000 was the matrix. The better of the two dissolution schemes described in the Supporting Information, method 1, was used. Probe concentration was kept constant at 0.68 mg/mL, regardless of molecular weight, while the matrix concentration ranged from 0 to 167 mg/g of pure water.

In their study of NaPSS rheology, Boris and Colby[25] combined their own results with small-angle X-ray scattering, viscosity, and diffusion literature data[26−32] to produce a helpful plot (their Figure 4a) of overlap and entanglement concentrations for NaPSS, without added salt, as a function of degree of polymerization. At a high degree of polymerization, practically any solution satisfies the overlap condition. This includes the pseudosolvent (Supporting Information) that contains LNaPSS-680000 probe; it is "self-overlapping". Using $N \approx 4800$ for our matrix polymer, Figure 4a of ref 25 shows that all but perhaps the lowest of our matrix-containing solutions well exceed the expected entanglement concentration, which nominally starts at about 3.5 mg/mL.

As shown in Figure 1, the diffusion of LNaPSS probes declines steeply with added matrix at first, but much

more slowly once well into the regime of matrix entanglement. A log−log plot (Supporting Information) hides this apparent two-step decline and shows that the concentration dependence of diffusion obeys $D \sim c_{\text{matrix}}^{-0.50\pm0.01}$ for the largest probe. As this probe is similar in molar mass to the matrix polymer, which has a very high mass, it is not surprising that the expectation of eq 2 is met. It is difficult to interpret the results for the smaller probes. The two-stage decline evident in Figure 1 is intriguing. Simulations by Chang and Yethiraj[22] suggest that the size of a polyelectrolyte with no added salt declines with concentration and then levels off as semidilute conditions are reached. We do not have viscosity data on our solutions, nor is the measurement of salt-free, low-shear-rate viscosities a trivial matter; however, Figure 3 of ref 25 shows that the viscosity should be at least 100 times greater than solvent values at our highest concentration (about 0.8 monomer mol/L), even for a NaPSS sample with half the molecular weight of our matrix material. Yet our three probe molecules retain 30%, 15%, and 6% (from lowest molecular weight to highest) of their zero-matrix diffusion values. It follows that very large deviations from Stokes−Einstein, bulk fluid behavior must occur. There is scant evidence for a nonmonotonic decline in diffusion,[22] although one point does bump up slightly from the trend in the lowest curve of Figure 1.

By comparing measurements at the same matrix concentration for the three probes, D is seen to depend on probe molecular weight. As discussed in the Background section, this observation bears on whether and how long LNaPSS molecules are involved in any loosely structured temporal aggregates that may form. LNaPSS can be more than a 100-fold less concentrated than the matrix polyelectrolyte, so the chance of incorporating with matrix NaPSS-990000 aggregates should exceed that of forming aggregates with other LNaPSS macromolecules. According to Sedlak, the apparent mutual[33] diffusion coefficient of NaPSS-990000 in the form of aggregates made from polymer with ~1 million molar mass in our concentration regime should be $\sim 1.0 \times 10^{-9}$ cm^2 s^{-1}. Thus, one might have expected a diffusion coefficient with about this value, independent of probe molecular weight, if the probes were well incorporated into abundantly present temporal aggregates. Our findings do not agree with this hypothesis but are consistent with the earlier suggestion[5] that the lifetime of a chain in a temporal aggregate is perhaps 0.05−20 s, i.e., shorter than the time scale of the FPR experiment as practiced in this study. For most of time during an FPR recovery, LNaPSS diffuses as a single chain.

The molecular weight exponent, β in $D_{\text{probe}} \sim M_{\text{probe}}^{-\beta}$, can be estimated at various matrix concentrations. Although there are only three probe molecular weights, at least they span a wide range suitable for a provisional attempt. The exponent β rises from about 0.67 ± 0.1 in the absence of matrix to about 0.95 ± 0.05 at the highest concentration (not shown). The zero-matrix value is consistent, given the broad uncertainty, with an expanded random coil. In 200 mM nitrate, 10 mM phosphate buffer adjusted to pH 7, β was estimated[5] to be 0.59 ± 0.05 in dilute solution, similar to the DLS results from Tanahatoe and Kuil[34] ($\beta = 0.61 \pm 0.04$ at 100 mM salt). The high-c_{matrix} limit ($\beta = 0.95 \pm 0.05$) resembles the pulsed field gradient NMR results[10] of Oostwal et al. in single-polyelectrolyte solutions ($\beta \approx 1$ over a range of concentrations).

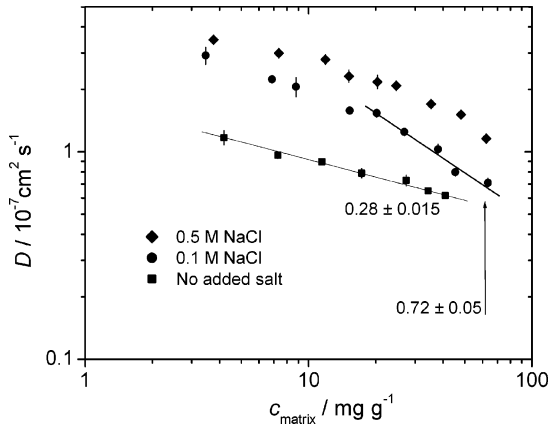

Figure 2. Effect of added matrix (NaPSS-990000) on the self-diffusion coefficient of LNaPSS-100000 (0.68 mg/mL) at various salt molarities, indicated.

c. Effect of Added Salt. Figure 2 shows sealed-cell FPR experiments on LNaPSS-100000 as a function of added matrix for three different salt conditions. At a given matrix concentration, the increased diffusion of LNaPSS with added salt can be understood qualitatively in terms of contraction of probe and matrix chains. (Even though the polymer chains do not obey the Stokes−Einstein relation, a small, salt-induced increase in water viscosity[35] must be overcome for the increase to occur.)

The increase is greatest at low matrix concentrations. At large matrix concentrations, added salt has less effect, possibly because the chains have provided enough counterions to "self-salt" the system or maybe because diffusion remains hindered despite chain contraction. The overall behavior is consistent with the trends implied by eqs 1−4 for single-polyelectrolyte solutions in the sense that the concentration dependence is generally enhanced by added salt, but scaling arguments fail to capture all the behavior when salt is present. For example, a line drawn through the last few points in the 0.1 M NaCl data set of Figure 2 does not fit the data at lower matrix concentrations.

d. Dialysis FPR Measurements. More about the effect of added salt on the diffusion of LNaPSS in a polymeric matrix was learned from dialysis FPR. The most important advantage of this approach is elimination of tedious procedures to prepare different samples with various salt concentrations. Dialysis FPR also minimizes sample drift and the probability of introducing differences while working up the samples. Another study showed that LNaPSS chains contract reversibly with added salt and established the time required to change conditions.[5] Figure 3 shows the effect of added salt on the self-diffusion of LNaPSS-167000 with or without matrix. Even a log−log representation was not linear, nor was a D vs $c_s^{1/2}$ plot. At any matrix concentration, the self-diffusion coefficient of LNaPSS-167000 increases with added salt. The presence of matrix at 7.8 mg/g, which exceeds the expected entanglement onset absent added salt but not by very much, reduces the diffusion coefficients across the board but more dramatically at low salt. In the opposite, high-salt limit, matrix polymer at 7.8 mg/g proves not much more restrictive to probe diffusion than no matrix at all. Perhaps probe and matrix contraction account for this; in any case, the rise in diffusion with salt is more dramatic than it was without matrix. The second matrix concentration shown, 29.7 mg/g, is deeper into the

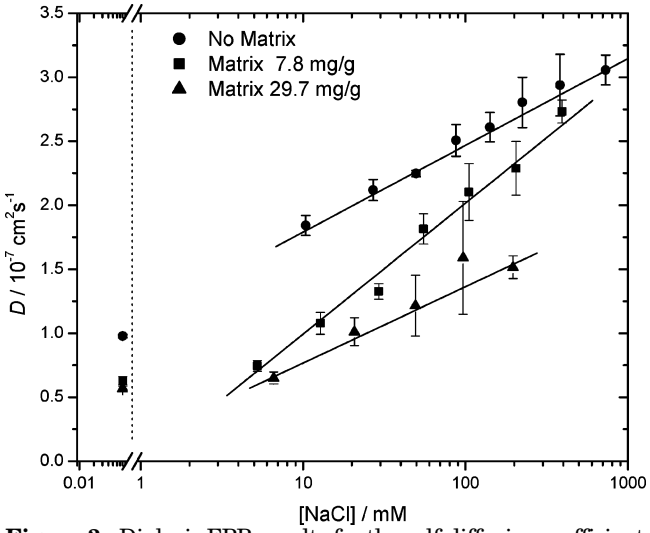

Figure 3. Dialysis FPR results for the self-diffusion coefficient of LNaPSS-167000 in a matrix of NaPSS-990000 as a function of added salt at fixed matrix concentrations, indicated.

entangled regime for the matrix at zero added salt. Apparently, this solution effectively impedes diffusion even at high salt; thus, the rate of increase of D with added salt is more modest than it was at 7.8 mg/g and similar to that in dilute solution. This is not the trend suggested by eqs 3 and 4 for single-polyelectrolyte solutions.

e. Effect of Dissolution Procedure. The behavior of LNaPSS in NaPSS-990000 matrix was affected by sample preparation. As discussed in Supporting Information, the hypothesized leveling of diffusion with matrix concentration, independent of molecular weight, can be observed when the samples are not properly aged. The implications for the structures that underlie the DLS slow mode are not obvious because DLS is more sensitive than FPR to their presence and because of the filtering extra steps normally taken to prepare solutions for DLS.

Conclusion. This first optical tracer diffusion study of bimodal polyelectrolyte solutions at low salt was designed to test a specific hypothesis concerning the residence of probe molecules in the structures that underlie the DLS slow mode. The hypothesis proved false, implying that large numbers of probe polyelectrolytes do not enter into the temporal aggregates established by the matrix or do not remain there for very long on the FPR time scale. This is consistent with other studies that compare the DLS and FPR behavior of polyelectrolytes at low salt.[5,14,18] Only in solutions that were not fully equilibrated was the hypothesized diffusion coefficient independent of probe molecular weight realized (Supporting Information).

Perhaps the byproducts of the study are more important than the original objectives concerning the slow mode. It has been learned that matrix retards LNaPSS diffusion strongly; however, as matrix concentration rises through the semidilute regime and beyond, the self-diffusion coefficient of LNaPSS declines more slowly. At equilibrium, in concentrated solutions containing matrix NaPSS with $M_w = 990\,000$, it was found that diffusion depends on probe molecular weight according to $D \propto M_w^{-0.95\pm0.05}$. The dependence of the probe diffusion on added salt was enhanced at an intermediate concentration of matrix polymer.

A stronger theoretical basis for diffusion in mixed polyelectrolyte solutions is required to guide additional experimental studies spanning a wider range of probe and matrix molecular weights. Those experiments can take advantage of improved labeling and purification practice.[36]

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Supporting Information Available: Details of labeling, fractionation, dissolution procedure, and effects thereof. This material is available free of charge via the Internet at http://pubs.acs.org.

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