Self-diffusion in solutions of a 20 base pair oligonucleotide: Effects of concentration and ionic strength

A. Wilk, J. Gapinski, and A. Patkowski
Faculty of Physics, A. Mickiewicz University, Umultowska 85, 61-614 Poznan, Poland

R. Pecora
Department of Chemistry, Stanford University, Stanford, California 94305-5080

(Received 28 July 2004; accepted 8 September 2004)

The long-time self-diffusion coefficients of a 20 base pair duplex oligonucleotide are measured as functions of 20-mer and added NaCl salt concentrations. The self-diffusion coefficients decrease monotonically with increasing 20-mer concentrations for the high-added salt sample and display non-monotonically decreasing 20-mer concentration dependences at lower added salt concentrations. The non-monotonic behavior is attributed to the opposing effects of the tendency to increase the interactions between 20-mers as the concentration is increased and to a decrease in the extent of the Coulomb forces as counterions from the 20-mer increasingly screen them. Attempts to account for the effect of the Coulomb forces on the self-diffusion coefficients by using effective dimensions in the hard rod theory give good agreement with experiment at the highest salt concentration studied. For the lower salt concentrations there appear to be two scaling regimes—one at low polyion concentration in which the high salt scaling of the rod dimensions by adding the Debye screening to the length and diameter of the rod is appropriate and one at high polyion concentrations where the scaling of the dimensions is the addition of 1/2 the Debye screening length. Estimates of the “overlap” concentration $C^* = 1/L_{	ext{eff}}$ indicate that the non-monotonic decrease occurs at concentrations lower than $C^*$. Finally, the fluorescence correlation spectroscopy self-diffusion coefficients measured here are compared with the mutual diffusion coefficients measured by dynamic light scattering. © 2004 American Institute of Physics.

[DOI: 10.1063/1.1811599]

I. INTRODUCTION

The diffusive rates of macromolecules and particles in nondilute suspensions are important factors in a wide variety of chemical processes in both industrial and biological systems. In such systems, macromolecules or particles are usually present at concentrations such that they significantly interact with each other. On the other hand, diffusive motions in infinitely dilute dispersions have been studied for many decades and, except perhaps for relatively small effects in systems with unscreened Coulomb forces, are largely understood. For rigid macromolecules and particles of regular shape—spheres, ellipsoids of revolution, and rods relations among the solution dimensions, the solvent viscosity and diffusion coefficients (both translational and rotational) are widely known. Measurements of the diffusion coefficients and viscosity are commonly used with these “hydrodynamic” relations to determine the solution dimensions of macromolecules and particles approximating these shapes. More recent advances in hydrodynamic modeling have provided computer programs to calculate the translational (and rotational) self-diffusion coefficients for large rigid molecules of arbitrary shape.

Rigid spherical particles have been extensively studied in nondilute liquid dispersions. There is much less known, however, about nondilute dispersions of nonspherical macromolecules and particles, especially charged systems in which Coulomb forces are important. Few measurements have been made, for instance, of the concentration dependence of the self-diffusion coefficients of highly charged rod-like molecules in solution. Most measurements on these systems are of the mutual diffusion coefficients using the dynamic light scattering-photon correlation technique (DLS). Measurements of self-diffusion have been done on rods with large aspect ratios and inevitably include complications of flexibility, or polydispersity or both. The most extensive measurements of the self-diffusion coefficients in a dispersion of rodlike polymers is of a series of uncharged helical polypeptides and do not include effects of long-range Coulomb forces.

We present here measurements of the long-time self-diffusion coefficients of a 20 base pair double-helical B-DNA oligonucleotide (20-mer) whose solution dynamics and structure have been previously studied in our laboratories by DLS, static light scattering, small angle x-ray scattering, and Fabry–Perot interferometry. The 20-mer is essentially monodisperse and is very rigid. No previous information on the self-diffusion of such well-defined systems over wide concentration and ionic strength ranges is available. We use fluorescence correlation spectroscopy (FCS), a technique that was first developed more than three decades ago, but has only recently come to be extensively applied to a wide range of problems. The renaissance of FCS is due to vast improvements in the experimental apparatus available. One of the most important of these is the...
use of confocal microscopy in commercial equipment to focus the incident laser beam to a very small volume and to accept only fluorescence emitted from this volume, thus greatly reducing unwanted background light and increasing sensitivity.

Other modern techniques that may be used to measure the long-time self-diffusion coefficient are fluorescence recovery after photobleaching and pulsed field gradient spin echo NMR. The major advantage of FCS over these other methods is its sensitivity. Self-diffusion coefficients may be measured at nanomolar concentrations of fluorophores and even at single molecule levels.

The 20-mer has been extensively characterized. Its solution dimensions have been determined at low 20-mer, high-added salt concentrations from DLS measurements of the rotational and translational diffusion coefficients. The dimensions, modeling the 20-mer as a rigid rod, are length \( L = 6.8 \text{ nm} \) and cross section diameter \( d = 2.0 \text{ nm} \), very close to the x-ray dimensions determined for the solid. The diameter represents the distance to the outer extent of the phosphate groups. Water of hydration is presumably contained mainly in the grooves of the double helix. The aspect ratio \( p = L/d = 3.4 \) is relatively modest. It is likely that rods of such low aspect ratios will not exhibit many of the dramatic entanglement effects predicted at high rod concentrations for rods of much larger aspect ratios (severe hindrance of diffusion perpendicular to the rod long axis, formation of mesophases, etc.).

Such well-characterized rigid rods can, however, be used to determine the effects of deviations from spherical shape on the behavior of colloid and polymer dispersions. The effects of Coulomb forces may also be readily determined. DNA is one of the most highly charged polymers known. The negative ion of the 20-mer has a charge of 42 electron charges. This charge is screened to varying degrees by counterions and added salt, so that a 20-mer in solution at high-added salt in a slightly basic solution may be expected to behave in many ways such as an uncharged hard rod. Under the conditions of the experiments reported here the effective charge of the polynucleotide was determined by our previous DLS experiments to be \(-7e\).

II. THEORY

Diffusion coefficients in nondilute dispersions depend upon the time scale of observation of the diffusion process. The short-time diffusion coefficient is the diffusion coefficient on a time scale short compared to the time it takes a particle to diffuse a distance comparable to its characteristic size (the radius for a sphere). The short-time self-diffusion coefficient is usually most strongly influenced by hydrodynamic interactions among the particles. The long-time self-diffusion coefficient is the diffusion coefficient determined at times long compared to the time it takes a particle to travel its characteristic dimension. On this time scale a particle is hindered by direct (nonhydrodynamic) interactions with other particles as it attempts to escape the “cage” formed by them. In FCS experiments on small polymers, the long-time self-diffusion coefficient is usually measured.

The self-diffusion coefficient (or more properly, the “interdiffusion coefficient”) in a three-dimensional system is the mean square distance traveled by a particle in a time \( t \), divided by \( 6t \). The mutual diffusion coefficient measures the decay of a concentration fluctuation and, unlike the self-diffusion coefficient, is proportional to a thermodynamic driving force (the osmotic compressibility) in the long wavelength limit. The mutual diffusion coefficient in this long wavelength limit is given by

\[
D_m = (1 - \Phi)\left(\frac{\partial \pi}{\partial c}\right)_{P,T} \frac{1}{\mu m},
\]

where \( \phi \) is the solute volume fraction and \( c \) the solute number concentration, the osmotic compressibility is denoted by \( (\partial \pi/\partial c)_{P,T} \) and \( f_m \) is the mutual friction coefficient. The exponent \( n = 0 \) or 1 depending on the frame of reference for \( f_m \). Vink, however, obtains \( n = 2 \). In any case, Eq. (1) gives the mutual diffusion coefficient as the product of a solution thermodynamic factor and the inverse friction coefficient.

The Einstein relation relates the self-diffusion coefficient to a friction coefficient for self-diffusion \( f_s \),

\[
D_s = \frac{k_B T}{f_s}.
\]

Note that Eq. (2) for the self-diffusion coefficient does not contain an explicit thermodynamic factor. The relative values and concentration dependencies of the mutual friction coefficient \( f_m \) and the self-friction coefficient \( f_s \) are outstanding questions in polymer and colloid dynamics.

The friction coefficients increase with increasing particle or macromolecule concentration for neutral particles and polyelectrolytes with high amounts of added salt. For systems with repulsive forces, the osmotic compressibility increases with increasing concentration. At infinite dilution \( D_s = D_m \). We have previously measured \( D_m \) for the 20-mer studied here. In dilute high salt solutions, Eimer and Pecora have shown that the Tirado–García de la Torre equations for rigid cylinders give a consistent description of the translational and rotational diffusion coefficients of oligonucleotides of low aspect ratio.

As the concentration is increased, the mutual diffusion coefficient usually increases (see, however, Ref. 48) while the self-diffusion coefficient is expected to decrease. For small volume fractions the self-diffusion coefficient may be expanded in a power series in the particle volume fraction \( \Phi \),

\[
D_s = D_0[1 - \alpha \Phi + O(\Phi^2)],
\]

where \( D_0 \) is the self-diffusion coefficient at infinite dilution. For the oligonucleotides the \( D_0 \) should be the same as the mutual diffusion coefficient extrapolated to zero volume fraction, and, as noted above, may also be calculated from the Tirado–García de la Torre relations and the rod dimensions.

The initial slope \(-\alpha\) Eq. (3) has been calculated for hard sphere colloidal particles in the absence of hydrodynamic interaction (HI) between the spheres (\( \alpha = 2 \)) and in the presence of HI (\( \alpha = 2.10 \)). This indicates that HI may not be very important for the long-time self-diffusion coefficient of rods of low aspect ratio. In any case, Dhont, Van Bruggen, and
Briels\textsuperscript{51} have performed a variational calculation of $\alpha$ for hard rods without HI. They find the dependence of $\alpha$ on the rod aspect ratio is given by

$$\alpha = 2 + \left( \frac{10}{3} \right) (p - 1) + \left( \frac{1}{3} \right) (p - 1)^2.$$ \hfill (4)

Note that when $p = 1$, $\alpha$ reduces to the correct value for a sphere without hydrodynamic interactions. Equation (4) is in good agreement with Löwen’s\textsuperscript{31} Brownian dynamics simulations on suspensions of hard rods.\textsuperscript{52} For the 20 base pair oligomer (20-mer) at high salt and the hydrodynamic solution dimensions given above, Eq. (4) yields $\alpha = 2.86$. Expressions for the coefficients of higher order terms in the expansion in Eq. (3) have not yet been reported. These terms could be important at some of the higher concentrations studied. We, however, neglect any such contributions in this work. In any case, the most novel behavior found here occurs at relatively low volume fractions.

Since the systems here are not hard rods, Eq. (4) is not expected to be a good approximation (except perhaps at high salt concentrations where there is considerable screening of the electrostatic forces. The Coulomb forces may often be accounted for in an approximate way by renormalizing the rod dimensions so that the rods act as hard rods but with larger effective dimensions than those obtained from hydrodynamics of the dilute rod system at high salt or dimensions determined from imaging techniques. The method we use here for doing this has been described previously.\textsuperscript{23} In short, the effective length $L_{\text{eff}} = L + \kappa^{-1}$ and $d_{\text{eff}} = d + \kappa^{-1}$ where $L$ and $d$ are the hydrodynamic dimensions and $\kappa^{-1}$ is the Debye screening length defined here as

$$\kappa^{-1} = 1/\sqrt{4\pi L_B (n_p Z_{\text{eff}} + 2 n_s)},$$ \hfill (5)

where $L_B$ is the Bjerrum length, which in water at 20°C is equal to 0.71 nm, $n_p$, $n_s$ are the number concentrations of polion and added salt, respectively, and $Z_{\text{eff}}$ is the effective charge of the polion.

Branka and Heyes\textsuperscript{43} and Kirchhoff, Löwen, and Klein\textsuperscript{54} have performed Brownian dynamics simulations on dispersions of charged rodlike particles. Branka and Heyes use a site-site Yukawa potential with two sites on a molecule with the distance between the two sites in a molecule that is a parameter of the model. Simulations were done for variable anisotropy of the particle diffusion coefficients. Their simulation corresponds to low salt, but the salt concentration dependence is not studied. Kirchhoff, Löwen, and Klein\textsuperscript{54} present a model that divides the rod into many sites and also use a Yukawa site-site intermolecular potential. The molecular length and charge are chosen to simulate a dispersion of tobacco mosaic virus (TMV) particles. A notable result of these simulations is that the long-time translational self-diffusion coefficient of the simulated TMV at zero added salt concentration is a nonmonotonic function of the polion concentration.

III. EXPERIMENT

A. Sample

The oligonucleotide used in this study is a 20 base pair synthetic B-duplex DNA with the primary structure of $5’$-CGT ACT AGT TAA CTA GTA CC-3’. The DNA was purchased from the Midland Certified Reagent Company, Midland, Texas. Catalog CO-1000, Lot 230303-0080B. It was synthesized by the phosphoramidite chemistry method and purified by anion-exchange high-pressure liquid chromatography. After purification, the pooled fractions were desalted by gel filtration. The product was supplied as the lyophilized sodium salt of DNA. The oligonucleotide is of greater than 99.9% monodispersity as verified by capillary gel electrophoresis with detection by ultraviolet absorption at 260 nm and mass spectroscopy. Melting curves show that the oligonucleotide is in double helical form under the solution conditions used in this study. The molecular weight is 13022 g/mol for the double helix. With 20 negatively charged phosphate groups in each polynucleotide chain, the double helix has 42 negative charges if fully ionized. In solution the effective charge is much less due to counterion condensation.\textsuperscript{23} The extinction coefficient used in the determination of the oligonucleotide concentration at 260 nm was assumed to be 260 (mM)$^{-1}$cm$^{-1}$=20 (mg/ml)$^{-1}$cm$^{-1}$, as usually used for a double helical form of DNA. All buffer solutions were made with fresh 18.2 MΩ water from a MilliQ-UV system. Buffer exchanges were performed 7–10 times from 4 to 0.1 ml using a Millipore concentrator to produce the desired salt concentration. Concentrations were measured by UV spectrometry at proper dilution (where absorbance is between 0.1 and 1) using the extinction coefficient at 260 nm given above. For FCS studies another batch of the same oligonucleotide labeled with a fluorescent dye (TAMRA-carboxytetramethylrhodamine) attached to the 5’ end were also purchased from Midland (Lot 872801-0094H).

B. Fluorescence correlation spectroscopy

FCS studies were performed using a Confocor2 (ZEISS, Germany), a commercial FCS spectrometer. A 5 mW 543 nm HeNe laser was used to excite the dye labeling the 20-mer. The nonlabeled sample after dialysis to appropriate buffer was concentrated to the desired maximum concentration and placed in the measuring chamber. The sample volume was not smaller than 50 μL to avoid substantial concentration change due to evaporation. Before the measurement a small portion of the labeled sample suspended in the same buffer was added to achieve the tracer concentration at a desired level of about 10 nM. Further concentrations were obtained through dilution with a 10 nM solution of labeled sample in appropriate buffer, so that the tracer concentration was maintained constant. Every two to three steps a small portion of the sample was taken out to check the concentration spectrometrically. Due to the relatively small chamber volume (0.5 ml), when the sample volume exceeded 0.4 ml, the next concentration was prepared in another chamber. One of the eight chambers in the sample holder was used to hold the 10 nM solution of the labeled sample, which served as a reference.
(infinite dilution). After each measurement the reference sample was also measured and only normalized results were further analyzed.

We used the software and theoretical model of the FCS correlation function implemented in the instrument software. The FCS correlation function $G(t)$ is given by

$$G(t) = 1 + \frac{1}{N} \left( \frac{T}{1-T} \exp(-t/\tau_1) + \sum_{i=1}^{n} \frac{A_i}{(1+t/\tau_i)\sqrt{1+c^{-2}(t/\tau_i)^2}} \right),$$

where $N$ denotes the average number of fluorophores in the illuminated volume, $\tau_1$ is the lifetime of the triplet electronic state of the fluorophore, $A_i$ represents the fraction of the $i$th component in a mixture of fluorophores ($\sum A_i = 1$), $c$ is the aspect ratio of the confocal volume, and $\tau_i$ is the correlation time corresponding to the diffusion time of the $i$th component through the normal cross section of the beam waist. The relation between the radius of the laser beam at the waist and the self-diffusion coefficient of the $i$th component and the transit time is

$$w^2 = 4 D_T \tau_i.$$

The experimental correlation functions initially were analyzed by means of a single-component model ($n = 1$) with a triplet contribution. Due to systematic errors in the short time region (up to $\sim 10 \mu$s) we decided to use a modified version of Eq. (6) where the triplet contribution has two components,

$$G(t) = 1 + \frac{1}{N} \left( \frac{T_1}{1-T_1} \exp(-t/\tau_1) + \frac{T_2}{1-T_2} \exp(-t/\tau_2) \right) + \frac{1}{(1+t/\tau)\sqrt{1+c^{-2}(t/\tau)^2}}.$$

The presence of another triplet-like process might arise by the richer chemical environment of the dyes that are no longer suspended freely in the solvent but covalently attached to the oligomers. The use of two components is a relatively good approximation of a continuous distribution of correlation times arising from transitions to and from the triplet state. We carefully checked that regardless of the fitting model the relative changes of the oligomer diffusion time were practically identical provided that the same model was used for the sample of interest and for the reference one. Even replacing the second triplet with a second diffusional component of unphysical correlation time ($\sim 6 \mu$s) leads to the same results. This is understandable because the correlation time of the oligomer diffusion is always longer than 100 $\mu$s—almost two decades away from the triplet contribution—and with the relative amplitude $\sim 10$ times greater than that of the triplet(s).

The laser power was maintained at very low level ($\sim 30$ $\mu$W corresponding to $\sim 40$ kW/cm$^2$) in order to avoid heat and photochemical effects due to absorption. The measurement time was typically set to 30 min divided into 1–2 min runs. Such long accumulation times were necessary in order to make the many parameter fits reliable.

Absolute measurements of diffusion properties using FCS require a calibration standard with a known diffusion coefficient that is labeled with the same dye as the sample of interest. The dye should be attached preferably in a chemically similar neighborhood as in the sample of interest to maintain its spectral properties. Calibration of the confocal volume is strongly wavelength dependent and might also be a function of the laser power because of the extremely high power density in the focus tuned to the absorption band of the dye. For larger particles the dependence of the effective confocal volume on the particle size must be taken into account.

Since in further analysis only relative changes were considered, attempts to obtain absolute values of the diffusion coefficients were restricted to simple comparison of the reference sample (diluted labeled oligomer) with rhodamine 6G ($D_0 = 2.8 \times 10^{-6}$ cm$^2$ s$^{-1}$) to ensure that the ratio of the correlation times corresponds to the ratio of the diffusion constants (measured in previous DLS studies for the oligomer). Such a test was necessary to confirm that the tracer represents the oligomer of interest and not oligomer fragments or aggregates. In all other measurements the ratio of actual diffusion coefficients to $D_0$ was assumed to be given by the reciprocal ratio of the correlation times measured for the particular sample and the reference sample. The calibration with rhodamine 6G was repeated every 2–3 h to ensure the system stability.

IV. RESULTS AND DISCUSSION

The self-diffusion coefficients for the 20-mer divided by the infinite dilution value ($D_0 = 1.07 \times 10^{-6}$ cm$^2$ s$^{-1}$) are plotted in Figs. 1(a) (linear) and 1(b) (ln) vs the weight concentration of 20-mer for four added salt concentrations. Also shown in Figs. 1(a) and 1(b) is the theoretical result of Dhont et al. for hard rods [Eqs. (3) and (4)] but expressed in terms of the weight concentration rather than the volume fraction. (The conversion assumes $L = 6.8$ nm and $d = 2$ nm). For the three low added salt concentrations the concentration dependence of the self-diffusion coefficient is more complicated. There is an initial steep decrease in $D$ with the behavior at the lowest salt concentration being the most striking. The decrease in $D$ is not monotonic. After passing through a relative minimum and a maximum, the decay is almost parallel to that at high salt. These features observed at very low polymer concentrations illustrate the advantages of FCS over alternative techniques to precisely measure diffusion coefficients at very low concentrations. A similar nonmonotonic decay is found in the simulations of Kirchhoff, Löwen, and Klein at zero added salt for a much longer rod.

We discuss the implications of these results in terms of renormalized hard rod models. Even though this approach is far from rigorous (especially at low added salt concentrations), it does give some insight into the effect of charges on the self-diffusion of rods in a very broad concentration range. Since the hard rod models are expressed in rod volume fractions, a key element of our analysis is the estimation of effective rod volume fractions for charged rods.
A. High added salt solutions

For the highest added salt concentration ($C_s = 200$ mM), the decrease in $D$ is initially linear but with a steeper slope ($\alpha = 6.7$) than that given by the hard rod formula of Dhont et al. ($\alpha = 2.86$ for $L/d = 3.4$), indicating a substantial contribution of Coulomb forces. The Debye screening length at 200 mM added salt is $\kappa = 0.67$ nm. This results in an aspect ratio $p = 2.8$ and an effective volume

\[ V_{\text{eff}} = 49.8 \text{ nm}^3, \]

which is larger than the hydrodynamic volume $V_h = 21.4 \text{ nm}^3$. The slope of a plot of $D/D_0$ vs the effective volume fraction yields $\alpha = 2.9$, in reasonable agreement with the prediction of $\alpha = 2.62$ by the Dhont et al. theory for hard rods of this aspect ratio. Thus, the renormalization of the rod dimensions in conjunction with the hard rod theory is a good approximation for the 200 mM added salt solution.

The experimental $\alpha$ at high salt obtained from Fig. 1(a) is consistent with the value obtained using PFG-NMR by Lapham et al. for a 24-mer at 100 mM added NaCl: $\alpha = 6.8$. They also obtain $\alpha = 4.4$ for a 12-mer and 8.3 for a 14-mer under the same conditions. The $\alpha$ for the 14-mer is larger than that for the 24-mer, which may indicate some systematic error in this value.

For a cylinder the fractional volume correction due to a small increase in diameter and length by $\Delta h$ is equal to

\[ k = \frac{4h}{d} \left(1 + \frac{\Delta h}{L} \right). \]

At 2 M ionic strength $k = 0.215$ nm, so the correction for the 20-mer $k = 44\%$ even at this high ionic strength, which is probably not experimentally attainable due to aggregation and precipitation of DNA molecules. Thus, it is not likely that results consistent with the theoretical prediction of Dhont et al. for hard rods could be obtained for small duplex oligonucleotides without using renormalized dimensions.

B. Low added salt solutions

At the lowest added salt solution the Debye screening length depends very strongly on the concentration of counterions and thus on the polion concentration. This behavior is shown in Fig. 2(a) where $\kappa^{-1}$ is plotted vs polion concentration for all added salt concentrations. This results in a nonmonotonic dependence of the effective volume fraction on the polion concentration, which is shown in a ln-ln plot in Fig. 2(b) and a linear-ln plot in Fig. 2(c). The maxima in...
Figs. 2(b) and 2(c) for the lower added salt concentrations result from a competition between an increase in the polyion concentration and a decrease in the screening length due to increasing counterion concentration. In other words, despite the increase in number concentration (increasing $C_p$), the strong decay of the effective dimensions may, for some combinations of $C_p$ and $C_s$, dominate the concentration dependence of the effective volume fraction. Of course, in the limit of high $C_p$, $\kappa^{-1}$ becomes small and the effective volume fraction follows the “true” volume fraction proportional to $C_p$. For all calculations of $\kappa$ we used $Z_{\text{eff}}=7$, a value obtained from fits to the mutual diffusion coefficient concentration dependence to prediction of the coupled-mode theory. 23
Although the measurements in Ref. 23 were performed for solutions of NaCl concentration in the range of 3–203 mM, we expect the value of the effective charge to not change very much at lower salt concentrations since the increase of the charge is limited by the value given by the Manning counter-ion condensation theory ($Z_{\text{eff}}=8$).

In order to test the simple scaling of the volume fraction using the effective size ($L_{\text{eff}}=L+\kappa^{-1}$ and $d_{\text{eff}}=d+\kappa^{-1}$) as often used in the literature, we plotted our experimental results for the self-diffusion coefficients as functions of the effective volume fraction $\Phi_{\text{eff}}$ in Fig. 3. If the scaling were correct, all the points should fall on the line defined by the Dhont et al. formula $D/D_0=1+2.86\Phi_{\text{eff}}$. As can be seen from Fig. 3, the points representing low salt data deviate strongly from the expected model line. In particular, at the lowest added salt concentration a very unusual shape of the curve is observed, which is due to the strong maximum in $\Phi_{\text{eff}}$ calculated in this way at the polyion concentration of about 0.1 ng/ml [Figs. 2(b) and 2(c)].

C. Scaling strategy

In order to find the scaling method that would lead to agreement with the Dhont et al. formula, we used our experimental results to calculate the effective volume fractions of hard rods that would give diffusion coefficients equal to those measured for different added salt concentrations. This effective volume fraction is calculated from

$$\Phi_{\text{eff}}=\left(1-\frac{D}{D_0}\right)^{-1/2.86}$$

and is shown in Fig. 4 (symbols) along with the lines representing the scaling used in Figs. 2 and 3. Note that the factor of 2.86 is the $\alpha$ calculated from the hydrodynamic dimensions of the rod [see Eqs. (3) and (4)]. Equation (9) does not take into account changes in the effective aspect ratio resulting from the change of the effective dimensions. These changes lead to a change in $\alpha$ from 2.86 to a value closer to that of a sphere ($\alpha=2$). Correcting for this would increase the values of the effective volumes at low salt (symbols) by relatively small amounts (at the most by a factor of 2.86/2 = 1.43). In any case, the $\kappa^{-1}$ correction at low added salt concentrations results in heavily overestimated effective volumes (up to an order of magnitude), which is consistent with the conclusion from the analysis of Fig. 3.

What are the effective dimensions that would give agreement with the $\Phi_{\text{eff}}$ obtained from use of Eq. (9)? It is clear from Figs. 3 and 4 that the effective dimensions $L_{\text{eff}}=L+\kappa^{-1}$ and $d_{\text{eff}}=d+\kappa^{-1}$ overestimate the effective volume at low added salt. We ask the question: how much should we increase the size of the rod to obtain an effective volume fraction equal to that calculated from Eq. (9)? The answer is the solution of the equation $\Phi_{\text{eff}}=\pi/4(d+x)^2(L+x)C_s$ for $x$ ($C_s$ is the rod number concentration). Figure 5 shows the values of $x$ (symbols) in comparison to the screening length $\kappa^{-1}$ (solid lines) for all added salt concentrations. For the three lower added salt concentrations it can be seen that the increased thickness due to the electrostatic forces is $\approx 1/(2\kappa^{-1})$ (dashed line), except at the lowest polyion concentrations, where it is close to $\kappa^{-1}$. The same conclusion may be drawn from Fig. 4, where the effective volume fraction calculated using $(1/2)\kappa^{-1}$ is also shown (dashed lines). Another way to check the applicability of this new scaling procedure is to replot the data from Fig. 3 using $\Phi_{\text{eff}}$ calculated using rescaled dimensions of adding $(1/2)\kappa^{-1}$ to the hydrodynamic length and diameter instead of $\kappa^{-1}$. The re-
results of this scaling are shown in Fig. 6. Note the surprisingly good fit— even for the lowest added salt concentration.

Since in Fig. 6 it is difficult to estimate the weight concentration corresponding to a given point, we plotted in Figs. 7(a) and 7(b) experimental data for \( C_s = 10^{-4} M \) also as a function of polyion concentration along with the lines obtained from the Dhont et al. equation using effective dimensions increased by \( \kappa^{-1} \) (dashed line) and \( (1/2)\kappa^{-1} \) (dotted line). In this representation there seem to be two concentration regimes where the experimental data obey different kinds of scaling. At the lowest concentrations, where added salt defines the ionic strength, \( \kappa^{-1} \) is the value providing the expected scaling of the volume fraction. At higher concentrations, where counterions dominate the ionic strength, addition of only half of \( \kappa^{-1} \) is sufficient to provide the correct scaling behavior. The same may be observed in Fig. 4, where data from different ranges of polyion concentration fit better to solid or dashed lines.

D. Overlap concentration (\( C^* \))

The “overlap concentration” is an important parameter in analyzing the dynamics and structure in polymer solutions. This concentration often signals the onset of a concentration region in which the polymers interact strongly. For rod solutions the overlap number concentration \( C^*_n = 1/L_{\text{eff}}^3 \) and the corresponding overlap weight concentration \( C^*_w = M_w \times (C^*_n)^{1/3} \) at a given added salt concentration, is not a straightforward task. In order to find the value of \( C^*_n \) at a given added salt concentration, we perform a “gedanken experiment.” We choose a very low polymer concentration so that it is lower than a hypothetical \( C^*_n \) calculated at the conditions defined by \( C_s, C_p, \) and \( Z_{\text{eff}} \). Then we increase \( C_p \) continuously until \( C^*_n \) calculated for that \( C_p \) equals to \( C_s \). Figure 8 illustrates this procedure for a 20-mer.

What is the \( C^* \) at a given added salt concentration? \( C^* \) is estimated here from the effective length determined to give the best fit to the Dhont et al. equation \( L_{\text{eff}} = L + (1/2)\kappa^{-1} \). The Debye screening length \( \kappa^{-1} \), of course, depends on both the added salt and the polyion concentrations so that determining \( C^* \) is not a straightforward task. In order to find the value of \( C^* \) at a given added salt concentration, we perform a “gedanken experiment.” We choose a very low polymer concentration so that it is lower than a hypothetical \( C^*_n \) calculated at the conditions defined by \( C_s, C_p, \) and \( Z_{\text{eff}} \). Then we increase \( C_p \) continuously until \( C^*_n \) calculated for that \( C_p \) equals to \( C_s \). Figure 8 illustrates this procedure for a 20-mer. The straight line in the figure is the line \( C^*_n = C_p \). It may be seen from the figure that despite huge enlargement of the effective dimensions of the 20-mer at low \( C_p \) and low \( C_s \), the true overlap concentration at given added salt concentration falls very close to \( C^* \) calculated from the hydrodynamic dimensions. The dependence of the true \( C^* \) on the added salt concentration falls very close to \( C^* \) calculated from the hydrodynamic dimensions.
concentration is shown in the inset of Fig. 8. This interesting finding is a result of the fact, that for this system, the ionic strength is to a large extent determined by the counter-ions released during solvation. Thus the effective dimensions of the molecule decrease rapidly with increasing polyion concentration. Hypothetically, larger effects would be expected for a less charged rod because of the smaller number of counterions and also for shorter rods because of a larger ratio of $\kappa^{-1/L}$ at low ionic strength.

Comparison of Figs. 8 and 1 show that the unusual nonmonotonic behavior of $D/D_0$ at the lower added salt concentrations occurs in the “dilute” regime much below the overlap concentration $C^*$. This finding is also supported by the results of small angle x-ray scattering\textsuperscript{23} on this sample which show that the position of the peak in the scattered intensity spectrum follows the $C_p^{1/3}$ dependence up to the concentration of 50 mg/ml at no added salt conditions.

We would like to make here a remark concerning the virial expansion of $D_s$ given by Eq. (3). The experimental data clearly show that the non-monotonic behavior of $D_s$ (and $\phi_{eff}$) occurs at relatively small effective volume fraction, which should justify the application of Eq. (3). Addition of the next highest term in Eq. (3) would, in any case, not explain the effects illustrated in Fig. 1.

Finally, Fig. 9 shows the mutual diffusion coefficients measured earlier by DLS (Refs. 23 and 24) for the 20-mer and the self-diffusion results from this work vs the weight concentration of the 20-mer at various added salt concentrations. According to Eq. (1), $D_m = k_B T f_m$ at infinite dilution, which should be the same as $D_s$ at infinite dilution. This is borne out within experimental error by the data in Fig. 9 for all salt concentrations. Note that the increase in $D_m$ and the decrease in $D_s$ with increasing concentration are faster the lower the added salt concentration. The strong increase in the osmotic compressibility in the light scattering case overrides any possible decrease in mutual friction for all the salt concentration studied. A comparison of the friction coefficients for self and mutual diffusion requires independent measurements of the thermodynamic factor in Eq. (1).

V. CONCLUSIONS

Fluorescence correlation spectroscopy has been used to measure the long-time translational self-diffusion coefficients of a 20 base pair oligonucleotide as functions of 20-mer and added NaCl salt concentrations. The self-diffusion coefficients decrease with increasing 20-mer concentrations for the high-added salt sample and this decrease is well described by the Dhont, Van Bruggen, and Briels hard rod theory\textsuperscript{21} with effective rod dimensions calculated by adding the Debye screening length to the hydrodynamic dimensions.

At lower added salt concentrations the self-diffusion coefficients decrease nonmonotonically with increasing 20-mer concentrations. The Brownian dynamics simulations of Kirchhoff, Löwen, and Klein\textsuperscript{22} for much longer charged rods also show such a non-monotonic decay for salt-free solutions. The nonmonotonic behavior is attributed to the opposing effects of the increasing polyion concentration and decreasing Debye screening length due to counterions supplied by the 20-mer. The effective volume calculated using the commonly used procedure of increasing the rod dimensions by the Debye screening length is much too high to explain the experimental results at the lower added salt concentration. Attempts to account for the effect of the Coulomb forces on the self-diffusion coefficients for the lower added salt concentrations by using effective dimensions in the hardrod theory give two scaling regimes—one at low polyion concentration where the dimensions scale as the Debye screening length and the other at high polyion concentration where the dimensions scale as one half the Debye screening length. Estimates of the overlap concentration $C^*$ even for lowest added salt concentrations are fairly close to the values estimated from hydrodynamic dimensions. The nonmonotonic decrease in the self-diffusion coefficients for the lower added salt concentrations occurs at concentrations well below our estimated $C^*$.

Values of the self (FCS) and mutual diffusion coefficients (DLS) for the 20-mer as functions of salt and polyion concentrations have been compared. As expected the mutual diffusion coefficient increases with increasing polyion con-
centration in contrast to the decrease (although nonmonotonic) of the self-diffusion coefficient.

ACKNOWLEDGMENTS

This work was supported in part by NATO Cooperative Linkage Grant No. PST. CLG979044.


Downloaded 22 Nov 2004 to 134.94.165.201. Redistribution subject to AIP license or copyright, see http://jcp.aip.org/jcp/copyright.jsp