Dynamic Light Scattering by Aqueous Solutions of Low Molar Mass DNA Fragments in the Presence of NaCl

Taco Nicolai and Michel Mandel*

Department of Physical and Macromolecular Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands. *Received June 9, 1988; Revised Manuscript Received November 14, 1988*

ABSTRACT: A systematic investigation has been performed on the polymer and NaCl concentration dependence of the quasi-elastic light scattering correlation function of homodisperse DNA consisting of 150 base pairs in aqueous salt solutions at 25 °C. An effective mutual diffusion coefficient could be determined which, at each NaCl concentration, increases linearly with DNA concentration. This increase is largely attributable to the effect of the second virial coefficient of the solutions. The value of this effective diffusion coefficient extrapolated to zero concentration does not depend on the salt concentration and seems to correspond to the translational diffusion coefficient of DNA molecules which do not deviate appreciably from a rodlike shape. For salt concentrations $C_s \leq 0.01$ M a slow mode appears in the correlation function which is probably due to the formation of loose aggregates.

Introduction

Dynamic light scattering has been found to be a useful technique for studying the behavior of macromolecules in solution, in particular the translational diffusion. Important concentration effects of the translational diffusion coefficient have been observed for *flexible* macromolecules when in solutions of the so-called *semidilute* regime. The mutual diffusion coefficient D_m measured for such systems has been found to increase considerably with increasing polymer concentration C and, in the case of flexible charged macromolecules, with decreasing salt concentration C_s. This is in contrast to what can be seen in the *dilute* regime, where D_m is only slightly increasing with C and, for charged macromolecules, also with C_s. These effects can be understood in terms of a dynamic network formation of flexible polymer chains above a certain critical concentration where the chains start to entangle. It has been predicted by de Gennes[1] that above that concentration D_m for uncharged flexible polymers should scale with $C^{0.75}$. This theory has been extended to polyelectrolytes by Odijk[2] and yields the same scaling law for D_m in solutions with an excess of low molar mass electrolyte. In the latter case the critical concentration depends not only on the molar mass but also on the ionic strength of the solution as a consequence of the charge interactions in these systems.

The dynamic network approach has been experimentally confirmed both for uncharged polymers such as polystyrene[3,4] and for strong polyelectrolytes such as poly(styrenesulfonates).[5,6] Recently questions have been raised about the possible occurrence of other modes in the semidilute regime besides the mode for which D_m scales as C^{ν} (with the exponent approximately equal to the theoretically predicted value 0.75).[7]

For a rodlike polymer the network theory obviously cannot be applied because entanglements cannot occur. A critical concentration has been predicted by Doi and Edwards[8] separating the concentrations for which the rods can rotate freely from the range where the solutions are still isotropic but exhibit strong repulsions between the rods causing restricted rotational diffusion. When the number concentration is much larger than L^{-3}, where L is the length of the rod, translational diffusion is possible only parallel to the rod axis, decreasing the mean value of D_m as compared to its value in the dilute regime.

A concentration dependence of D_m has been observed both for uncharged and for charged rodlike macromolecules. The increase of D_m with increasing concentration has been found for rigid poly(γ-benzyl L-glutamate) in dimethylformamide (DMF) and has been ascribed to the compensation of a decreasing self-diffusion coefficient by an increase through the second virial coefficient.[9] The concentration dependence of D_m of short DNA fragments, which may be considered to be near the rod limit, has also been investigated.[10] It was concluded that the large decrease of D_m with increasing C_s is in qualitative agreement with the coupled ion diffusion theory developed by Lee and Schurr,[11] although large quantitative deviations appear.

When the macromolecular concentration is increased, the intensity correlation function determined in dynamic light scattering often starts to deviate considerably from a single-exponential decay, making a determination of D_m more difficult. This behavior has been found for flexible polymers at high concentrations[12] and for flexible polyelectrolytes at moderate concentrations, especially when little or no low molar mass electrolyte is added.[13–15] In the case of rodlike polymers and polyelectrolytes similar effects have been noted.[9,10,16] Usually deviations from single-exponential decay have been found to increase with decreasing salt concentration in polyelectrolyte solutions, although it has been suggested that in a semidilute solution of 200-base-pairs-long DNA a gel like phase occurs with a subsequent deviation from a single exponentiality of the intensity correlation function and that such a phase is stabilized by the presence of added salt.[17]

Here we shall present a systematic investigation of the concentration dependence of D_m of homodisperse DNA consisting of 150 base pairs at various salt concentrations between 0.002 and 0.5 M NaCl. It has been shown that the behavior of DNA of this length seems to be, in many aspects, that of a rigid rod.[16–19] The correlation functions, which have been measured, have been fitted to a cumulant expansion and a bimodal exponential function. Mutual diffusion coefficients at constant C_s have been found to increase linearly with the DNA concentration. The dependence of D_m on the salt concentration can, for the main part, be explained by the influence of C_s on the second virial coefficient, which had been measured previously.[19] Increasing deviations from single-exponential behavior of the intensity correlation function has been observed upon lowering the salt concentration and increasing DNA concentration. The influence of the temperature on D_m has been determined by measuring correlation functions at different temperatures between 10 and 40 °C, for two DNA solutions differing both by C and C_s. No significant temperature dependence, besides that due to the viscosity of the solvent and the change in the energy of thermal

0024-9297/89/2222-2348$01.50/0

motion, has been revealed, however.

Theory

In homodyne dynamic light-scattering experiments the normalized intensity correlation function $g^{(2)}(\tau)$ can be determined, which is related to the normalized field correlation function $g^{(1)}(\tau)$ by the Siegert relation for scattered light obeying Gaussian statistics:

$$g^{(2)}(\tau) \equiv \frac{\langle I(0)\, I(\tau)\rangle}{\langle I\rangle^2} = 1 + |g^{(1)}(\tau)|^2 \quad (1)$$

Here $I(\tau)$ is the intensity of the scattered light at time τ, and angular brackets stand for an averaged value. For homodisperse, noninteracting, small isotropic particles the field correlation function is an exponential function of time:

$$g^{(1)}(\tau) = \exp(-\Gamma\tau) \quad (2)$$

where the decay rate Γ is related to the mutual diffusion coefficient D_m and the length of the scattering vector q by

$$\Gamma = q^2 D_m \quad (3)$$

with

$$q = (4\pi n/\lambda_v) \sin(\theta/2) \quad (4)$$

Here n is the refractive index of the solution, λ_v the wavelength of the incident light in vacuo, and θ the angle of observation of the scattered light with respect to the direction of propagation of the incident beam.

For polymers with cylindrical symmetry the normalized field correlation function in the absence of interactions and polydispersity effects is given by[20]

$$g^{(1)}(\tau) = \exp(-\Gamma_1\tau) + K \exp(-\Gamma_2\tau) \quad (5)$$

where the relaxation rates Γ_1 and Γ_2 are given by

$$\Gamma_1 = q^2 D_m \quad (6a)$$

$$\Gamma_2 = q^2 D_m + 6D_r \quad (6b)$$

with D_r the rotational diffusion coefficient of the polymer. The relative contribution of the second exponential in eq 5 depends on the value of K, which is proportional to the square of the ratio of the electric polarizability anisotropy and the mean polarizability. In the case of low molar mass DNA, K is smaller than 0.001 (ref 20), which means that the influence of the rotational diffusion coefficient on the correlation function is negligible and that eq 5 reduces to eq 2 under ideal conditions.

The mutual diffusion coefficient represents the response of the system to concentration fluctuations and can be related to the concentration dependence of the osmotic pressure Π,[21] the force which opposes concentration fluctuations:

$$D_m = (M/N_{Av}f)(1 - \upsilon C)(\partial\Pi/\partial C) \quad (7)$$

Here f is the friction coefficient of the polymer of molar mass M at the given concentration C, N_{Av} is Avogadro's constant, and υ is the specific volume of the particle. If we use a virial expansion for the osmotic pressure, which is also possible for polyelectrolyte solutions in the presence of an excess of low molar mass salt, and represent the friction coefficient by $f = f_0\phi_f(C)$, where f_0 is the friction coefficient at infinite dilution and $\phi_f(C)$ represents a function of concentration which tends to unity for $C \to 0$, then we may write for the mutual diffusion coefficient the following expression:

$$D_m = D_0\{(1 - \upsilon C)(1 + 2MA_2C + \ldots)\}\phi_f^{-1} \quad (8)$$

Here A_2 is the second virial coefficient (in mol L g^{-2} if C is expressed in g L^{-1}) determined at constant chemical potential of the low molar mass salt in the case of a polyelectrolyte solution. The value of the mutual diffusion coefficient at zero concentration D_0 equals the translational diffusion coefficient of the individual macromolecules as given by the Einstein equation

$$D_0 = k_B T/f_0 \quad (9)$$

with k_B the Boltzmann constant and T the temperature in kelvin. Theoretical expressions for the translational diffusion coefficient D_0 of cylinders with length L and diameter d have been derived by Broersma[22] and Tirado and Garcia de la Torre:[23]

$$D_0 = (k_B T/3\pi\eta L)\{\ln p + \tfrac{1}{2}(y_\perp + y_\parallel)\} \quad (10)$$

where $p = L/d$, η is the viscosity of the solvent, and $y_\perp$ and $y_\parallel$ are end-effect corrections. According to Broersma, these corrections may be written as

$$y_\perp = 0.5 + 4.2\{1/(\ln 2p) - 0.39\}^2 \quad (11a)$$

$$y_\parallel = -0.58 + 7.4\{1/(\ln 2p) - 0.34\}^2 \quad (11b)$$

whereas according to Tirado and Garcia de la Torre

$$y_\perp = 0.839 + 0.185/p + 0.233/p^2 \quad (12a)$$

$$y_\parallel = -0.207 + 0.98/p - 0.133/p^2 \quad (12b)$$

Tirado et al.[24] have compared both sets of end-correction equations with various experiments on DNA fragments and have concluded that a better agreement with experimental values is obtained when eq 12 are used.

The friction coefficient f that appears in eq 7 is the same friction coefficient that may be obtained from sedimentation coefficients determined in ultracentrifuge sedimentation experiments. This coefficient is related to the molar mass and the specific volume of the polymer, the density of the solution ρ, and the friction coefficient:

$$s = M(1 - \upsilon\rho)(N_{Av}f)^{-1} \quad (13)$$

The sedimentation coefficient s changes with polymer concentration through the concentration dependence of the friction coefficient only (provided the solutions are dilute enough for $\upsilon\rho$ the remain constant):

$$s = s_0\phi_f^{-1}(C) \quad (14)$$

Here s_0 is the sedimentation coefficient at infinite dilution, which may be put in the following form with help of eq 9, 13, and 14:

$$s_0 = M(1 - \upsilon\rho)(N_{Av}f_0)^{-1} = D_0M(1 - \upsilon\rho)(N_{Av}k_BT) \quad (15)$$

Materials and Methods

Mononucleosome DNA has been prepared from chicken erythrocyte DNA by the procedure described by Shindo et al.[25] as reported previously.[19] The molar mass of the DNA fragments as determined by static light scattering was $(1.10 \pm 0.13) \times 10^5$ g mol^{-1}, which corresponds to a fragment of roughly 150 base pairs.[19] Solutions have always been prepared by dissolving freeze-dried DNA in Millipore filtered and deionized water containing NaCl (of analytical grade) to the desired salt concentration. The solutions have been made "dust free" by repeated filtration through Millipore filters (0.22 μm). Values of DNA concentrations have been determined spectrophotometrically at 260 nm, where an extinction coefficient of 20 L g^{-2} cm^{-1} has been used.[25] Concentrations have always been determined after the experiment to take into account possible losses caused by filtration.

The intensity autocorrelation functions have been measured with a Malvern 4300 photocorrelation spectrophotometer in-

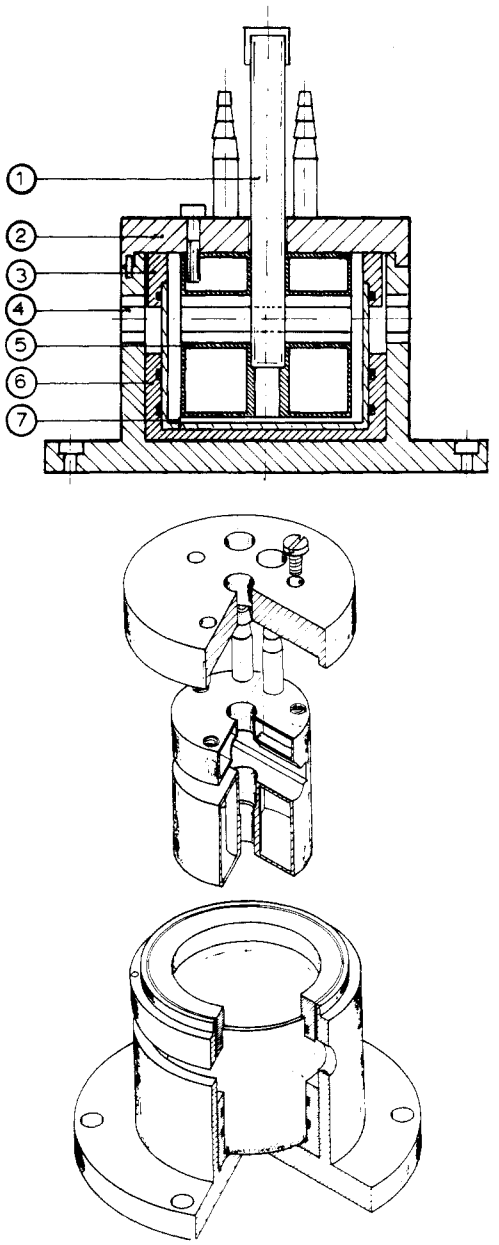

Figure 1. Schematic representation of the thermostated sample holder: (top) overall view; (bottom) cross section: sample tube in quartz (1); the materials used are poly(oxymethylene) deldrin (2, 3, 6), brass (4, 5), and glass (7).

corporating a type K7023/2cm 96-channel Malvern digital correlator. The laser beam was provided by a Spectra Physics Series 2000 argon ion laser at the wavelength λ_v = 514.5 nm. The sample holder has been changed to ensure that the temperature of the sample is stable within 0.1 °C and to avoid as far as possible temperature gradients in the cell. The following construction has been used (see Figure 1). A metal container is placed in a glass holder. Through the metal container water is pumped that is kept at constant temperature by an external thermostated bath. The toluene in the glass holder is in close contact with the metal container, and the glass holder is covered with isolating material so that the temperature of the toluene remains stable. The cell that contains the sample is over full length enclosed by the metal container, thus reducing the possibility of temperature gradients in the cell. All measurements have been performed at 25 °C unless stated otherwise.

The correlator is connected to a PDP-11 minicomputer to control its operation and to analyze the data. A dust discrimination procedure similar to the one described by Alon and Hochberg[26] has been used to minimize influences of parasitic scatterers that may have escaped elimination by filtration. This is hard to avoid in the case of aqueous solutions.

Sedimentation experiments have been performed at 25 °C in a Beckman Spinco E analytical ultracentrifuge equipped with both Schlieren optics and UV detection at the Department of Biochemistry, Leiden University.

Data Analysis of the Dynamic Light-Scattering Experiments

The measured intensity autocorrelation functions $Y(\tau)$ in the homodyne mode may, for solutions of homodisperse, noninteracting isotropic macromolecules, be written as follows (see also eq 1 and 3:

$$Y(\tau) = C' \exp(-2\Gamma\tau) + B \tag{16}$$

where C' is a factor depending on the experimental setup and B the base line (ideally equal to unity if Y would represent the normalized autocorrelation function). If parasitic scatterers such as dust particles are present in the scattering volume, the correlation function will be disturbed. Because these parasitic scatterers in general move very slowly as compared to the dissolved macromolecules, in first approximation only the base-line value will be affected and will increase above the value unity. The perturbation by dust can therefore be reduced by treating B as an additional fit parameter. However most of the measured correlation functions could not well be fitted to eq 16, although the deviations of the experimental points with respect to the least-squares fit to a single exponential remains relatively small. Therefore the correlation functions have been fitted by using a cumulant expansion or a double-exponential function (see hereafter).

The cumulant method has originally been developed to describe correlation functions of polydisperse systems[27] or systems presenting a (continuous) distribution of decay rates. However, the cumulant expansion has also been found useful in analyzing correlation functions of systems with strongly interacting particles[5,28-30] which show small deviations from eq 16. The experimental correlation function is then expanded in the following way:

$$Y(\tau) = C' \exp(-k_1\tau + (2K_2/2!K_1^2)(K_1\tau)^2 - (2K_3/3!K_1^3)(K_1\tau)^3 + \ldots) + B \tag{17}$$

where K_i/K_1^i is the ith normalized cumulant defined in a general way by

$$K_i/K_1^i = (-1/K_1)^i \left(\frac{\partial^i |\ln g^{(1)}(\tau)|}{\partial \tau^i} \right)_{\tau=0} \tag{18}$$

Here $g^{(1)}(\tau)$ is the normalized field correlation function. In the case of a distribution of decay rates, the ith cumulant can be identified with the ith moment of the distribution, the first cumulant corresponding to the mean decay rate. In the general case K_1 may be considered related to an effective diffusion coefficient D_{eff} if it is linearly dependent on q^2, i.e., if the following relation is satisfied:

$$K_1 = 2D_{eff}q^2 \tag{19}$$

The experimentally found correlation functions have been fitted to an expansion with two or three cumulants. The difference on the value of K_1 obtained by a fit with three cumulants as compared to that from a fit by two cumulants was generally about 5% only. Because of this small difference and because the third normalized cumulant is usually much smaller than the second one, it is justified not to consider four cumulants in the fit. In a few cases only the value of the normalized second cumulant was smaller than the third one. In these case the result of a fit to an expression with two cumulants yielded values for

D_{eff} that were more consistent with the value of D_{eff} from other correlation functions.

When the presence of two distinct time scales in the correlation function could be observed visually, a double exponential was also tried as a fit function:

$$Y(\tau) = (C'_1 \exp(-\Gamma_1\tau) + C'_2 \exp(-\Gamma_2\tau))^2 + B \quad (20)$$

The correlation functions have been fitted to eq 17 or 20 by a linear least-squares technique using the Marquardt–Levenberg algorithm.[31] The quality of a fit was judged from (a) the standard deviation of the experimental points with respect to the fitted curve and (b) the distribution to the differences between the experimental points and the calculated values according to the fit function used. The former should not be larger than the estimated experimental error on the values of the measured points; the latter should not reveal systematic deviations with respect to zero, i.e., the difference should be randomly distributed around zero. Often a quality factor Q is used as a test for this purpose. This factor is defined as follows:

$$Q = 1 - \{\sum(y_i - \bar{y}_i)(y_{i+1} - \bar{y}_{i+1})\}\{\sum(y_i - \bar{y}_i)^2\}^{-1} \quad (21)$$

where y_i is the value of the ith experimental point and $\bar{y}_i$ its calculated value according to the fitted curve. The summation includes all the data of a given curve. Ideally $Q = 1$ for data for which the deviations with respect to the fitted curve are perfectly randomly distributed, but often a value $Q > 0.7$ is found to be satisfactory. We have observed, however, that visual inspection of a difference plot is much more sensitive in detecting systematic deviations of the experimental points from the fit curve than considering the value of Q. More specifically, if only the first few points of the experimental correlation function deviate strongly from the fitted curve, this is *not* reflected in the value of Q but is easily revealed by looking at the difference plot.

Both fitting procedure have been tested on homodisperse samples of polystyrene latex particles, mixtures of polystyrene latices of two different sizes, analogous systems with silica in toluene, and computer-simulated data to which random noise was added.[32] The cumulant analysis yielded accurately the expected values of the decay rate when a single decay was simulated or when measurements on homodisperse samples have been used. When a bimodal function was simulated (with equal contributions of the two exponentials) or measurements on a mixture of two different particles were considered (with relative contributions of the larger particles varying between 0.75 and 0.50), a fit to a double-exponential function yielded values for the fit parameters that deviate considerably from the expected values unless the noise on the data was very small (much smaller than 1%). Such low noise values are in practice often difficult to achieve, particularly in aqueous solutions. Except when the noise on the data is very small, a cumulant expansion with three cumulants fits the data equally well. It seems therefore impossible to judge purely from the quality of the fit whether the experimental correlation function is bimodal or not.

Results

Correlation functions have been measured for solutions of various DNA concentrations C (in g L^{-1}) at several NaCl concentrations C_s (in mol L^{-1}). Measurements were performed at five or more different angles between 30 and 90°. Each experiment was performed immediately after filtration of the solution, because it has been observed that the frequency and the amplitude of the fluctuations in the mean intensity of the scattered light increase in time just as if the concentration of parasitic scatterers in the solution increases. This behavior is particularly noticeable at lower salt concentrations and has also been observed in static light-scattering experiments.[19] The fluctuations increase until a stationary state is reached after a few hours. When the solution is then filtered again, the intensity of the scattered light returns to the level found after the first filtration, but after a short time fluctuations reappear again as before. If this procedure is repeated several times, each time the same behavior is observed.

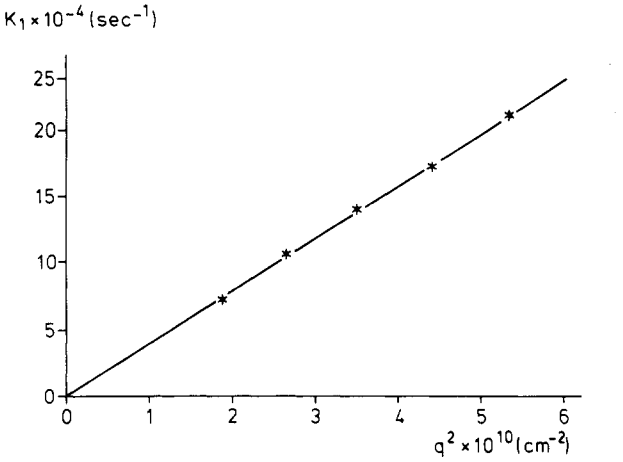

Figure 2. The q^2 dependence of the first cumulant K_1 of a DNA solution, C = 1.5 g L^{-1}, C_s = 0.02 M.

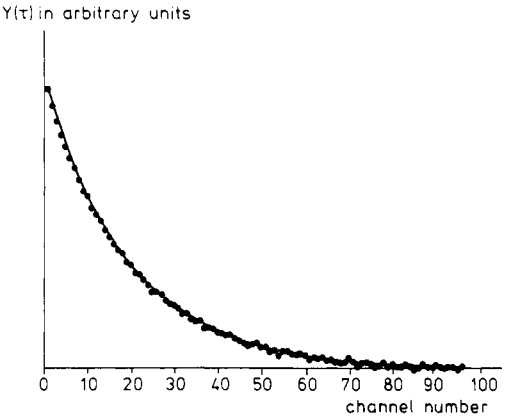

Figure 3. Intensity correlation function $Y(\tau)$ of a DNA solution, C = 1.5 g L^{-1}, C_s = 0.02 M, at an angle of observation of 50°. The drawn curve is the best fit according to a cumulant expansion.

The first cumulants, as obtained by the cumulant expansion analysis, for each series of measurements at constant C and C_s have been plotted against q^2 and yielded straight lines. From the slope, calculated by a least-squares linear fit, an effective diffusion coefficient has been derived according to eq 19. The DNA concentration dependence of the effective diffusion coefficient has been determined for six different NaCl concentrations between 0.5 and 0.002 M at 25 °C. At relatively high salt concentrations ($C_s \geq$ 0.02 M) and low DNA concentration ($C < 3$ g L^{-1}) the correlation function was essentially single exponential. Values of the normalized second cumulant were smaller than 0.1 in all these cases.

Each plot of the first cumulant K_1 vs q^2 yields a statistically acceptable straight line with an intercept that is zero within experimental error. In Figure 2 an example is given of the angular dependence of K_1 in this regime. If the DNA concentration is increased and the salt concentration decreased, the correlation function starts to deviate from a single exponential. No sudden transition from a regime where the correlation function deviates considerably from the simple exponentiality could be de-

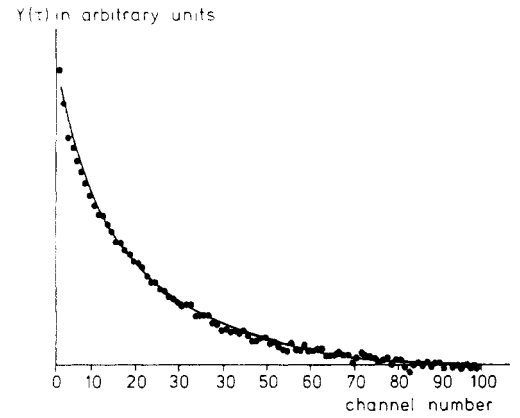

Figure 4. Intensity correlation function $Y(\tau)$ of a DNA solution, C = 3.7 g L^{-1}, C_s = 0.005 M, at an angle of observation of 80°, where the correlation function strongly deviates from a single exponential. The drawn curve is the best fit to a cumulant expansion.

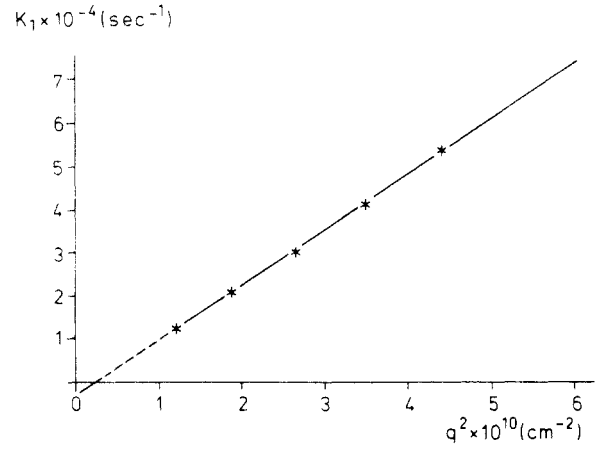

Figure 5. The q^2 dependence of the first cumulant K_1 of a DNA solution, C = 3.7 g L^{-1}, C_s = 0.005 M; regime where the correlation function strongly deviates from a single exponential.

tected, however. In Figure 3 an example is represented of a correlation function typical for the first regime, and in Figure 4 an example typical for the second regime. The sampling time was always chosen in such a way that the last few points were almost horizontal. The use of shorter sampling times made little difference to the value of the first cumulant. When a much larger sampling time was used, a lower value for the first cumulant was found in the second regime. Deviations from single-exponential behavior manifested themselves in four ways: (1) increasing values for the normalized second and third cumulants, up to a value of $K_2/K_1^2 = 0.5$; (2) appearance of a *negative intercept* in the K_1 vs q^2 plot; (3) fluctuations in the mean intensity of the scattered light increasing in both frequency and amplitude with time, as if parasitic scatterers are slowly formed in the solution; (4) increasing base-line values. Also a very fast decay could be detected, the value of which was difficult to characterize properly because it contributed only to the first few points of the correlation function even at very short sampling times. By omission of the first few points of the correlation function, usually less than five, the influence of this fast decay could be minimized. It cannot be excluded that this very fast decay is an artifact due to the use of short sampling times (1 μs). In fact it has been observed with other systems as well, particularly with aqueous solutions.

In Figure 5 an example of the angular dependence of K_1 vs q^2 is given where the negative intercept of the least-squares straight line can be seen. It is clear, however, that

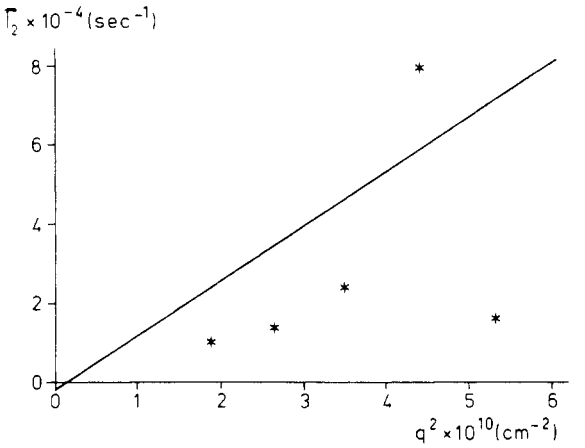

Figure 6. Typical example of the q^2 dependence of the slow mode relaxation rate Γ_2 exhibiting large fluctuations with the scattering angle. DNA solution with C = 1.7 g L^{-1}, C_s = 0.005 M.

over a wide range of angles (20–90°) there is no curvature. Possibly this curvature occurs at lower angles which are not well accessible with the equipment available and are highly inaccurate because of backscattering and scattering from dust particles. To check the existence of instrumental artifacts eventually responsible for the negative intercepts, measurements on a solution of homodisperse polystyrene latex in water have been performed right after an experiment with a DNA solution exhibiting the behavior just discussed. For the latex system, however, the K_1 vs q^2 plot did not show a significant intercept. Clearly such a negative intercept reflects some behavior of the DNA solutions under the conditions considered. The value of the negative intercept in the K_1 vs q^2 plot, whenever observed for DNA solutions, does not depend on the salt concentration or the DNA concentration in a systematic way. Its magnitude remains relatively small in all cases where it exceeds the experimental error. The intercept shown in Figure 5 is representative for its order of magnitude in general.

Correlation functions that exhibit a clear deviation from single exponentiality have also been fitted to a double-exponential function, eq 20. If a floating base line was used, no consistent values for the two decay rates could be obtained. If a fixed value B = 1 was assumed, in many cases a fit of equal quality compared to a fit based on a cumulant expansion has been found. One decay rate turned out to be linear in q^2; it was always the fastest one. Values of an effective diffusion coefficient have been calculated from the slope of this fast decay rate vs q^2. These values are comparable to, albeit somewhat larger, than the values derived by using the first cumulant in a cumulant expansion. The intercepts of the lines Γ_1 vs q^2 were in most cases smaller than for the corresponding lines K_1 vs q^2. The second mode is slow compared to the first one, but it was practically impossible to determine accurate values for Γ_2 and the related slow effective diffusion coefficients, because the values of Γ_2 showed an eratic behavior when changing from one angle to another (see Figure 6). The relative contribution of the second slow mode to the correlation function also fluctuated strongly but in general increased with increasing DNA concentration and decreasing salt concentration.

There are probably two reasons for the large uncertainties in the parameters obtained from a bimodal fit. First, the noise on the data (for the solutions investigated generally of the order 0.1–1%) could not be reduced to a level necessary for an accurate bimodal fit. This is due to the relatively low scattering intensity of the DNA solutions at low salt concentrations. Second, the slow mode

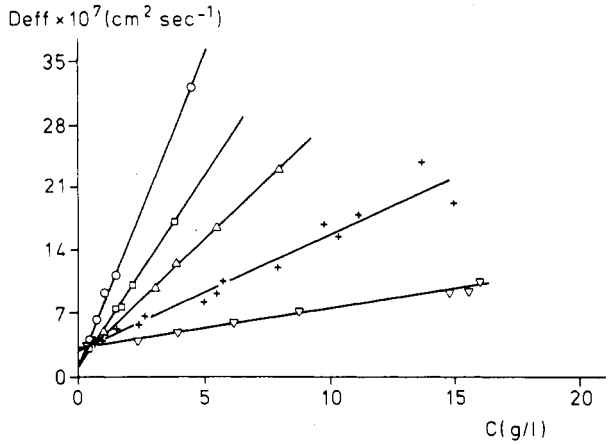

Figure 7. Concentration dependence of the effective diffusion coefficient D_{eff} obtained from a cumulant analysis at various NaCl concentrations: 0.1 (▽), 0.002 (+), 0.01 (△), 0.005 (□), 0.002 M (○).

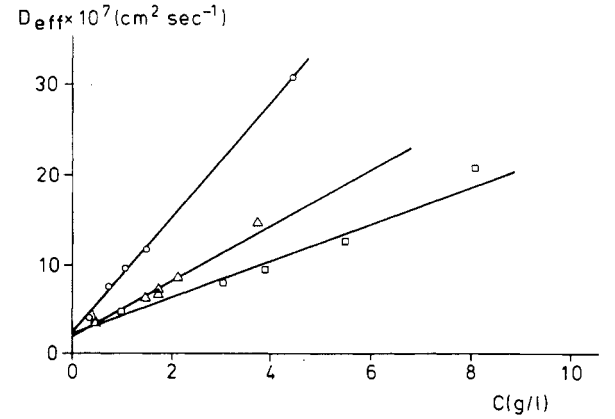

Figure 8. Concentration dependence of the effective diffusion coefficient D_{eff} obtained from a bimodal analysis, at various NaCl concentrations: 0.01 (□), 0.005 (△), 0.002 M (○).

does not present one well-defined decay, even when the sampling times were chosen to be so large that the fast mode cannot contribute anymore to the correlation functions, for the values of the effective diffusion coefficients derived at various angles fluctuated strongly, often by more than a factor of 10.

Values of the effective diffusion coefficient obtained from a cumulant expansion are represented in Figure 7 as function of DNA concentration at various salt concentrations. Errors in the individual values of D_{eff} as obtained from the linear least-squares fit of K_1 vs q^2 are a few percent, except at low salt and high DNA concentrations where the errors are larger (up to 10%) due to the low scattering intensity. Note that at lower salt concentrations the DNA concentrations had to be limited to lower values because of increasing experimental difficulties and the strong decrease of the relaxation rate with increasing C observed at low values of C_s. As can be seen, D_{eff} increases linearly with the DNA concentration at fixed C_s for the DNA concentrations investigated.

For the three smallest salt concentrations, values of D_{eff} coefficients have also be derived from the fast mode of the bimodal analysis. These values, represented as function of C in Figure 8 have been summarized in Table I and compared to the corresponding values derived from the first cumulant in the cumulant expansion fit. Errors in the values of D_{eff} obtained by the former method are usually larger than for the values according to the latter, for reasons stated above. No bimodal analysis has been applied to the solutions for which $C_s \geq 0.02$ M because for these solutions the correlation functions could be fitted to a cumulant expansion for which the second normalized cumulant was sufficiently small to consider these functions as single exponentials.

Table I
Effective Diffusion Coefficient (10^{-7} cm^2 s^{-1}) of Aqueous DNA Solutions at Low Concentrations

	C_s = 0.002 M		C_s = 0.005 M		C_s = 0.01 M	
C, g L^{-1}	$D_{eff}{}^a$	$D_{eff}{}^b$	$D_{eff}{}^a$	$D_{eff}{}^b$	$D_{eff}{}^a$	$D_{eff}{}^b$
0.33	2.8	4.4				
0.40					3.1	3.8
0.43			2.6	4.2		
0.71	4.9	8.0				
1.00					4.0	5.0
1.04	7.0	10.0				
1.45	8.4	12.2	5.8	6.5		
1.7			6.0	7.2		
2.1			7.7	9		
3.0					7.5	8.5
3.7			12.7	15		
3.85					9.3	10
4.4	23.4	31				
5.45					12.2	13
6.0					16.9	21

[a] Obtained from K_1 of a cumulant analysis. [b] Obtained from Γ_1 of a bimodal analysis.

Table II
Values of D_{eff} of Aqueous DNA Solutions, Corrected for Changes in the Solvent Viscosity η and Temperature T at Various Temperatures (Values of $\eta D_{eff}/T$ in 10^{-12} g cm s^{-2} K^{-1})

	C = 1.8 g L^{-1}, C_s = 0.5 M	C = 15.0 g L^{-1}, C_s = 0.02 M	
T, °C	$\eta D_{eff}/T^a$	$\eta D_{eff}/T^a$	$\eta D_{eff}/T^b$
10	9.3	36.9	57.6
15	8.9		
25	9.9	41.8	51.0
35	9.6		
40	9.4	38.0	49.4
45	9.4		

[a] Obtained from K_1 of a cumulant analysis. [b] Obtained from Γ_1 of a bimodal analysis.

For several solutions the temperature dependence of D_{eff} between 10 and 40 °C has been determined. The values thus obtained have been corrected for changes in the viscosity of the solvent and changes in the thermal energy k_BT to detect whether there are other factors determining the translational diffusion. For a solution containing a high salt concentration the temperature dependence of the thus corrected value of D_{eff} was found to be negligible (see Table II). For a solution containing less salt and at a high DNA concentration, the corrected values of D_{eff} obtained through a cumulant expansion analysis still do not show a clear trend in the temperture dependence. Corrected values, obtained from the fast mode in a bimodal analysis of the correlation functions for the same solutions, seem to show a slight decrease with increasing temperature, which is however not sufficient to draw any definite conclusions.

Sedimentation coefficients have been determined at three different DNA concentrations (0.004, 6.5, and 10.5 g L^{-1}). Measurements have been performed at a high salt concentration (C_s = 0.5 M) to avoid Donnan effects. It may be assumed, however, that the measured concentration dependence, as shown in Figure 9, is approximately valid for lower salt concentrations as well, as the dimensions of the rodlike DNA molecules are not expected to be much influenced by the salt concentration. As can be seen from Figure 9 the change of s with concentration is practically

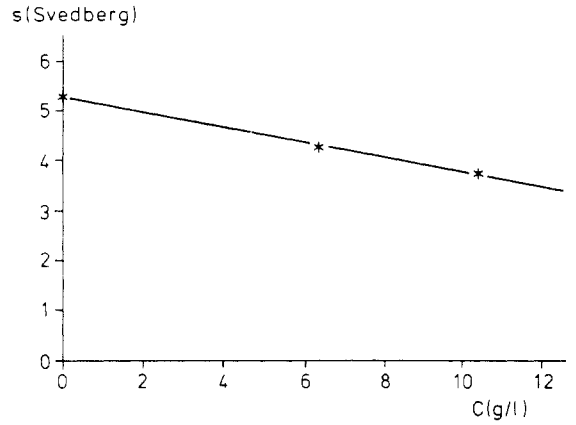

Figure 9. Concentration dependence of the sedimentation coefficient s for DNA solutions in 0.5 M NaCl.

Table III
Effective Diffusion Coefficients D_0 (10^{-7} cm^2 s^{-1}) at Infinite Dilution

C_s, M	$D_0{}^a$	$D_0{}^b$
0.002	1.1 ± 0.3	3.1 ± 0.4
0.005	1.1 ± 0.2	2.0 ± 0.7
0.01	2.0 ± 0.2	3.7 ± 0.5
0.02	2.7 ± 0.4	
0.1	3.0 ± 0.1	
0.5	3.3 ± 0.05	

[a] Obtained by using values of D_{eff} from K_1 in a cumulant fit. [b] Obtained by using values of D_{eff} from Γ_1 in a bimodal fit.

linear, so that for the range covered the following expression holds to a good approximation:

$$\phi_f \simeq 1 + k_f C \tag{22}$$

The following values have been derived from the linear plot of s vs C: $k_f = 0.03 \pm 0.01$ L g^{-1}, $s_0 = 5.33 \pm 0.05$ S (with s_0 representing the sedimentation coefficient at infinite dilution).

Discussion

As can be seen from Table I, the values of D_{eff} obtained from K_1 in the cumulant expansion analysis are systematically lower than the corresponding values obtained by applying a bimodal fit. This is not surprising because the latter have been derived by using the fast mode with the higher effective diffusion coefficient. If we consider K_1 to depend on the *mean value* of both diffusion coefficients, then necessarily it must be smaller than the larger of the two, as found. It may therefore be concluded that the more pronounced deviations of the correlation function from a single exponential as observed at the three lowest salt concentrations by the increasing value of the normalized second cumulant are consistent with the appearance of the slow mode, the diffusion coefficient of which also affects the mean value of D_m derived from K_1.

As both the values of D_{eff} obtained either from K_1 or Γ_1 depend linearly on the concentration C, it is possible to determine by simple extrapolation the value of the effective diffusion coefficient at infinite dilution D_0. These values, determined with the help of a linear least-squares fit, are represented in Table III. They show a considerably dependence of D_0 on C_s if all the values considered are derived by using D_{eff} from the cumulant analysis of the correlation functions. On the other hand, when these values for $C_s \geq 0.02$ M only are combined with the values of D_{eff} from Γ in the bimodal fit for $C_s \leq 0.01$ M, no significant C_s dependence is revealed, as is to be expected for particles that would behave as rigid rods. This is further confirmation that the values of D_{eff} derived from K_1 at the lowest salt concentrations are not to be interpreted as the mutual diffusion coefficients of single DNA molecules. If we use the combination of the values for D_0 derived from values of D_{eff} obtained through a cumulant analysis for the three highest salt concentrations with the values for D_0 derived from the values of D_{eff} as determined from the bimodal fit, the mean value is $\bar{D}_0 = (3.0 \pm 0.6) \times 10^{-7}$ cm^2 s^{-1}, which is in excellent agreement with the value of D_0 calculated from the sedimentation coefficient at infinite dilution s_0 (using $M = 1 \times 10^5$ g mol^{-1}, $\upsilon = 0.56 \times 10^{-3}$ L g^{-1} (ref 34), and $\rho = 1.00$ g cm^{-3}), i.e., $D_0 = (3.04 \pm 0.05) \times 10^{-7}$ cm^2 s^{-1}.

Table IV
Comparison between Experimental and Calculated Values of k_d (L g^{-1})

C_s, M	$k_d{}^a$	$k_d{}^b$	$k_d{}^c$
0.002	4.7 ± 1.3	2.1 ± 0.3	3.6 ± 0.1
0.005	2.5 ± 0.5	1.7 ± 0.7	1.2 ± 0.05
0.01	0.95 ± 0.07	0.6 ± 0.1	0.55 ± 0.05
0.02	0.33 ± 0.05		0.37 ± 0.05
0.1	0.090 ± 0.004		0.09 ± 0.03
0.5	0.002 ± 0.002		0.01 ± 0.03

[a] Obtained by using D_{eff} from K_1 of the cumulant analysis. [b] Obtained by using D_{eff} from Γ_1 of the bimodal analysis. [c] Calculated according to eq 23 with values of A_2 from static light scattering,[19] $\upsilon = 0.56 \times 10^{-3}$ L g^{-1},[33] and $k_f = 0.03 \pm 0.01$ L g^{-1} (see text).

The experimentally determined value of D_0 may be compared to theoretical estimates for the translational diffusion coefficient of cylinders as calculated with the help of eq 10–12 and assuming the DNA molecules to have a length of 50 nm (values based on a rise per base pair of 3.3 nm (ref 32)) and a diameter of 2.5 nm. These values are $D_0 = 2.9 \times 10^{-7}$ cm^2 s^{-1} according to the Broersma formula and $D_0 = 2.6 \times 10^{-7}$ cm^2 s^{-1} according to Tirado and Garcia de la Torre. The former is in good agreement with the mean value derived from the light scattering results and the value derived from s_0. The value calculated with the help of the equation proposed by Tirado and Garcia de la Torre is approximately 10% lower but still consistent with the value $\bar{D}_0$ from the light scattering, considering the inaccuracy on the latter. In any case there is no evidence from these data that at the higher salt concentration some *bending* of the DNA fragments would occur with a subsequent increase of D_0. The bending suggested by Elias and Eden[33] from the values of the rotational diffusion coefficients of the same kind of DNA fragments would not be revealed by our measurements. The absence of a well-defined temperature dependence of D_{eff} at high salt concentration, after correction for the change in T and η, is also not in favor of bending to occur at the salt concentrations considered. The slight decrease of D_{eff} with temperature at the lower salt concentration but a much higher DNA concentration should be attributed to other effects. The temperature dependence results presented here are too fragmentary to allow any statements on their nature, however.

If we consider the effective diffusion coefficient to represent a mutual diffusion coefficient, the following concentration dependence of D_{eff} can be predicted by using eq 8 and 22:

$$D_{eff} = D_0[1 + (2MA_2 - \upsilon - k_f)C + ...] = D_0(1 + k_d C + ...) \tag{23}$$

Values of k_d obtained from a linear least-squares fit of D_{eff} vs C have been collected in Table IV with, at the lowest concentrations, the use of D_{eff} obtained from K_1 of the cumulant analysis as well D_{eff} derived from Γ_1 of the bimodal analysis. It may be noted that the latter are sys-

tematically lower. In the same table we have compared these experimental values of k_d with values of k_d calculated according to eq 23. The values of the second virial coefficients used in this calculation have been the values determined by static light-scattering experiments,[19] whereas k_f has been obtained from the sedimentation experiments and has been given above as well as the value of υ taken from literature. (In fact the influence of υ on k_d is negligible and that of k_f is small, particularly at the lower salt concentrations). The comparison between the experimental and the calculated values of k_d is quite satisfactory if, again, at the lower salt concentrations the values of k_d as derived from the effective diffusion coefficient obtained from the fast mode in the bimodal analysis are considered. Only for $C_s = 0.002$ M the discrepancy between calculated and experimental values is significant. At this very low salt concentration the intensity of the scattered light is weak and the inaccuracy on the experimental value therefore high.

It seems justified to accept the existence of a slow mode for solutions with $C_s \leq 0.01$ M. Though the value of the diffusion coefficient corresponding to the slow mode and its relative contribution to the correlation function cannot be determined with sufficient accuracy, it is well established that the value of that diffusion coefficient is at least more than 1 order of magnitude smaller than the diffusion coefficient corresponding to the fast mode which can be considered to represent the mutual diffusion coefficient of the individual rodlike DNA fragments. As for the origin of the slow mode we can, at present, only speculate. The occurrence of such a slow mode in the correlation function is not specific for the low molar mass DNA investigated here and may well be a general polyelectrolyte effect. It has been observed for several other polyelectrolytes. In the case of poly-L-lysine[11,35] and poly(styrenesulfonates)[13,14] a sharp transition from a region where the fast decay dominates the correlation function to a region where the slow one is preponderant has been observed upon lowering the salt concentration at constant polyelectrolyte concentration. Only for a very small salt concentration range are the two distinct decay rates detectable. In the case of low molar mass DNA, however, the two modes seem to coexist over a broader range of salt concentrations.

According to Fried and Bloomfield[17] a gellike phase is formed in solutions containing low molar mass DNA. This gellike phase has been assumed to be stabilized by the presence of salt. The observation of these authors that the contribution of the scattering by the DNA in the gel phase increases with increasing salt concentration is in contrast with our results and other dynamic light-scattering experiments on low molar mass DNA.[10,16] To explain results of steady-state fluorescence polarization experiments on short DNA fragments, Hand and Kearns[36] have assumed that loose aggregates are formed. Schmitz and Ramsey[35] have also suggested that temporal aggregates occur in solutions of poly-L-lysine at low salt concentrations by sharing of counterions by neighboring polyelectrolytes. Drifford and Dalbiez[13,14] have proposed that for solutions of poly(styrenesulfonates) at low salt concentrations, diffusion of short structured domains due to interchain interaction with a large correlation length might cause the slow mode in the correlation function. The slow mode observed by Koene and Mandel[15] for several poly(styrenesulfonate) samples in aqueous solutions *without salt* in the semidilute regime has been attributed to a reptation-like motion of the more flexible polyelectrolyte chains in a dynamic network in view of the concentration and molar mass dependence observed.

The present results on the nearly rodlike DNA fragments can, qualitatively, be explained by the presence of small amounts of loose aggregates or local ordering on a short length scale, appearing in the solution for $C_s \leq 0.01$ M or at high DNA concentrations. The fact that the slow mode cannot be characterized by a well-defined decay may thus be due to the fact that the size of these aggregates (or ordered domains) is highly heterodisperse and depending on the history of the solution. The occurrence of such aggregates could also be deduced from the static light-scattering experiments[19] on the same systems. There it was found that these aggregates are probably destroyed by filtration through Millipore filters (0.22 μm), possibly by shear effects. In fact, when the time-averaged scattered intensity was measured on flowing, continuously filtered solutions, no evidence for such aggregates could be detected. However, aggregates start to form immediately after filtration, but the process reaches equilibrium only very slowly as follows from the time effects observed. Because the molar mass of these aggregates is high, a small amount of such aggregates is enough to seriously influence the correlation function. An increasing influence of scattering from aggregates at lower angles could also explain the negative intercepts in the angular dependence of the first cumulants at small salt concentrations without affecting the linear dependence of K_1 on q^2 at the higher angles. At salt concentrations $C_s \geq 0.02$ M, where the correlation functions are well described by single exponentials, the concentration or the size of the aggregates probably decreases to such an extent that the correlation function determined by the diffusion of the individual macromolecules is only slightly affected. The temperature independence of the corrected effective diffusion coefficients at the higher salt concentration is consistent with the absence of appreciable amounts of aggregates.

It may be surprising from the point of view of the wormlike chain model that DNA fragments with a contour length approximately equal to its persistence length (at high ionic strength) exhibit a behavior that can be interpreted as that of a rigid rod. This has already been found for the second virial coefficient[19] and has been confirmed by the present study of the translational diffusion coefficient. It should, however, be taken into account that the wormlike chain is the simplified model based on the continuous space curve limit of an equivalent chain with local free rotation. Therefore it is quite possible that rather bulky molecules such as the DNA fragments considered reach the rod limit faster with decreasing contour length than predicted by the model.

Conclusions

Parasitic effects, probably attributable to the formation of aggregates, make it difficult to analyze accurately the intensity autocorrelation function of the DNA fragments investigated over a wide range of C_s values, particularly at low salt concentration ($C_s \leq 0.01$ M). Nevertheless, several conclusions may be drawn from the present study.

No anomalous DNA concentration effects have been observed over a large range of concentrations. An effective diffusion coefficient could be determined that depends linearly on the DNA concentration over a wide range. The concentration dependence of the effective diffusion coefficient and the appreciable change of this dependence with decreasing salt concentration may, to a large extent, be attributed to the salt concentration dependence of the second virial coefficient. Furthermore, the values of the effective diffusion coefficient extrapolated to zero concentration correspond to the translational diffusion coefficient of the DNA fragments that do not deviate appre-

ciably from a rodlike shape. This is in agreement with what has been concluded from the salt dependence of the second virial coefficient determined by static light scattering.[19]

At low salt concentrations the intensity correlation functions contain a contribution of a slow mode that is probably due to the (time-dependent) formation of loose aggregates or local ordering on a short length scale.

Acknowledgment. These investigations have been carried out under the auspices of the Netherlands Foundation for Chemical Research (SON) and with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). We are indebted to Dr. C. W. A. Pley (Department of Biochemistry, Leiden University) for his help in performing the sedimentation experiments.

References and Notes

(1) de Gennes, P. G. *Macromolecules* **1976**, *7*, 587.
(2) Odijk, T. *Macromolecules* **1979**, *12*, 688.
(3) Adam, M.; Delsanti, N. *Macromolecules* **1977**, *10*, 1229.
(4) Roots, J.; Nystrom, R. *J. Polym. Sci., Polym. Phys. Ed.* **1981**, *19*, 979.
(5) Koene, R. S.; Mandel, M. *Macromolecules* **1983**, *16*, 220.
(6) Koene, R. S.; Nicolai, T.; Mandel, M. *Macromolecules* **1983**, *16*, 227.
(7) Brown, W. *Macromolecules* **1985**, *18*, 1713; **1986**, *19*, 1083, 2002.
(8) Doi, M.; Edwards, S. F. *The Theory of Polymer Dynamics*; Clarendon Press: Oxford, 1986.
(9) Russo, R. S.; Karasz, F. E.; Langley, K. H. *J. Chem. Phys.* **1985**, *80*, 5312.
(10) Fulmer, A. W.; Benbassat, J. A.; Blomfield, V. A. *Biopolymers* **1981**, *20*, 1147.
(11) Lee, W. I.; Schurr, J. M. *J. Polym. Sci., Polym. Phys. Ed.* **1975**, *13*, 873.
(12) Brown, W. *Polymer* **1984**, *25*, 680.
(13) Drifford, M.; Dalbiez, J. P. *Biopolymers* **1985**, *24*, 1501.
(14) Drifford, M.; Dalbiez, J. P. *J. Phys. Lett.* **1985**, *46*, L-311.
(15) Koene, R. S.; Mandel, M. *Macromolecules* **1983**, *16*, 973.
(16) Mandelkern, M.; Dattagupta, N.; Crothers, D. M. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *87*, 4294.
(17) Fried, M. G.; Blomfield, V. A. *Biopolymers* **1981**, *20*, 2141.
(18) Elias, J. G.; Eden, D. *Macromolecules* **1981**, *14*, 410.
(19) Nicolai, T.; Mandel, M. *Macromolecules* **1989**, 22, 438.
(20) Berne, B.; Pecora, R. *Dynamical Light Scattering*; Wiley: New York, 1976.
(21) Yamakawa, H. *Modern Theory of Polymer Solutions*; Harper and Row: New York, 1974.
(22) Broersma, S. *J. Chem. Phys.* **1960**, *32*, 1626.
(23) Tirado, M. M.; Garcia de la Torre, J. G. *J. Chem. Phys.* **1979**, *71*, 2581.
(24) Tirado, M. M.; Garcia de la Torre, J. G. *J. Chem. Phys.* **1984**, *81*, 2047.
(25) Shindo, H.; McGhee, J. D.; Cohen, J. S. *Biopolymers* **1980**, *19*, 523.
(26) Alon, Y.; Hochberg, A. *Rev. Sci. Instrum.* **1975**, *46*, 388.
(27) Koppel, D. *J. Chem. Phys.* **1972**, *57*, 4814.
(28) Phillies, G. D. J. *Macromolecules* **1976**, *9*, 447.
(29) Ackerson, B. *J. Chem. Phys.* **1978**, *69*, 684.
(30) Hess, W. *Light Scattering in Liquids and Macromolecular Solutions*; Plenum: New York, 1980.
(31) Nash, J. C. *Compact Numerical Methods*; Hilger: Bristol, United Kingdom, 1979.
(32) (a) Nicolai, T. Thesis, Leiden, 1987. (b) Nicolai, T.; Mandel, M., to be submitted for publication.
(33) Elias, J. G.; Eden, D. *Biopolymers* **1981**, *20*, 2364.
(34) Gray, H. B.; Hearst, J. E. *J. Mol. Biol.* **1968**, *35*, 111.
(35) Schmitz, K. S.; Ramsey, D. J. *Biopolymers* **1985**, *24*, 1247.
(36) Hard, T.; Kearns, D. R. *Biopolymers* **1986**, *25*, 1519.

Viscosity of Dilute Polyelectrolyte Solutions: Concentration Dependence on Sodium Chloride, Magnesium Sulfate, and Lanthanum Nitrate

J. Cohen and Z. Priel*

Department of Chemistry, Ben-Gurion University, Beer-Sheva 84105, Israel.
Received June 21, 1988; Revised Manuscript Received October 24, 1988

ABSTRACT: The reduced viscosity of polyelectrolyte solutions has been measured by using fully sulfonated sodium-neutralized polystyrene to which magnesium sulfate or lanthanum nitrate has been added. The high accuracy and sensitivity of the experimental setup enables us to perform measurements at extremely low polymer and salt concentrations. It was found that increasing the charge of the cation from monovalent (Na^+) to divalent (Mg^{2+}) and trivalent (La^{3+}) shifts the maximum position of the reduced viscosity toward higher polymer concentrations and decreases the absolute value of the reduced viscosity. A good agreement between experimental findings and theoretical predictions was obtained.

Introduction

Early investigations of the anomalous viscosity of polyelectrolyte solutions without added salt appeared to suggest that the reduced viscosity would increase without bound as one lowers the polyion concentration.[1] However, a careful investigation of the dilute solution behavior revealed that the apparently unbounded rise of the reduced viscosity is always followed by a maximum and normal polymer behavior which is recovered at the extrapolated limit of zero polyelectrolyte concentration.[2] It has been argued that the observed behavior is a consequence of the screening of long-range intermolecular interactions by the residual electrolyte in the solution.[2] This explanation was supported by the observation of "regular" neutral polymer behavior upon isoionic dilution of polyelectrolyte solutions.[3,4] Most of the experimental work dealt with the existence of the maximum that appeared at relatively low polymer concentrations and therefore was close to the limit of the accuracy of the measuring systems. On the other hand, there are no systematic measurements, as far as we know, of a given polyelectrolyte vs chemical and physical parameters.

We have developed an apparatus that enables us to perform accurate measurements of the shear viscosity of low ionic strength, dilute polyelectrolyte solutions down to polymer concentrations below 1 part per million.[5,6] A

0024-9297/89/2222-2356$01.50/0