Dynamics of DNA in Polyacrylamide Solutions. An Electrical Birefringence Study

Konstantin Starchev, Jean Sturm,* and Gilbert Weill

Institut Charles Sadron, C.N.R.S. and University Louis Pasteur, 6 rue Boussingault, F-67083 Strasbourg, France

Received July 23, 1998; Revised Manuscript Received November 13, 1998

ABSTRACT: The electrophoretic transport of λ-DNA in solutions of linear polyacrylamide (7×10^5 Da) has been investigated by electric birefringence. Three regimes depending on the concentration of polyacrylamide have been determined: at high concentration, a gellike regime shows a behavior that is compatible with reptation theory, and the birefringence signal shows the characteristic overshoot when the electric field is applied. At low concentration, the results are consistent with the formation of a 1−1 entangled complex of DNA and polyacrylamide. Between, a crossover regime is characterized by a threshold field above which the overshoot appears. It is shown that the threshold is correlated with the field where the lifetime of the entanglements of polyacrylamide becomes longer than the time of deformation of the DNA in the field.

Introduction

Capillary electrophoresis in buffers containing hydrophilic linear polymers has been shown to be effective in the separation of both double-stranded and single-stranded DNA.[1−3] This technique combines the advantages of free-solution capillary electrophoresis, in terms of automation, speed, reproducibility, and on-line detection, with the resolving power of gel-based methods. Considerable efforts are currently focused on the use of entangled polymer solutions as sieving medium. The separation mechanism in entangled solutions[4,5] is generally assumed to depend on the concentration in a way comparable with the Ogston model[6] and biased reptation models[7,8] developed for permanent gels. The role of the limited lifetime of the entanglements has been addressed by Duke and Viovy.[5,9]

Recently, the experimental results of Barron et al.[10−12] have demonstrated that separation can also be achieved in dilute unentangled solutions of hydroxyethylcellulose (HEC) as well as other linear water-soluble polymers.[13−15] The separation is found to be 5−10 times faster than in entangled polymer solutions. However, an extended network does not exist below the entanglement threshold, and the classical approaches developed in the gel case[6−8] cannot be transcribed to the dilute solution case. Barron et al. proposed an alternative mechanism for DNA separation in dilute HEC solutions[10] assuming that, when a DNA and a HEC molecule encounter, they undergo a transient entanglement coupling. Hence, DNA molecules are forced to drag HEC molecules along with them, resulting in a decrease of the DNA electrophoretic mobility. Separation occurs because the formation of entanglements between the DNA and the polymer molecules depends on the size of the DNA molecule.

Videomicroscopy of DNA in solutions of HEC[16,17] indeed demonstrates entanglements of DNA with one or several discrete HEC molecules but is unable to estimate the rates of formation and dissociation as well as the dynamics of the process. The same technique, applied to T2-DNA in dilute solutions of high molecular weight polyacrylamide, reveals a rotatory "waltzing motion",[18] but the description remains qualitative. A theoretical model for the formation and dissociation of the entangled complex that fits with the DNA molecular weight and HEC concentration dependence of the electrophoretic mobility has been developed,[19] based on the competition between the electrophoretic force acting on DNA and the additional drag forces due to the entangled HEC molecules. As a result, one expects that, even in dilute polymer solutions, the DNA is deformed in the direction of the field, making the solution optically anisotropic.

In this paper we explore the electric birefringence of λ-DNA molecules in high molecular weight polyacrylamide solutions throughout the dilute and semidilute (entangled) range of concentrations, as a way to characterize the extent and dynamics of deformation in different regimes. Linear dichroism of YOYO stained T2-DNA in dilute and semidilute solutions of polyacrylamide has been reported recently by Carlsson et al.[20] Our work provides, from a more quantitative analysis of our results, some new interpretation of the different regimes.

Materials and Methods

The birefringence instrument consists of a 12 mW He−Ne laser, two 10 mm Glan-Thomson prisms as polarizer and analyzer crossed at 45° with respect to the field, a standard square spectrometer quartz cell with 1 cm optical length fitted with two horizontal parallel electrodes 8 mm apart, a $\lambda/4$ plate placed after the cell for linear detection, and a photodiode as detector. The signal was sent to a 14-bit AD converter (DATEL PC-414) with 8K memory capacity and then processed by a PC microcomputer. The rise and decay of the signal was analyzed using a Marquardt−Levenberg nonlinear fitting algorithm. The computer also drives the rotation of the analyzer with a stepping motor (one step = 10^{-3} deg) and a home-built high-voltage generator. This generator can produce squared and reverse pulses up to 1000 V. However, in the present investigation the field strength was limited below 100 V/cm to prevent the sample from heating. The resolution of the birefringence setup was 2×10^{-5} deg per bit/channel. It was found that the signals are reproducible within 1×10^{-4} deg optical retardation. In the cases of low signal/noise ratio, up to 100 accumulations were used. The intrinsic time constant of the birefringence setup was less than 10 μs (measured from the relaxation of acetone). Usually 2048 points were used for recording the response to a square pulse. The fitted relaxation

10.1021/ma981163z CCC: $18.00
Published on Web 01/05/1999

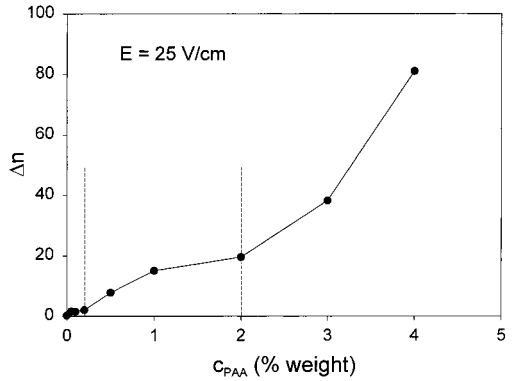

Figure 1. Steady-state electric birefringence of DNA in polyacrylamide solutions (7×10^5 Da) as a function of the polyacrylamide concentration; $E = 25$ V/cm, 0.01 M TBE buffer. The birefringence value is in arbitrary units.

times were reproducible with less than 10% standard error for signals of 10^{-3} optical retardation (after accumulation).

A Tris borate EDTA buffer, pH 8.3, 0.01 M, was used as solvent to meet the conditions of electrophoresis. Due to the unsufficient purity of commercial high molecular weight polyacrylamides, PAA was obtained from the polymerization of acrylamide (ultrapure, ICN) in the buffer. A solution containing 4% acrylamide was degassed by nitrogen bubbling; then 0.8 μL of TEMED and 0.8 μL of ammonium persulfate (from a stock solution at 25%) per milliliter of acrylamide solution were added. The polymerization was complete after mixing for 2 h. The intrinsic viscosity of PAA was measured in a capillary viscometer, $[\eta] = 300$ mL/g, corresponding to a molecular weight around 7×10^5 Da, a contour length $\cong 2 \times 10^4$ Å, a radius of gyration $\cong 250$ Å, and an overlap concentration $c^* \approx 0.3\%$. The molecular weight distribution has not been studied, but from former studies, using the same polymerization process, the ratio M_w/M_n is expected to be equal to 2.[21] The dynamic shear modulus of the 4% solution has been measured with a Haake RV20/CV20 rheometer. The 4% stock solution was diluted to the final concentration with buffer. No birefringence could be observed from these PAA solutions for fields below 100 V/cm. They present however a positive birefringence at larger fields. λ-DNA (48 500 bp, contour length $\cong 1.65\ 10^5$ Å, radius of gyration $\cong 5000$ Å) was purchased from Sigma. DNA was added to the polyacrylamide solution to a concentration of 20 μg/mL.

Results and Discussion

A negative birefringence signal was obtained for all PAA concentrations due to the orientation of the DNA base pairs perpendicular to the field. Figure 1 shows the steady-state birefringence as a function of PAA concentration at $E = 25$ V/cm. At zero PAA concentration the small negative birefringence in solution is always detected. It is attributed to the orientation resulting from the ionic polarization of the DNA double layer.[22]

The increase of the birefringence with PAA concentration reveals three domains: (i) At very low concentration, there is a finite increase which levels off around $c \approx 0.2\%$. (ii) A second increase takes place between 0.2% and 2% with a downward curvature. (iii) Above 2% the birefringence increases again with an upward curvature. It is interesting to note that the overlap concentration of the PAA, $c^* \approx [\eta]^{-1} \approx 0.3\%$, is inside of the second domain.

The field dependence of the birefringence has been found to follow the E^2 Kerr prediction up to 0.5% PAA (Figure 2). Indeed symmetry considerations upon electric field reversal impose that the contribution to the birefringence of one DNA polyacrylamide entangled

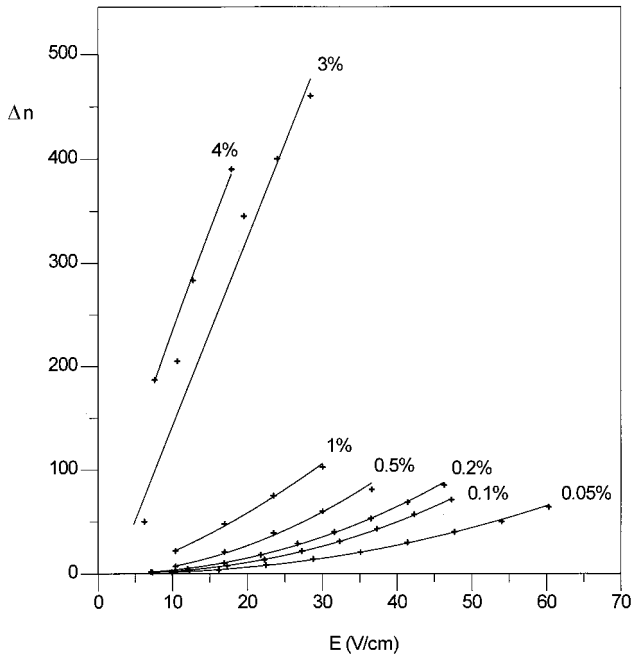

Figure 2. Field dependence of the steady-state electric birefringence of DNA at several concentrations of polyacrylamide (7×10^5 Da); 0.01 M TBE buffer.

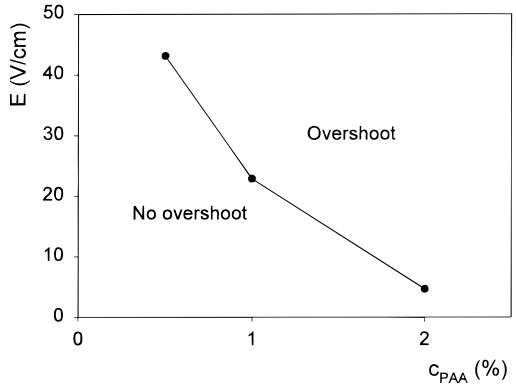

Figure 3. Threshold field for the apparition of an overshoot as a function of the polyacrylamide concentration (percent weight).

"complex", through deformation and orientation, is proportional to E^2 at low field. Such an overall E^2 dependence implies therefore that the number of "complexes" is field-independent. Above 2% the field dependence is predominantly linear in the range 5−20 V/cm. In this range of PAA concentrations, an overshoot is always present in the rise of the birefringence, as found for DNA in agarose gels,[18,19] indicating a gellike behavior. At concentrations between 0.5% and 2% we have observed that the monotonic rise of the birefringence at low field transforms into an overshoot at high fields. A schematic "phase diagram" giving the threshold field as a function of the PAA concentration is given in Figure 3. At very low concentrations there is no overshoot, whatever the field, and the rise of the birefringence can be fitted by a sum of two exponentials. In all three regimes the decay of the birefringence cannot be fitted by a single exponential, but a superposition of two exponentials with time constants τ_1 and τ_2 and relative amplitudes A_1/A_2 is always satisfactory. This contrasts with what has been observed for DNA in agarose gel where the decay is fitted by the superposition of a fast stretched exponential with a field-dependent exponent

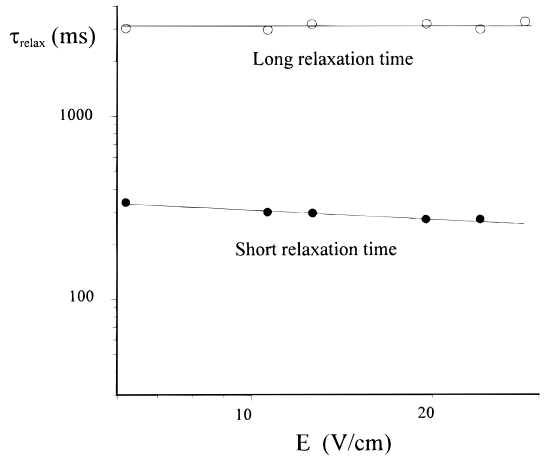

Figure 4. Field dependence of the long decay time constant in the gellike regime.

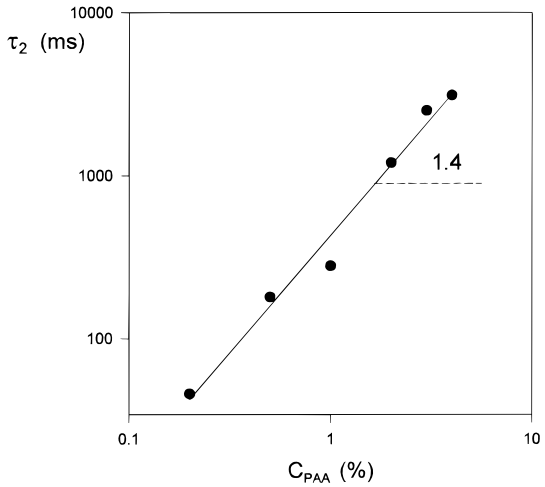

Figure 5. Concentration dependence of the long decay time in the gellike regime. Concentration of PAA is given in percent weight.

and time constant and of a slow exponential with a field-independent time constant.[23,24] This is related to overstretching, i.e., an increase in the number of pores occupied by the DNA chain or, in other words, to the increase of the tube length. The fast decay corresponds to the relaxation of the tube length to its equilibrium value and the slow one to the reptation of the relaxed molecule out of the oriented tube. Overstretching therefore requires that the total DNA contour length is much larger than the equilibrium tube length or the pore diameter a much larger than the DNA persistence length P. In semidilute solutions the role of a is played by the screening length ξ, and overstretching is therefore not expected for $\xi < P$. Scaling laws for $a < P$ have been predicted by Semenov et al.[25]

Having indicated the qualitative features that substantiate the existence of three regimes, we now proceed to a more detailed and quantitative analysis of the results in respectively the high concentration "gellike", crossover, and dilute regimes.

Gellike Regime: $c_{PAA} > 2\%$ ($M_w = 7 \times 10^5$ Da). For DNA in agarose gels, where the pore size a is larger than the DNA persistence length (50 nm), the overall orientation has been shown to increase with increasing pore size.[26] In this regime (Figure 1), the overall orientation increases with increasing concentration, i.e., with decreasing pore size. In PAA solutions, the pore size a is related to the screening length $\xi \propto (c/c^*)^{-3/4}$.[29] Values of ξ for PAA solutions are given in ref 20; for $c = 1\%$, $a \approx 11$ nm, much smaller than the DNA persistence length. In this constrained situation, the smaller the pore size, the more extended is the DNA conformation and the resulting orientation in the electric field. We therefore do not expect any significant overstretching. This agrees with the biexponential relaxation of birefringence mentioned above where the two time constants are found essentially field independent (Figure 4). We cannot know however whether the biexponential decay reflects the presence of internal modes or a distribution of pore sizes and conformation. Moreover, values of τ_1 are more sensitive than τ_2 to the fitting procedure. We therefore focus the discussion on the concentration dependence of the longest relaxation time τ_2 which is expected to reflect the reptation time. As, from reptation theory, it is predicted to vary with a^{-2}, one expects a $c^{1.5}$ dependence. The values of τ_2 from the gellike regime are plotted in Figure 5 together with those derived in the intermediate regime, for fields above the overshoot threshold. They appear compatible with the reptation prediction.

In the study of the birefringence of DNA in high concentration agarose gels, it has been found that the overshoot disappears at high concentrations, as expected if the pore size becomes comparable to the DNA persistence length. From what is said above, the presence of an overshoot in the gellike regime may appear inconsistent since the overshoot is always present and takes place at a time τ_{ov} which varies roughly as E^{-1} in a way resembling what is found in permanent gels with pore sizes larger than the DNA persistence length. One must however consider that the semidilute solution has an internal dynamic due to both facts that, compared to an agarose gel, the PAA chain is flexible and the chain entanglements mobile. It is in that respect interesting to compare the values of τ_{ov} to the terminal relaxation time of the PAA solution. This is considered in the next section, dedicated to the intermediate regime.

Crossover Regime: $0.5\% < c_{PAA} < 2\%$ ($M_w = 7 \times 10^5$ Da). The most striking effect in this regime is the existence of a threshold field above which the overshoot appears. Above this field, τ_{ov} again varies as E^{-1}. This dependence is similar to that observed in a permanent gel. The apparition of the overshoot seems therefore to be connected to the rate of formation of J conformations with extended arms[27] which increase with E and to the lifetime of the entanglements. Below the threshold field the entanglements yield, and no overshoot is observed. It is therefore natural to compare the observed values of τ_{ov} at the threshold field with the lifetime of the entanglements as measured from the terminal time derived from rheology. Measurements of the frequency dependence of the dynamic moduli G' and G'' have been carried out on a 4% PAA solution, and the terminal time τ_t has been calculated as the inverse of the frequency at which $G'(\omega)$ and $G''(\omega)$ cross at low frequency:[28] $\omega \approx 0.1$ Hz $\Rightarrow$ $\tau_t = \omega^{-1} \approx 10$ s. The values of τ_t at other concentrations have been calculated using the scaling law $\tau_t \propto c^{1.5}$.[29] The results are reported in Table 1. The correlation is very good and suggests that overshoot takes place when the lifetime of the entanglements is much longer than the time of deformation of the DNA in the field. This gives a basis to the phase diagram of Figure 3 which should now be checked using PAA of

Table 1. Comparison of the Overshoot Time at the Threshold Field and the Terminal Time of the Semidilute Polyacrylamide Solution

c_{PAA} (%)	τ_{ov} (ms)	τ_{max} (ms)
0.5	275	440
1.0	560	1250
2.0	2913	3500

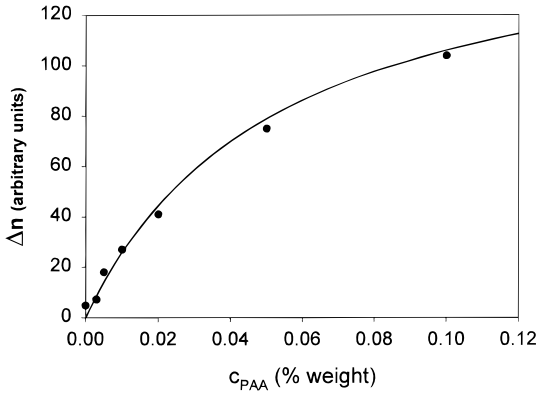

Figure 6. Steady-state electric birefringence of DNA in dilute polyacrylamide solutions. The fit assumes the formation of a 1−1 DNA−polyacrylamide complex ($\Delta n \propto kc/(1 + kc)$); $E = 25$ V/cm.

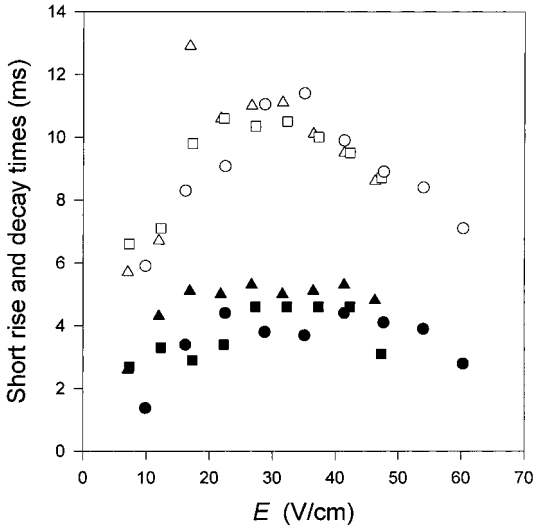

Figure 7. Short rise (open symbols) and decay (closed symbols) times of the DNA electric birefringence in dilute polyacrylamide solutions: $c = 0.05\%$ (○); $c = 0.1\%$ (□); $c = 0.2\%$ (△).

different molecular weight. In these conditions, we check also that the decay times are essentially independent of the electric field above the threshold field (where the ratio A_1/A_2 increases more rapidly), and we can assume that the long time constant τ_2 reflects the reptation time of the DNA molecule in a permanent gel, allowing to report them in Figure 5 to check the concentration scaling law.

Dilute Regime; $c_{PAA} < 0.2\%$ ($M_w = 7 \times 10^5$ Da). The most remarkable features of the steady-state birefringence in this regime are firstly the shape of the dependence with the polyacrylamide concentration at constant electric field (Figure 6). Neglecting the contribution of bare DNA, it can be fitted, for linear polyacrylamide concentrations lower than 0.1%, i.e., well below c^*, with $\Delta n \propto kc/(1 + kc)$ corresponding to the fraction

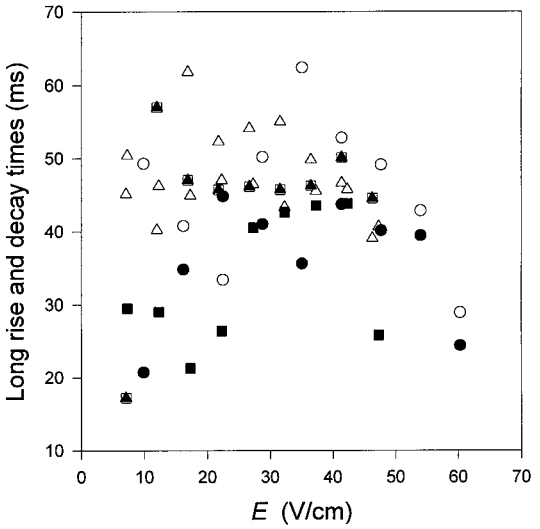

Figure 8. Long rise (open symbols) and decay (closed symbols) times of the DNA electric birefringence in dilute polyacrylamide solutions: $c = 0.05\%$ (○); $c = 0.1\%$ (□); $c = 0.2\%$ (△).

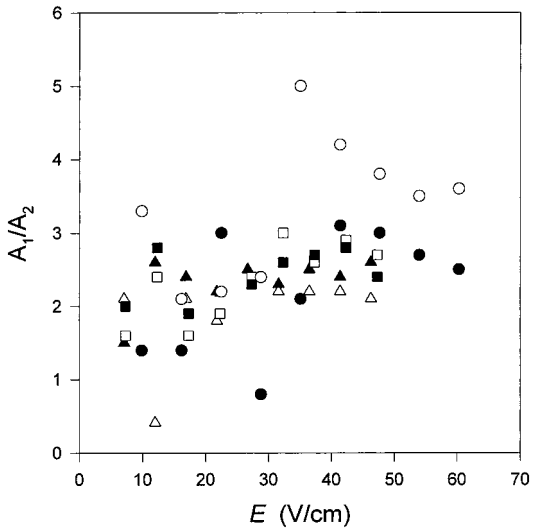

Figure 9. Ratio of the amplitudes of the biexponential short and long rise (open symbols) and decays (closed symbols) of the DNA electric birefringence in dilute polyacrylamide solutions: $c = 0.05\%$ (○); $c = 0.1\%$ (□); $c = 0.2\%$ (△).

of entangled DNA, assuming an equilibrium

$$\text{DNA} + \text{LPA} \Leftrightarrow \text{DNA}\cdots\text{LPA}$$

A second feature is the E^2 dependence at constant polyacrylamide concentration (Figure 2).

This is consistent with the entanglement of one bare DNA molecule with one polyacrylamide molecule, with a field-independent equilibrium constant, and the subsequent orientation (deformation) resulting from the competition between the electric forces acting on the DNA and the drag force amplified by the presence of entangled polyacrylamide. In their model of capillary electrophoretic separation of DNA using ultradilute polymer (HEC), Hubert et al.[19] assume that both the collision time between a DNA and a polyacrylamide molecule and their disentanglement time are proportional to the electrophoretic velocity, i.e., to the electric field. This corresponds, in terms of an equilibrium theory for the formation of the complex, to rates of formation and dissociation that are both proportional to E, making their ratio k independent of E.

Further arguments for the formation of a predominantly 1−1 complex at concentrations well below c^* can be found in the analysis of the rise times and decay times of the birefringence. If complexes with more than one polyacrylamide molecule would be formed when increasing the polyacrylamide concentration, the characteristic times would be expected to change appreciably. As mentioned above, the rise and decay have been fitted with two exponentials. The two rise times τ_1 and τ_2, the two decay times τ'_1 and τ'_2, and the corresponding ratios of amplitudes A_1/A_2 and A'_1/A'_2 are plotted in Figures 7, 8, and 9 as a function of the electric field for different concentrations of polyacrylamide in the dilute regime. There is no significant dependence on the concentration, implying a similar "complex" at all concentrations. The short time constants τ_1 are significantly larger than τ'_1, while τ_2 and τ'_2 are very close. These times are evidently connected with the process of deformation, and it should be interesting to study their variation using DNA and polyacrylamide of different molecular weights and reduced polymolecularity.

Conclusion

A crossover between a dilute regime and a gellike regime has been clearly identified for the electric birefringence of λ-DNA in polyacrylamide solutions, in connection with DNA electrophoresis. The crossover depends not only on the overlap concentration but also on the electric field. This can be understood semiquantitatively if one considers that, at higher fields, the electrophoretic transport becomes fast as compared to the time of reorganization of the entanglements in semidilute solutions. Above the crossover, the concentration dependence of the longer characteristic decay time of the birefringence fits the predicted concentration dependence for the DNA reptation time. In the dilute regime, the concentration dependence of the birefringence can be understood as resulting from a dynamic equilibrium between bare DNA and a 1−1 DNA−polyacrylamide complex. The lack of higher complexes may be due to the fact that the high molecular weight linear polyacrylamide and the λ-DNA do not differ sufficiently in size, a condition which probably does not correspond to optimized separation in ultradilute solutions. A combination of electric birefringence experiments and of measurements of electrophoretic mobility of DNA in dilute and semidilute polymer solutions appears, as in permanent gels, promising to understand the underlying mechanisms of electrophoretic separation, particularly for dilute polymer solutions.

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MA981163Z