Electric Birefringence Measurements in Aqueous fd Virus Solutions

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ABSTRACT: Aqueous solutions of rodlike fd virus particles (length l = 880 nm, diameter d = 9nm) are examined by the transient electric birefringence method. A special experimental setup is used, which allows a very sensitive variation of the screened Coulomb interaction between the particles. The birefringence signal is measured as a function of the virus particle concentration and the ionic strength of the solution. For low applied electric field strength it is demonstrated that anomalous birefringence is an effect of the steric and electrostatic interactions between the fd virus particles. It is shown that this behavior is correlated to a decrease of the rotational diffusion constant D_R. In the case of high ionic strength the deduced values of D_R are in agreement with the theory of Teraoka and Hayakawa[1] up to a concentration of $8c^*$ (c^* = 1 particle/length^3 is the overlap concentration); at larger concentrations the values are significantly higher than predicted.

Introduction

Polyelectrolytes represent a class of macromolecules that carry a large number of elementary charges distributed along them. Some polyelectrolytes are stiff rodlike particles like tobacco mosaic viruses (TMV). Polyelectrolytes can also be flexible as in the case of most of the synthetic polyelectrolytes. Between these two extrema ranges the fd virus investigated in this paper, which is a semiflexible filamentous bacteriophage of length l = 880 nm, diameter d = 9 nm, and very low polydispersity.[2] On its surface approximately 10^4 ionizible acid groups are located. In aqueous solutions these give rise to a negative charge of up to 500 e^- on each virus, which in highly deionized water leads to a long-range Coulomb interaction. The interaction can be screened by adding salt (NaCl) or Tris/HCl to the solution. The strength of the electrostatic as well as that of the steric interaction has a strong influence on the electric birefringence.

In dilute solutions polyelectrolytes can be easily oriented in the direction of an electric field. This behavior is well understood. It originates in the very strong anisotropy of the ionic polarizability in comparison to the atomic polarizability or the effect of a permanent dipole moment. Applying an electric pulse to the solution results in a time-retarded buildup of the birefringence Δn until a stationary value Δn_0 is reached. At the end of the pulse the birefringence decays with a characteristic time constant, from which the rotational diffusion constant D_R and the length of the polyelectrolytes can be calculated.

At higher concentrations deviations from that normal behavior occur. A second effect is observed that has an opposite sign to that of the first one. This behavior has been generally called the anomaly of the electric birefringence. The effect was studied by many authors on rodlike particle systems like aqueous solutions of micelles,[3] TMV,[4-7] and bentonites[8] and flexible species like NaPSS.[9] Several models and interpretations for this effect were given.

Asai and Watanabe[4] studied this anomaly on TMV solutions in a concentration range between $0.2c^*$ and $3.5c^*$. They observed anomalous birefringence at low field strengths above a concentration of $0.8c^*$. The strength of the anomalous signal depended on the concentration of the added salt. Therefore they concluded that the anomaly msut be an effect of the electric and steric interaction between the rodlike macromolecules. They suggested that the anomaly is caused by the appearance of a permanent dipole contribution perpendicular to the long axis of the virus in concentrated solutions. However they did not explain how this dipole moment can be related to steric or electric interactions between the macromolecules. No detailed measurements of the rotational diffusion coefficient were presented.

Hoffmann et al.[3] found anomalous birefringence in aqueous solutions of rodlike micelles above the overlap concentration. They interpreted the results by a buildup of an induced dipole moment perpendicular to the rod axis caused by the overlap of the double layers of the rods. However, it is difficult to interpret the data, because the length of the micelles in the solution changes with concentration.

The fd virus is an excellent system to study this anomaly because of its low polydispersity, relatively large stiffness, high charge, and therefore high electrostatic interaction and low aggregation rate. These parameters beside others enter also the structural and diffusion properties accessible to static (SLS) and dynamic (DLS) light-scattering experiments.[10-12] One difficulty in dynamic light-scattering investigations is the occurrence of different contributions in the correlation function like diffusive, bending, and rotational modes. They can be reduced by deriving values for the rotational diffusion constant from birefringence measurements. For fd particles those are only available for high ionic strength.

The aim of this paper is therefore on the one hand to supply a set of rotational diffusion coefficients as function of ionic strength and particle concentration. On the other hand we wish to show that the effect of the anomaly of the electric birefringence is strongly related to the interaction between the rods. Therefore the transient electric birefringence (TEB) apparatus was combined with a tube pump system which allows the variation of the ionic strength, i.e. the interaction of the particles at fixed concentration in a very sensitive way. Ionic strengths down to 10^{-6} M could be achieved.

In this paper results at small electric fields (4×10^2–2×10^4 V/m) are presented.

Experimental Section

Materials. A stock solution (9 mg/mL) of fd virus particles (length l = 880 nm, diameter d = 9 nm, MW = 16.4×10^6, about 10^4 ionizable groups on the surface) was prepared with the help

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of Professor I. Rasched (University of Konstanz) following a method of Marvin et al.[13] Therefore *Escherichia coli* bacteria were infected with the fd virus. After 8 h at 37 °C the fd virus bred rapidly by a factor of 1000. The reproduction was stopped, and the viruses were separated from the bacteria by several steps of precipitation and centrifugation. Finally the fd virus solution was ultracentrifuged in a CsCl gradient and dialyzed against a solution of 0.01 M Tris/HCl to obtain a pure stock solution. The samples were prepared by diluting the stock solution with highly purified water (R = 18 MΩ). To eliminate dust and other large particles, the samples were centrifuged at 2000 rpm for 10 h before each measurement. From SLS measurements and electron microscopy we conclude that the samples are almost free of aggregates and virus fragments. The actual concentrations of the samples were determined by their UV absorption at $\lambda = 269$ nm (extinction coefficient $\epsilon = 3.84$ cm^2/mg), using a Beckmann spectrometer (DU-64, Darmstadt, FRG).

Tube Pump System. In a closed circuit, including the birefringence cell and the conductivity cell, the samples were deionized by pumping them with a tube pump through mixed-bed ion-exchange resin (MB3, Serva Diagnostics, Heidelberg, FRG) until the desired conductivity was achieved.[14] When the exchange process was continued, the conductivity became constant in time, and the suspension could be considered free of small ions other than H^+ or OH^-. The minimal ionic strength, which can be reached, depends on the particle concentration. Alternatively, NaCl or Tris/HCl was added to the suspensions to obtain higher conductivities, i.e. smaller Coulomb interaction. All measurements were carried out at 20.0 ± 0.2 °C. Conductivity measurements were performed using a Knick conductometer (Knick, Berlin, FRG).

Transient Electric Birefringence Apparatus. The electric birefringence apparatus was a commercial instrument (spectrometer DB 10, Suck, Siegen, FRG) and similar to that described elsewhere.[15] The pulse length of the electric field was adjusted such that a steady-state value of the signal was reached and ranged between 2×10^{-2} and 2×10^{-1} s^{-1}. The rotational diffusion constant D_R was determined from the decay of the birefringence signal after a rectangular pulse had been applied. In the case of a field-free monoexponential decay D_R was deduced by using the formula

$$\Delta n(t) = \Delta n_0 \exp(-6D_R t)$$

where $\Delta n(t)$ is the decay of the electric birefringence and Δn_0 is the steady-state value.

Results and Discussion

From literature the following quantities concerning the rotational properties for fd virus particles are known:[16-18] The magnetic birefringence of fd virus solutions (concentration range 0.02–2 mg/mL, i.e. 0.5–50c^* in 0.01 M Tris/HCl) is positive. Shear-aligned solutions of fd viruses are positively birefringent with respect to the direction of shear. Therefore fd viruses orient parallel to the magnetic field and in the case of positive electric birefringence fd viruses orient parallel to the electric field too. In the dilute range at high ionic strength the rotational diffusion D_R of the fd virus was determined by the transient electric birefringence (TEB) technique to be 20.9 ± 0.3 s^{-1}.[2]

The behavior of fd viruses in aqueous solution in the electric field depends on different factors: the particle concentration, the interaction between the particles, and the applied electric field strength.

In our investigations the fd virus solutions were examined up to a concentration of 40c^* at high salt concentration and therefore completely screened Coulomb interaction and up to 10c^* at minimal ionic strength. At higher concentrations a phase transition between the liquidlike and a crystalline phase occurs. The concentrations are usually divided in two parts, the dilute range ($c < c^*$) and the semidilute range ($c > c^*$). In our case it seems useful to introduce a third, intermediate concentration range.

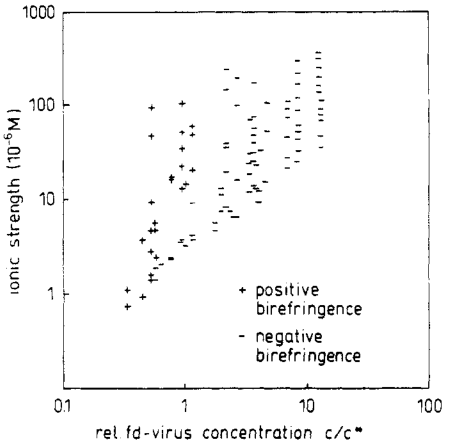

Figure 1. Sign of the electric birefringence signal of aqueous fd solutions at low applied field strength (4×10^2–2×10^4 V/m) as a function of the virus concentration and the ionic strength.

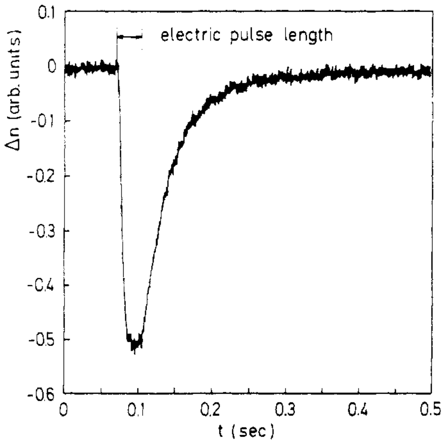

Figure 2. Typical anomalous birefringence signal of fd virus at concentration 1c^*, ionic strength 4.5×10^{-6} M, and electric field strength 6×10^3 V/m.

In the dilute range ($c < 0.5c^*$), even without added salt, effects of particle–particle interaction are not observed. In static light-scattering experiments a liquidlike order caused by electrostatic interactions is observed also below $c = 0.5c^*$ at minimal ionic strength. This seems not the case in our experiment, possibly since we are not able to reach quite the same small ionic strength due to the experimental setup. In the semidilute range ($40c^* > c > 1.2c^*$) the particles are sterically interacting, and at low salt concentration they can additionally interact via their screened Coulomb potentials. In the third range, the intermediate range ($0.5c^* < c < 1.2c^*$), no steric interaction is observed, but at low salt concentrations the particles are electrostatically interacting.

Figure 1 shows the sign of the birefringence signal at low applied electric field strengths (4×10^2–2×10^4 V/m) depending on the virus concentration and the ionic strength of the solution. We find that anomalous birefringence exists only when the fd particles are able to interact with each other via screened Coulomb or hard-core interaction. Above a concentration of 1.2c^*, even at ionic strengths of 10^{-2} M, the birefringence is negative. A typical negative birefringence signal is shown in Figure 2.

If we go, in Figure 1 for a fixed ionic strength, from very low particle concentration to the semidilute concentration range, we see the sign changing from positive to negative at a certain concentration. At very low particle concentration corresponding to a positive sign the single particle behavior is measured. This means that the anomaly of the birefringence is not a phenomenon of a single rod in

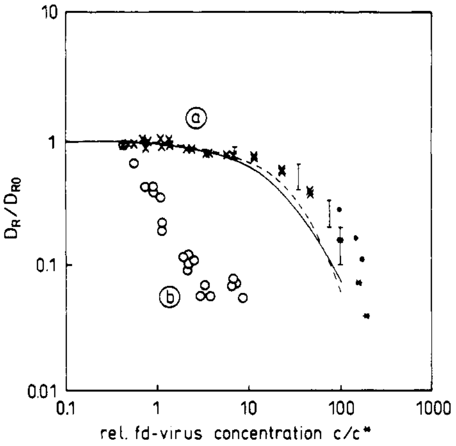

Figure 3. Normalized values of the rotational diffusion constant D_R/D_{R0} (D_{R0} = 21.7 ± 0.5 s^{-1}), as a function of the fd virus concentration: (a) Key: (X) high ionic strength (10^{-2} M); (—) theory of Teraoka et al.;[1] (- - -) Monte Carlo simulations by Doi et al.;[19] (I) values determined by dynamic light scattering by Fujime et al.;[23] (●) values determined by transient electric birefringence by Maguire et al.;[24] (*) values determined by transient magnetic birefringence by Nakamura et al.[25] (b) Key: (O) minimal ionic strength.

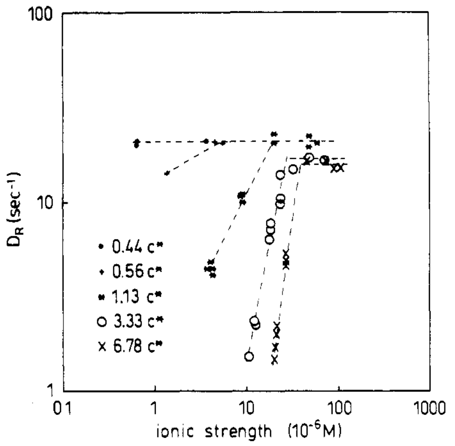

Figure 4. Rotational diffusion constant D_R as a function of the ionic strength for different fd virus concentrations. The dashed lines serve to connect the data points.

a solution at a fixed ionic strength: above a certain particle concentration it is a collective phenomenon of the interacting rods in the solution. Therefore the negative sign is not an effect of change of the optical anisotropy of a single particle. We can be rather sure that the optical anisotropy of the particles remains constant when the particle concentration increases while the ionic strength remains constant. Therefore it is very probable, that when we observe anomalous birefringence the interacting fd virus particles prefer to orient perpendicular to the applied electric field at low field strength ($c > 0.5c^*$).

Figure 3, curve a, shows the dependence of D_R on the fd concentration at high ionic strength (10^{-2} M), here the particles interact via hard-core repulsion. Below c^* a value of D_{R0} = 21.7 ± 0.5 s^{-1} for the rotational diffusion constant is measured, which is in agreement with the results of Newman et al.[2] Up to a concentration of about $8c^*$ the values are following the curve derived by Doi et al. using Monte Carlo simulations,[19] and they are in agreement with the predicted relationship between D_R and D_{R0} by Teraoka and Hayakawa:

$$D_R/D_{R0} = (1 + b^{-1/2}(nL^3))^{-2}$$

where $b = 1.35 \times 10^{-3}$ is a numerical factor and n is the number of solute molecules per unit volume.[1,20]

The measured concentrations are not high enough to obtain the high-concentration behavior of the formula above predicted by Doi and Edwards:[21]

$$D_R/D_{R0} = b(nL^3)^{-2}$$

Above a concentration of $8c^*$ the values for D_R lie significantly above the values predicted by Teraoka and Hayakawa and the Monte Carlo simulation. Corresponding deviations were also observed for other systems.[22] Our measured D_R values are in agreement with those obtained by Fujime,[23] Maguire,[24] and Nakamura,[25]

The dependence of D_R on the fd concentration at minimal ionic strength is completely different (Figure 3, curve b). In the dilute concentration range, the particles are not interacting and we find the same value for D_R as in the former case. In spite of the very increased Debye screening length the steric values (length, diameter) of the particles seem to be the same as for very small Debye screening length. Therefore the effect of the different ionic strength cannot be seen in the behavior of one single particle. It is observed in the interaction between the particles which is very well demonstrated in the intermediate and semidilute concentration range. Here, caused by the electrostatic interaction at very low ionic strength, the D_R values are decreased with regard to the values measured at high ionic strength. The combined effect of steric and electrostatic interaction hinders the rotational diffusion in a drastic manner. At concentrations above $3c^*$ the D_R values measured in the presence of electrostatic interaction are over 1 order of magnitude smaller than those measured with only steric interaction. The constant values above $3c^*$ might be seen in the light of the near phase transition at about $10c^*$.

Figure 4 is another representation of the data. It shows the dependence of D_R on the ionic strength of the solutions. Each curve belongs to a certain fd concentration. The respective point on the left side is identical with the data in Figure 3 at the smallest ionic strength, the respective point on the right side corresponds to the experimental results in Figure 3 at high ionic strength. At the smallest measured concentration, $c = 0.44c^*$, no particle–particle interaction seems to be present and therefore D_R is constant. At higher particle concentrations first electrostatic interaction sets in at low ionic strength, and then, above $1.2c^*$, also steric interaction, as stated before.

Anomalous birefringence is correlated with a decrease of the rotational diffusion constant D_R. One might argue that these two effects are caused by planar aggregates. But this is very unlikely. From SLS experiments[10] at low ionic strength we know that there exist no aggregates without electric field. The here described anomalous birefringence is a low electric field effect and disappears at higher fields. It seems very improbable that field-induced planar aggregates exist only at low electric fields and disappear at higher field strengths.

Despite the various efforts in exploring the electric birefringence behavior of rodlike particles in aqueous solutions, no convincing model is available to explain the anomalous birefringence behavior. Very likely an induced dipole moment arises perpendicular to the rod axis, which tries to orient the rod axis perpendicular to the external electric field. Hoffmann et al.[3] have developed a picture to show how such a dipole could be formed. They also try to explain that induced dipoles parallel to the external field will not occur. This is an effect of the overlap of the ion clouds above c^*. We observe the anomaly already at $c = 0.5c^*$. However, the ionic strength is 2–3 orders of magnitude lower. In this case the ionic clouds are much more extended so that the explanation still might hold.

It should be mentioned that anomalous magnetic birefringence was not observed even at high fd virus concentrations.[16] This is obvious, if we consider it solely as a collective effect of electric dipole moments induced by the external electric field.

Additionally, we would like to draw attention to the following fact: One difference between normal and anomalous behavior that occurs between an orientation of the rods parallel or perpendicular to the electric field is that in the second case the particles are still able to orient in a plane. In this case no parallel orientation between the particles is necessary. Anomalous behavior is correlated to particle–particle interaction. Therefore it might be possible that the particle–particle interaction tends to hinder a parallel orientation between the rods, as it is obvious for two repulsive interacting rods, and therefore anomalous behavior is preferred.

Conclusions

The decay of the electric birefringence of fd particles in aqueous solution has been measured at small electric fields ($\leq 2 \times 10^4$ V/m) over a wide range of concentrations. The ionic strength varied from 10^{-6} to 10^{-2} M. It was demonstrated that normal birefringence occurs only if interaction between the macromolecules is negligible, that is at sufficient small particle concentration. Above about $1.2c^*$ steric and electrostatic interactions lead always to an anomalous effect. In between, the sign of the birefringence depends on the strength of the electrostatic interaction. We conclude that anomalous behavior of electric birefringence is characteristic to aqueous solutions of interacting rodlike particles.

The decay curves are single exponential at the used field strengths. A complete set of rotational diffusion coefficients D_R was derived therefrom, which can now be used as input for a better description of the available dynamic light-scattering data.

The decrease of D_R at high ionic strength with increasing steric interaction measured by Fujime,[23] Maguire,[24] and Nakamura[25] could be confirmed. A drastic reduction of more than 1 order of magnitude is found with increasing electrostatic interaction.

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