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Electric birefringence of polytetrafluoroethylene particles in agarose gels

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Abstract

Electric birefringence studies of strongly elongated, rod-like particles of polytetrafluoroethylene (PTFE) in agarose gels show that the negative effect observed by semi-diluted aqueous suspensions at low frequencies and at low electric field strengths (the so called 'anomaly') disappears. The absolute value of the low frequency effect increases 3–4 times and the amplitude of modulation decreases faster compared to that of the suspensions. This together with decreased decay relaxation times in gels make the possibility that the PTFE particles orientation in gels is not due to dipolar but to electrophoretic orientation mechanism quite probable. Similar change in the orientation mechanism could be expected also for suspensions of higher concentrations. The further elucidation of the orientation mechanism using fractions with lower polydispersity, broader ranges of experimental conditions (particle concentration, ionic strength and composition, electric field strengths, frequencies, etc.) could be of interest for several fields: colloid electro-optics and especially that of concentrated colloids, pulsed field gel electrophoresis of DNA (and especially its sinusoidal biased field variant) and of nucleoprotein complexes and for the gel research.

Keywords: Electric birefringence; Fibers; Agarose gel; Electrophoretic orientation; Dipolar orientation; Electro-optic anomaly

1. Introduction

The conventional electro-optical properties of suspended charged colloidal particles in d.c. and a.c. electric fields are related to their optical anisotropy and ionic polarizability anisotropy dispersion, arising from the response of their clouds of counterions with the frequency and from their rotational diffusion constants. The general theory of the electro-optic behaviour of colloid particles can be extended to polyelectrolyte molecules such as short rigid or long flexible double-stranded DNA fragments or Polystyrene sulphonate molecules of narrow distribution of length [1]. High electric fields are than requested. It has, however, been shown that the long flexible polyelectrolyte molecules can be oriented in gels with mesh sizes much smaller than their contour length at very low electric fields. The orientation plays a major role in the electrophoretic separation of large DNA fragments. It has been explained by the bias reptation mechanism [2,3] and electrophoretic orientation model [4]. It seems conversely

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that the study of the electro-optics of rigid colloidal particles in gel with a mesh sizes in the order of the particle length would be of interest.

A suspension of polytetrafluoroethylene (PTFE) particles in an agarose gel could be regarded as an appropriate model. Depending on their polymerization conditions the PTFE particles can appear in the form of needle-like crystalline fibres. Their electric properties arise from the adsorption of fluorinated charged surfactants at the interface. Their optical properties are related to their nearly perfect crystallinity due to polymer chains aligned along the long axis of the particles.

There have been several important studies on the dielectric, electro-optic and scattering properties of PTFE particles which have been carried out mainly by the groups of Foster [5,6], Hoffmann [7–9] and Degiorgio [10,11], respectively. PTFE aqueous solutions appear very similar to the inorganic colloidal particles such as clays, oxides, etc. In particular they show at rather low concentration a reversal of the sign of their electric birefringence. From positive at

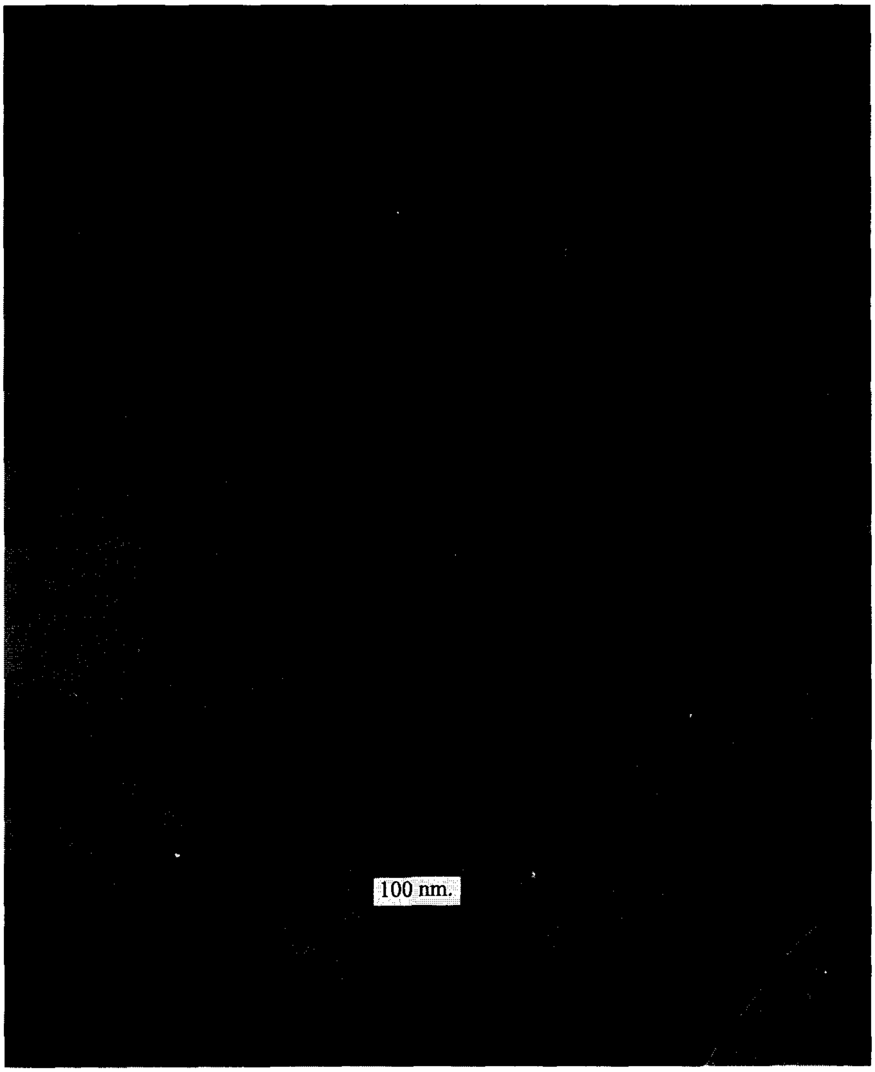

Fig. 1. Electron microscope photograph of a PTFE suspension. The final magnification is 90000x. Scale bar: 100 nm.

very low concentrations to negative at much higher concentrations. This is interpreted as resulting from an orientation of large aggregates in direction perpendicular to the electric field applied. One recovers, however, the positive birefringence when high frequency a.c. fields are used. The aggregation process and the orientation of the aggregates are not yet fully understood.

Due to unavoidable turbidity of the agarose gels the study at very low concentrations of isolated particles cannot be carried out. It appears, however, worthwhile to compare the electro-optics at higher concentrations of PTFE particles in the absence or presence of agarose gels of different concentrations (mesh size) with the double preoccupation to follow the influence of the gel on the state of aggregation and on the mechanism of orientation.

2. Experimental

2.1. Method

The basic part of the measurements of the electric birefringence Δn was made with a classical type electric birefringence apparatus [12], including as a light source a 1 mW He–Ne laser. A 1-cm quartz spectrophotometric cell with two Pt electrodes separated at 6 mm was used.

A $\lambda/4$ plate was placed between the cell and the analyser with its slow axis oriented at $3\pi/4$ with respect to the electric field direction, which gave the possibility to follow the sign of the birefringence and to increase the sensitivity of the measurements. The recorded signal was further used to calculate the electric birefringence, following the procedure described in Refs. [12] and [13].

The magnitude of the variation of the steady-state component and of the amplitude of the oscillatory component of the electric birefringence Δn at any frequency v is [6]:

$$\Delta n^{t} = \Delta n^{st,n} + \Delta n^{\text{osc}}. \qquad (1)$$

Rise and decay times τ_r and τ_d were approximately estimated by taking the times for which the electric birefringence has reached $(1-1/2.72)$ part of the stationary value or has fallen 2.72 times from the stationary value respectively.

2.2. Materials

The polytetrafluoroethylene fibres were received as a gift from Prof. H. Hoffmann, University of Bayreuth. The fibres were prepared by emulsion polymerization and stabilized from flocculation by hydrophobic surfactants (perfluoroalkanoic acids), which are supposed to be nearly quantitatively (95–99%) adsorbed on the polymer surface [14]. The fibres were quite polydispersed with a maximum number of particles of length between 100 and 1200 nm (number average length 210, weight averaged length 540 nm [14]). The number of particles is strongly decreasing with increasing length. Only single particles of dimensions about 2000 nm were found. The thickness of the particles is 30–35 nm and could go up to 100 nm. Some of the particles of greater thickness seemed to be side by side aggregates. So the shorter thicker particles could be better described as a 'contamination' with a considerable number of small spherical particles with diameters mainly between 50 and 150 nm (see the electromicroscope photograph on Fig. 1). The degree of crystallinity has been determined [14] to be 95% which is a remarkably high value for a polymer latex particle of a density of 2.31 g cm^{-1}. This might be interpreted as a indication that the polymer chains are fully oriented along the particle axis.

The overlap threshold concentrations C^* and C^{**} in number of particles per unit volume are defined as:

$$C^* = 1/L^3$$

$$C^{**} = 1/L^2 d,$$

where L is the longest particle dimension and d is the shorter dimension of the rod-like particle. The calculated weight averaged values for C^* and C^{**} were 0.52% and 8.9%, respectively. The experimental concentrations of the PTFE particles in the solution and in the agarose gels were 6%, 1% and 0.5%. These concentrations are above and/or near to the estimated overlap threshold concentration C^* (0.52%).

The conductivities (k) of the samples of 6%, 1% and 0.5% PTFE solutions and in gels were considerably scattered about 1.3, 0.6 and 0.5 mS cm^{-1} respectively.

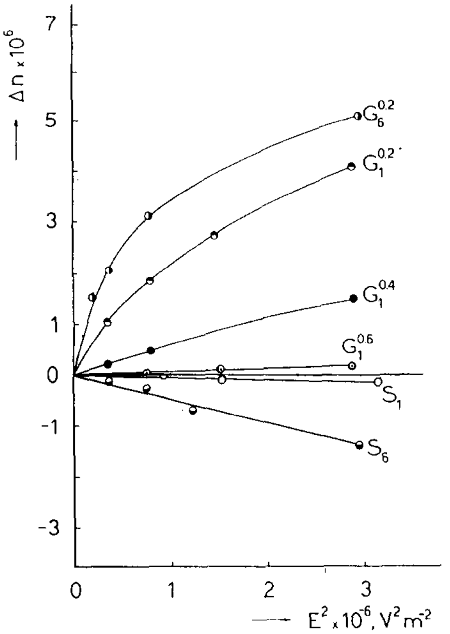

Fig. 2. Dependence of Δn on the square of the d.c. electric field strength (E) for PTFE-solutions and PTFE–agarose gels. Curves G_x^y are for PTFE–agarose gels of $x = 1\%$ and 6% PTFE and $y = 0.2\%$, 0.4% and 0.6% agarose. Curves S are for PTFE-solutions of 1% and 6% PTFE, respectively; k ca.1 mS/cm.

The agarose was purchased 'electrophoresis grade' from Bethesda Research Laboratories. For diluted agarose gels (in general less than 1% agarose) the effective pore size depends only on the gel concentration [15]. In the present study the empirical formulas introduced by Serwer [16] and Stellwagen [17,18,20] were used:

$$r = 118/A^{0.74}\,(\text{Serwer}), \tag{2}$$

$$r = 25 + 70/A\,(\text{Stellwagen}), \tag{3}$$

$$r = 137/A^{0.5}\,(\text{Stellwagen}), \tag{4}$$

where r is the average radius in nm of the gel's pore size and A is the percentage of the agarose in the gel. As size standards were used solid spheres, random DNA coils and for DNA restriction fragments for (2), (3) and (4), respectively. The experimental concentrations of the agarose in the agarose gels studied were 0.2%, 0.4% and 0.6%. The calculated from the formulas (2), (3) and (4) r were in the range 388–306 nm, 232–217 nm and 172–177 nm, respectively. The behaviour of the agarose gel matrix under the action of pulsed electric field depends on the field strength and pulse duration [18]. Thus at short pulses (10–1000 ms) and high electric field

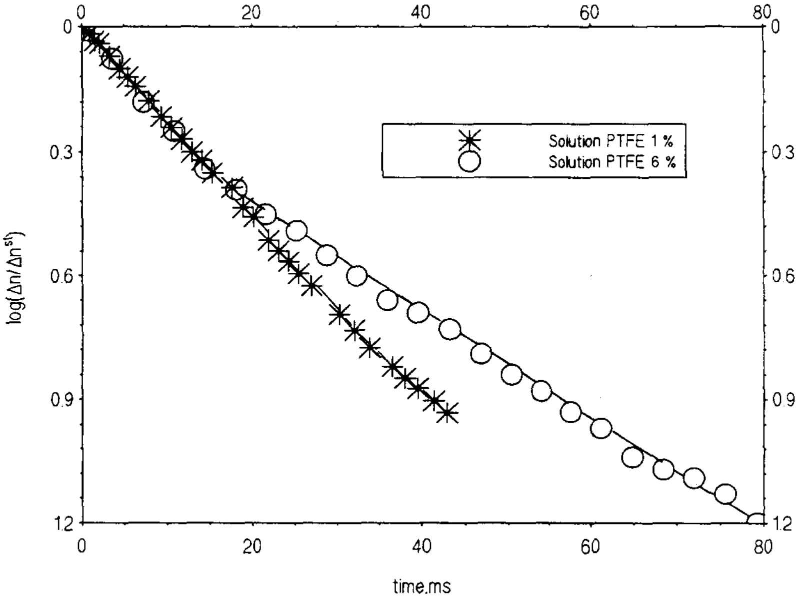

Fig. 3. Dependence of $(\Delta' n/\Delta^{s\prime} n)$ on time (t) for PTFE solutions. Curves S_1 and S_6 are for 1% and 6% PTFE, respectively; d.c. electric field $E = 17$ V/cm, k ca. 1 mS/cm.

strength (1–10 kV/cm) on the one side and long pulses (0.5–2 s) and low electric field strength (10–100 V/cm) on the other gel shows an electric birefringence effect. In this study, the rectangular electric pulses of the order of 20 V/cm and 10 ms in duration were applied. There were no reproducible electric birefringence signals from the gels alone (without PTFE) detected.

2.3. Sample preparation

The PTFE–aqueous solutions were prepared from a stock 10% w/v PTFE aqueous solution. The PTFE–agarose gels were obtained by gelation of agarose solution in the presence of PTFE particles. For each essay 1% agarose solution was prepared from a single stock 1% agarose gel. The 1% (w/v) agarose stock gel was prepared by dissolution of agarose in distilled water at a 100°C oil bath for 30 min. Then the agarose solution was transferred to a water bath at 50°C for 30 min for thermal equilibration. After compensation for the evaporated water, the gelation of the agarose solution was carried out at 37°C in a thermostat for 30 min. The agarose stock gel was kept under 4°C. The gel contained 0.02% sodium azide added just before gelation. For each essay the appropriate quantity was taken from the agarose stock gel and melted at 70°C in water bath. The obtained agarose solution was equilibrated for 30 min at 50°C. For varying the agarose and PTFE concentrations, each sample was prepared by mixing calculated volumes from the melted agarose stock gel (i.e. from 1% agarose solution), from the PTFE-stock solution and from distilled water. The final volume of each sample was 2 ml. After mixing, the sample was allowed to stand at 50°C for 30 min and the mixture was poured into the birefringence cell. The electrode assembly was inserted into the cell and the cell was thermostated at 37°C for 30 min. The gel was cooled to room temperature for another 30 min and the birefringence measurements were carried out.

3. Results

3.1. PTFE particles in aqueous solutions

The d.c. electric field strength dependence of Δn for the 1% and 6% solutions displayed in Fig. 2 follows the Kerr law up to E about 17 V cm^{-1}. The negative value indicate that the mean orientation of the particles is perpendicular to the field as already reported in this range of concentration. Δn is not proportional to the concentration as it is expected for a colloid system. The field free decay reported in Fig. 3 is nearly monoexponential with a rotational time increasing with the concentration (Table 1) indicating an increasing interaction. The rise and decay times are similar for the 1% solution. The frequency dependence of the steady state value of the electric birefringence at the 13 V cm^{-1} is displayed on Fig. 4. It turns from a large negative effect at low frequency to a smaller positive effect at

Table 1

Decay relaxation times (τ_d) and ratio rise–decay relaxation times (τ_r/τ_d) for PTFE-solutions and PTFE–agarose gels

PTFE, %	τ_d, ms	τ_r/τ_d
PTFE-solutions		
1	17 ± 3	1 ± 0.2
6	33 ± 3	0.15 ± 0.07
PTFE – agarose gels		
1	5 ± 2	9 ± 6
6	36 ± 24 [*]	0.7 ± 0.5 [*]

[*] Bigger variations are partly due to different undershoots of different pulses and to the different gel concentrations.

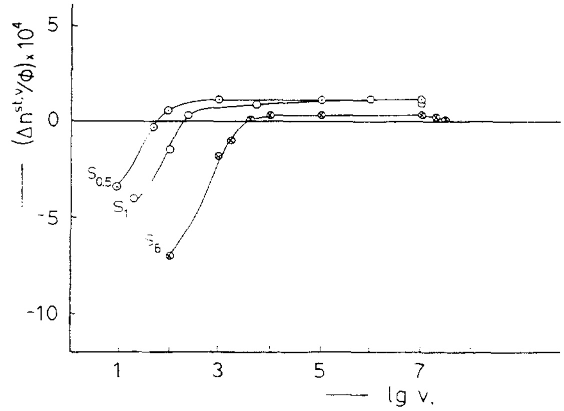

Fig. 4. Dependence of the reduced to unity volume fraction Δn ($\Delta n/\phi$) for PTFE-solutions on log ν. Δn^{st} is the steady-state value of Δn for a.c. electric field (E) of frequency ν; ϕ is the volume fraction, equal to $2\cdot10^{-4}$, $4\cdot10^{-4}$ and $2.4\cdot10^{-3}$ for 0.5%, 1% and 6% PTFE, respectively. Curves S are for PTFE-solutions of 0.5%, 1% and 6% PTFE, respectively. $E = 13$ V/cm, k ca. 1 mS cm^{-1}.

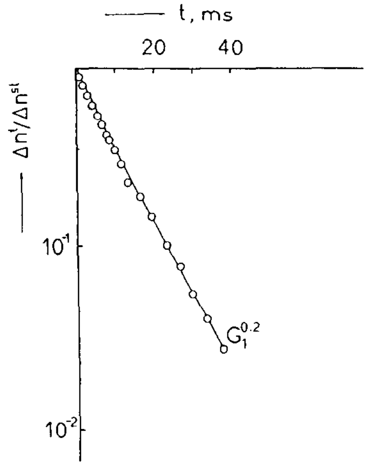

Fig. 5. Dependence of $(\Delta n'/\Delta n^{st})$ on time (t) for PTFE–agarose gel of 1% PTFE and 0.2% agarose; d.c. electric field $E = 17$ V cm^{-1}, k ca. 1 mS cm^{-1}.

higher frequency (up to 30 MHz). This is in agreement with the results of Kramer an Hoffman [7] and Angel [14]. The frequency at which Δn changes sign increases with increasing concentration

3.2. PTFE particles in agarose gel

The d.c. electric birefringence of 1% PTFE 0.2%, 0.4%, 0.6% agarose gels as well as 6% PTFE and 0.2% agarose gel are displayed on Fig. 2. The electric birefringence is large and positive but does not follow the Kerr law. Δn is not proportional to the concentration at a given agarose gel concentration (see $G_1^{0.2}$ and $G_6^{0.2}$ in Fig. 2) and it seems that for a given PTFE concentration it decreases with the concentration of agarose gel. The field free decay of the 1% PTFE–0.2% agarose gel is displayed on Fig. 5. It is nearly monoexponential with smaller τ_d as for the corresponding agarose free solution suggesting that, in agreement with the positive sign of the birefringence, Δn arises from individual particles or from aggregates with the particle axes oriented along the field. The decay time is not significantly dependent upon the agarose concentration and the rise time is much longer than the decay time (Table 1). The situation seems more complicated for the 6% PTFE–0.2% agarose gel where the decay time is very highly non exponential and much longer than for the

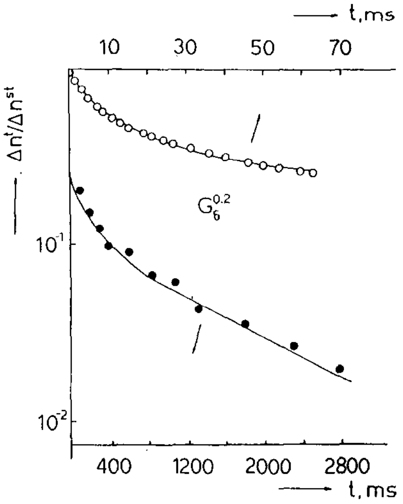

Fig. 6. Dependence of $(\Delta n'/\Delta n^{st})$ on time (t) for PTFE–agarose gel of 6% PTFE and 0.2% agarose; d.c. electric field $E = 17$ V cm^{-1}, k ca. 1 mS cm^{-1}. The filled points are related to the lower scale.

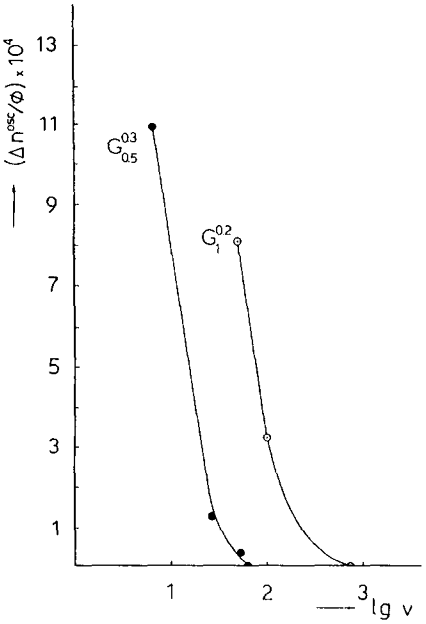

Fig. 7. Dependence of the reduced to unity volume fraction Δn $(\Delta n/\phi)$ for PTFE–agarose gels on log ν. Δn^{osc} is the amplitude of the oscillating component of Δn for a.c. electric field E of frequency ν; ϕ is the volume fraction equal to $2\cdot 10^{-4}$, $4\cdot 10^{-4}$ and $2.4\cdot 10^{-3}$ for 0.5, 1 and 6% PTFE, respectively. Curves G_x^y are for PTFE–agarose gels of $x = 0.5$, 1 and 6% PTFE and $y = 0.2$ and 0.3% agarose. $E = 13$ V cm^{-1}, k ca. 1 mS cm^{-1}.

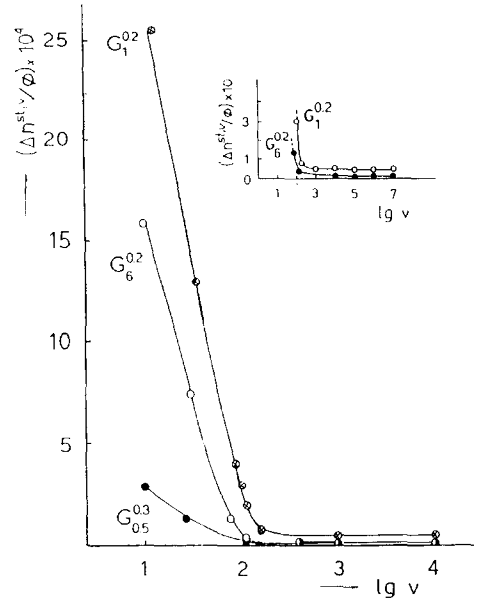

Fig. 8. Dependence of the reduced to unity volume fraction Δn ($\Delta n/\phi$) for PTFE–agarose gels on log ν. Δn^{st} is the steady-state value of Δn for a.c. electric field E of frequency ν; ϕ is the volume fraction, equal to $2\cdot 10^{-4}$, $4\cdot 10^{-4}$ and $2.4\cdot 10^{-3}$ for 0.5, 1 and 6% PTFE, respectively. Curves G_x^y are for PTFE–agarose gels of $x = 0.5$, 1 and 6% PTFE and $y = 0.2$, 0.3 and 0.4% agarose. $E = 13$ V cm^{-1}, k ca. 1 mS cm^{-1}. On the insert, the frequency dependence up to 10 MHz are given.

1% solution (Fig. 6). The frequency dependence of the amplitude of the electric birefringence is displayed on Fig. 7. The fast decay in the 10^1 – 10^3 Hz range depending on the samples fit with the inverse of the rotational decay time. The frequency dependence of the amplitude of the steady state component of the birefringence is displayed on Fig. 8. It shows a very fast decay in the same range as Δn, a puzzling result, since Δn is expected to remain high in the range of frequency between the inverse of the rotational relaxation time of the particles and of the inverse of the characteristic decay time of the large scale fluctuations of the counterions in the particle double layers. Measurements of the rise and decay time using a.c. pulses is made difficult by the complicated pattern of the rise and decay showing undershoots and overshoots as shown on Fig. 9. Generally there is a fast build up and slower decay of a smaller negative effect and a slow build up and a faster decay of a bigger positive effect.

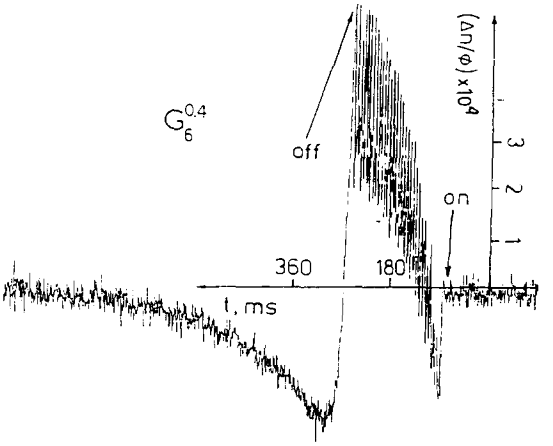

Fig. 9. Dependence of Δn on the time (t) for PTFE–agarose gel of 6% PTFE and 0.4% agarose; $\nu = 87$ Hz, $E = 13$ V cm^{-1}, k ca. 1 mS cm^{-1}.

4. Discussion

Two main facts emerge from the data presented above. First, in the presence of agarose gel positive electric birefringence is observed at all frequencies. This could be attributed to the displacement of the equilibrium between single particles (or small aggregates) with positive birefringence at low frequency and large aggregates with negative birefringence at low frequency in favour of the former. It is, however, puzzling that 0.2% agarose seems more efficient than higher concentrations. This may, however, reflect a complex process involving the thermodynamic interactions but also the topological constraints imposed by the agarose mesh size. The second fact is that if one accepts that the positive birefringence of the PTFE in agarose gel is essentially due to single particles or small aggregates, its electric field and frequency dependence should match those found from electro-optical studies of very low concentration solution [19]. This has been shown, in particular by electric light scattering [19] to obey Kerr law up to 300 V cm^{-1} and to present a slow decrease of the static part of the electro-optic effect up to 10^4–10^5 Hz which contrasts our finding that the n becomes negligibly small at frequency above 10^3 Hz.

One possible explanation could be that the orientation of the particles is not simply the result of the torque acting on the particle but of a coupling of the orientation and translation in the array of obstacles represented by the agarose gel fibres. Electrophoretic mobility measurements have been carried out on the PTFE particles leading to a value varying from 4×10^{-4} cm^2 V^{-1} s^{-1} in pure water to 3×10^{-4} cm^2 V^{-1} s^{-1} in 10 M NaCl. This implies for fields of about 10 V cm^{-1} at a frequency of 100 Hz a maximum displacement of their centres of mass of the order of few 100 nm, corresponding approximately to the mesh size of the gel. The low frequency dispersion of Δn could than be interpreted as resulting from 'collision' between the PTFE particles and the agarose gel fibres. In d.c. fields, the same argument could be used to explain the departure from the Kerr law.

The study of the orientation of charged colloidal particles in gel will always be faced with the double problem of the equilibrium between single particles aligning in the field and aggregates aligning perpendicular to the field, a phenomenon which has been shown to be very general [6,7]. But one can expect that using simpler colloids whose charge is not dependent from adsorbed surfactant as in PTFE (which may in the gel also re-equilibrate) and a more controlled ratio of length to mesh size can help to check the above speculations. Conversely, gel electrophoresis of large rigid particles may than be helpful in the separation of particles of more homogeneous length.

Acknowledgements

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