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Polymer 41 (2000) 559–564

polymer

Rotational dynamics of agarose aggregates in water/DMSO mixtures

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Received 25 November 1998; received in revised form 1 February 1999; accepted 10 February 1999

Abstract

The dynamic behaviour of agarose aggregates grown in binary mixtures of water and dimethyl sulphoxide (DMSO) has been investigated by Transient Electric Birefringence (TEB). Different agarose concentrations in various water/DMSO compositions have been studied. The birefringence relaxation has been fitted by means of a stretched exponential with an exponent of 0.5 which suggests highly size-polydispersed aggregates. The rotational diffusion coefficient, $\langle D_{\mathrm{rot}} \rangle$, has been seen to depend strongly upon the composition of the binary solvent and to increase markedly with increasing electric field. The gradient $\mathrm{d}\langle D_{\mathrm{rot}} \rangle/\mathrm{d}E$ has been thus considered a relevant parameter. For low agarose concentrations $\mathrm{d}\langle D_{\mathrm{rot}} \rangle/\mathrm{d}E$ displays a maximum for a solvent composition in the vicinity of $f_{\mathrm{DMSO}} = 0.17$ (mole fraction). Also, $\mathrm{d}\langle D_{\mathrm{rot}} \rangle/\mathrm{d}E$ varies as a power of concentration whose exponent first increases with increasing DMSO proportion and then levels off at nearly the same mole fraction (i.e. $f_{\mathrm{DMSO}} = 0.17$). The results are discussed in the light of the occurrence of a ternary complex (agarose/water/DMSO), a model of organization within the agarose fibres recently proposed from thermodynamics investigations.

Keywords: Agarose; Binary mixtures of water and dimethyl sulphoxide (DMSO); Transient Electric Birefringence (TEB)

1. Introduction

The thermoreversible gelation of agarose still remains a topical subject in spite of the numerous investigations carried out these past thirty years see for instance Ref. [1]. Former conclusions on the molecular structure (i.e. the double helix) and on the gelation mechanism, that were drawn from diffraction experiments combined with energy calculations [2,3], are now being seriously questioned. Foord and Atkins [4], and later Guenet et al. [5] have produced arguments against the double helix. Similarly, gelation is now believed to take place through the alignment of chains as the persistence length of the latter in the sol state is incompatible with the intertwinning process considered so far [5] (i.e. double helix formation). Also, recent thermodynamic investigations by differential scanning calorimetry have suggested the existence of ternary complexes in gels prepared from binary solvents such as water/dimethyl sulphoxide [6]. Clearly, the molecular structure of the chains and of the fibrils constituting the gels are still in need of further elucidation.

As has been shown for poly[vinyl chloride] (PVC) thermoreversible gels much can be gained from a study on the finite-size aggregates rather than on the infinite-size network [7–9]. Such a study can be undertaken by means of Transient Electric Birefringence [10] (TEB). This experimental technique is mostly used to probe rotational dynamics of different kinds of assemblies in solution see for instance Ref. [11]. During the past decade, TEB has been used to study a large variety of systems such as biomolecules, microemulsions, surfactant micelles, viruses, polyelectrolytes, liquid crystals and aggregates of different sorts. In the present article, we report on a study of the rotational dynamics of agarose aggregates in a binary solvent: water/dimethyl sulphoxide (water/DMSO). In water strong aggregation occurs unlike what is observed in DMSO. In the binary mixture aggregation and gelation usually are seen to take place at room temperature as soon as the molar fraction of water becomes larger than $f_{\mathrm{H_2O}} \approx 0.5$ [5,12]. The present investigation was primarily intended for studying the variation of shape and size of the aggregates as a function of the binary solvent composition. Unexpected results have been found that may have a direct bearing on the existence of a ternary complex.

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PII: S0032-3861(99)00211-6

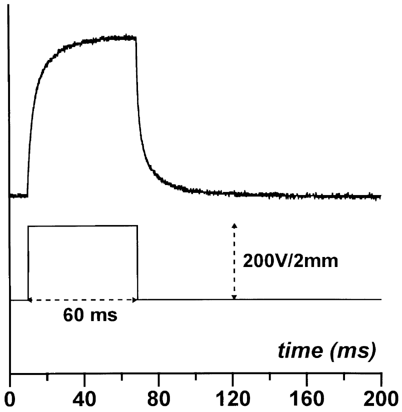

Fig. 1. Typical TEB signal from agarose solution (upper curve) together with the corresponding electric field pulse (lower curve).

2. Experimental

2.1. Set up

The TEB measurements were performed using a He–Ne laser operating at $\lambda = 632.8$ nm. The laser beam was polarized at 45° with respect to the horizontal optical bench prior to crossing a thermostated cell containing the solution. The electric field was delivered in the cell by two parallel, steel-made electrodes aligned vertically and spaced by a gap of 2 mm. After crossing the sample cell, the laser beam was directed through a $\lambda/4$ plate, and then through an analyzer before eventually reaching the detection photodiode. The analyzer was vertically oriented. The experiment consisted in submitting the sample to a square electric pulse and then in recording the intensity at the detection photodiode as a function of time. In order to improve the precision of the measurements, the laser beam was splitted just before the first polarizer and the intensity of the unaffected beam was subtracted after appropriate calibration from the signal measured in the detection photodiode. The overall time resolution of the set up was typically ≈ 1 μs. The samples were allowed to rest for about 10 min between two consecutive shots, and were systematically discarded after 5 shots had been performed. The effect of field strengh was also studied. Whenever this quantity was increased a new sample was used in order to get rid of memory effects.

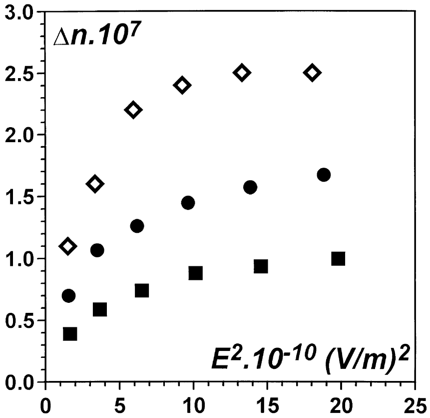

Fig. 2. Typical static birefringence for an agarose solution versus the square of the electric field for different mole fractions of DMSO, f_{DMSO}: ◇ pure water; ● = 0.1; ■ = 0.2; $C = 0.05$ g/l.

2.2. Sample preparation

The agarose sample was supplied by Hispanagar (Burgos, Spain). Its molecular weight was $M_v = 1.12 \times 10^5$ as determined by viscometry measurements (see Ref. [5]). The residual water content was found to be 12.7%. The sulfate content as given by the manufacturer was 0.1%. The methyl content as measured by proton NMR was lower than 0.7%. No L-galactose 6-sulfate was detected by ^{1}H NMR and ^{13}C NMR.

The dimethyl sulfoxide was purchased from Aldrich and was used without further purification. The binary solvent was prepared with double distilled water.

Solutions of the desired concentrations were prepared in 20 cc flasks by gently adding agarose powder to the binary solvent while stirring, and then by heating to the appropriate temperature. It usually took about 20–30 min to obtain homogeneous solutions. These solutions were quenched to room temperature and subsequently aged for a minimum of one day prior to investigation by TEB.

For the sake of comparison, PVC aggregates were prepared from diethyl oxalate (DEO) solutions. PVC was supplied by Rhovyl SA and had the following averaged molecular weights:

$M_w = 1.2 \times 10^5$ with $M_w/M_n \approx 2$.

DEO was purchased from Aldrich and was used without further purification. Aggregates were produced from homogeneous solutions obtained at 150°C, and then quenched to room temperature.

3. Results and discussion

The agarose concentrations used in this study always stood below the critical gelation concentration ($C_{gel} \approx 1$ g/l) [13], and were typically in the range 0.03 g/l $\leq C \leq$ 0.2 g/l. Under these conditions, only finite-sized aggregates are produced whose size is an increasing function of the agarose concentration and diverges to infinity at C_{gel}. This implies that interactions between aggregates grow on approaching C_{gel}. It is known from electron microscopy investigations that agarose aggregates are fibre-like structures (linear and branched) formed by parallel associations of agarose chains [14] (bundle-like association).

A typical dynamic birefringence signal obtained for a dilute aqueous solution containing agarose aggregates is plotted in Fig. 1. Aggregates align along the electric field while the electric pulse is applied which results in the solution becoming birefringent. The birefringence, $\Delta n(t)$,

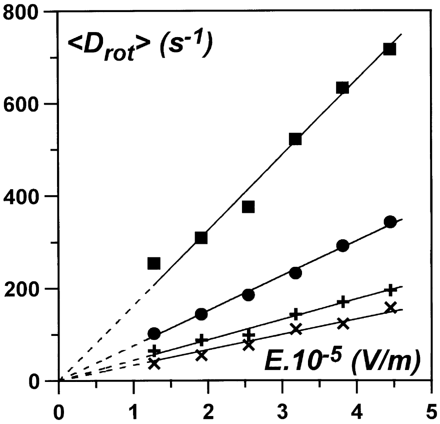

Fig. 3. Typical variation of D_{rot} as a function of the magnitude of the electric field. $C = 0.03$ g/l (■); $C = 0.05$ g/l (●); $C = 0.08$ g/l (+); $C = 0.1$ g/l (×) ($f_{DMSO} = 0.2$).

increases until a saturation value Δn_o is reached. Once the electric field is switched off, the birefringence signal decays as thermal motion disorientes again the aggregates. The dynamical relaxation of the birefringence curve is therefore related to the size and shape of the particles and the interactions between them, if any.

The variation of the static birefringence, Δn_o, as a function of the square of the applied electric field, E^2, is plotted in Fig. 2 for agarose aggregates in different water/DMSO mixtures. Δn_o is a function of the applied electric field which can be described by O'Konski's theory [15] for monodisperse, diluted rod-like particles. Δn_o is then proportional to $\Phi(\beta, \gamma)$, a function which depends upon the permanent and the induced dipoles of the particles, i.e. β and γ, respectively, as well as upon the electric field, E. In the limit of low fields

$$\Phi(\beta, \gamma) \propto E^2 \tag{1}$$

while for high fields $\Phi(\beta, \gamma)$ saturates. As can be seen in Fig. 2, the static birefringence reaches saturation for high values of the field, thus exhibiting the general behavior of the orientational function, $\Phi(\beta, \gamma)$. Fitting data of Fig. 2 with O'Konski's equation can provide information about the permanent and induced dipoles of the aggregates. Here, it cannot be proceeded this way, and the reason why will become apparent in what follows.

For dilute monodisperse particles, the relaxation of the birefringence is expressed through a simple exponential decay [16]. Here, we are dealing with fibre-like particles that are known to be polydisperse both in length and in cross-section. It has been shown that the TEB relaxation behavior can be described by means of a stretched exponential for dilute solutions consisting of highly polydisperse rigid rods [10]:

$$\Delta n(t) = \Delta n_o \exp\{-(t/\tau)^{\alpha}\} \tag{2}$$

where τ is the characteristic time which is related to the rotational diffusion coefficient, D_r through [10,17]:

$$\tau = \frac{1}{6D_r}. \tag{3}$$

The mean rotational diffusion coefficient $\langle D_{rot} \rangle$ is then written for polydispersed systems:

$$\langle D_{rot} \rangle = \frac{\alpha D_r}{\Gamma(1/\alpha)} \tag{4}$$

where Γ is the gamma function.

In the present case, the best fits have invariably yielded $\alpha = 0.5 \pm 0.02$, with conspicuous disagreement whenever significant departure from this value was tried. The value $\alpha = 0.5$ is consistent with highly polydisperse, cylinder-like aggregates. From Eq. (4), $\langle D_{rot} \rangle$ is therefore written:

$$< D_{rot} >= 0.5 D_r. \tag{5}$$

For solutions of highly polydisperse aggregates, one may wonder whether the characteristic time τ derived at low fields describes unambiguously the dynamics of the whole system (for instance only a fraction of the aggregates may undergo proper orientation). This led us to perform experiments in a large range of electric field values. As can be seen from the results plotted in Fig. 3 we have observed a conspicuous dependence of the rotational diffusion coefficient $\langle D_{rot} \rangle$ on the electric field for all the agarose solutions studied (α remained 0.5 ± 0.02 independent of the field strength). Qualitatively speaking, increasing the strength of the electric field entails a decrease of the characteristic time which corresponds to an accelerated dynamics. The dependence of $\langle D_{rot} \rangle$ on E in the investigated range of electric fields can be described, to a first approximation, with a straight line going through the origin. This does not obviously mean that at $E = 0$ gives $\langle D_{rot} \rangle = 0$, so that it is quite likely that at very weak fields some finite value should be attained. This suggests that there exists a critical field E_c below which $\langle D_{rot} \rangle$ becomes invariant or nearly invariant.

One could assume off-hand that this dependence of $\langle D_{rot} \rangle$ on E arises merely from a non-uniform orientation of the aggregates if, at low fields, only the larger aggregates are properly oriented while the smaller ones are not. Augmenting the field strength would then orient a growing fraction of smaller aggregates resulting in an increase of $\langle D_{rot} \rangle$. The exponent α of relation Eq. (2) should, however, gradually decrease from 1, value that corresponds to a narrow particle size distribution, down to 0.5, value that corresponds to a broad size distribution of the aggregates. Yet, as stated above, the exponent α found experimentally is strictly field-invariant and found to be 0.5.

In order to find out whether the effect is genuine and pertains to agarose solutions or arises from some unaccounted artifacts a parallel study has been carried out on morphologically similar aggregates. For such a study a good candidate turns out to be poly[vinyl chloride] (PVC) that also forms highly size-polydispersed fiber-like aggregates in organic solvents [7–9] (here diethyl oxalate, DEO,

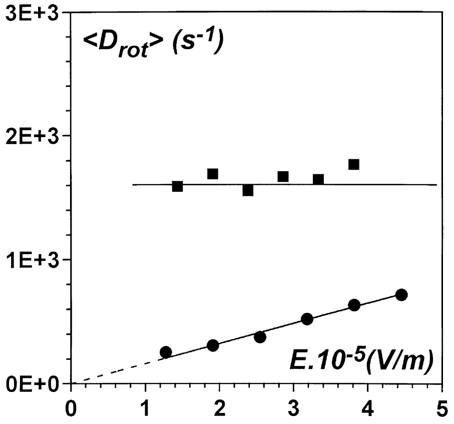

Fig. 4. Variation of $D_{\rm rot}$ as a function of the magnitude of the electric field for PVC aggregates produced from 5 g/l solutions (■). For the sake of comparison the result for agarose aggregates is also shown. $C = 0.03$ g/l, $f_{\rm DMSO} = 0.2$ (●).

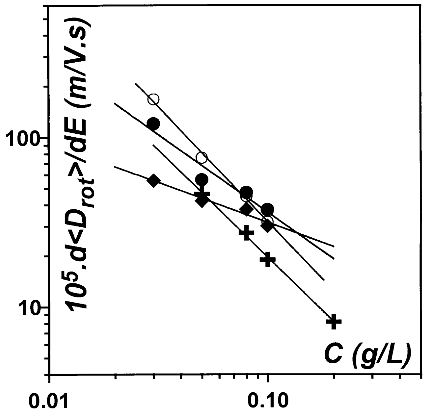

Fig. 6. Variation of $dD_{\rm rot}/dE$ as a function of agarose concentration for different molar fractions of DMSO, $f_{\rm DMSO}$: ◆ = pure water; ● = 0.1; ○ = 0.2; + = 0.37. Solid lines are best fits with power laws.

was chosen). Admittedly, PVC and agarose differ in nature. Here we just intend to test the assumption of non-uniform orientation for aggregates that microscopically present a very similar morphology. Although PVC concentrations are higher than those used for preparing agarose aggregates, the relevant parameter is $C/C_{\rm gel}$ which is approximately of the same order of magnitude in both systems (for PVC [7], $C_{\rm gel} \approx 0.025$ g/cm^3, so that $C/C_{\rm gel} \approx 0.2$). Otherwise, PVC is quite appropriate as the dipolar characteristics are very similar to those of agarose. Here again $\alpha = 0.5$ was obtained for PVC aggregates, and found to be non-variant with the field strength. Conversely, it was observed that $\langle D_{\rm rot}\rangle$ does not show any significant dependence on the electric field for this system (see Fig. 4). This outcome together with the invariance of α therefore suggest that the dependence of $\langle D_{\rm rot}\rangle$ on E is an intrinsic property of the agarose/water/DMSO systems.

Another origin for the field-dependence effect remains worth examining. So far we have considered the aggregates to be under strictly dilute solution conditions. This assumption might not be totally correct in the sense that intermolecular distances may be of the order of the aggregates size which would result in non-negligible interactions between neighboring aggregates with a possible effect on the dependence of $\langle D_{\rm rot}\rangle$ on E. We, however, note that the lower the preparation concentration, and correspondingly the weaker the intermolecular interactions, the stronger the effect. The effect of interactions can be further tested by studying solutions initially prepared at given concentration, and then subsequently diluted. An agarose solution prepared at 0.1 g/l has been diluted to 0.03 g/l while keeping the binary solvent composition constant (namely, a DMSO mole fraction $f_{\rm DMSO} = 0.2$). The variation of $\langle D_{\rm rot}\rangle$ as a function of E for this diluted solution is shown in Fig. 5. Data for the initial solution of 0.1 g/l together with those for a solution originally prepared at 0.03 g/l are also displayed. It can be seen that the slope of the dependence of $\langle D_{\rm rot}\rangle$ on E, we name this quantity $d\langle D_{\rm rot}\rangle/dE$, does not change significantly upon dilution. The diluted solution data are simply shifted upwards with respect to the original solution. The parameter $d\langle D_{\rm rot}\rangle/dE$ therefore characterizes a given concentration, and appears to be uncorrelated to the interactions between aggregates. Conversely, the intercept of $\langle D_{\rm rot}\rangle$ vs E depends markedly upon the dilution.

The parameter $d\langle D_{\rm rot}\rangle/dE$ depends upon the concentration of preparation of the aggregates as it differs whether the 0.03 g/l solution is obtained through dilution or not. This most certainly arises from the fact, already reported for other systems (such as PVC aggregates for instance) that the size and shape of the aggregates is determined by the preparation concentration [8].

Under usual conditions, i.e. when the characteristic relaxation time does not depend upon the applied electric field, $\langle D_{\rm rot}\rangle$ is plotted as a function of polymer concentration to find out whether there exists any power law variation whose exponent can be related to the aggregates structure [10]. Presently, this is not directly possible due to the strong

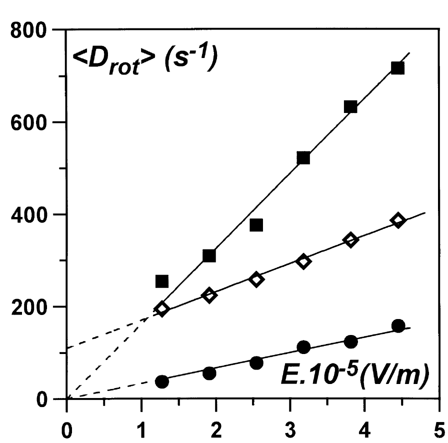

Fig. 5. Effect of dilution on the value of $D_{\rm rot}$. (●) = starting solution $C = 0.1$ g/l; (◇) the same system after dilution to 0.03 g/l; (■) = solution originally prepared at 0.03 g/l. Straight lines are best fits.

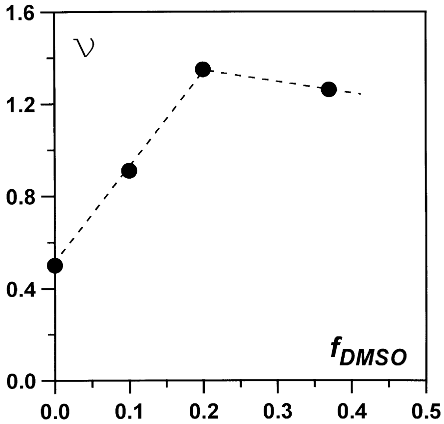

Fig. 7. Variation of ν (exponent of dD_{rot}/dE vs $C_{agarose}$) as a function of the DMSO molar fraction. Dotted line is a guide for the eye.

dependence of $\langle D_{rot}\rangle$ on E. There is no reason to take those values of $\langle D_{rot}\rangle$ at low fields that do not necessarily represent the equilibrium value characterizing the aggregates. Conversely, as discussed above, $d\langle D_{rot}\rangle/dE$ appears as a characteristic of a given ternary system agarose/water/DMSO. In Fig. 6 are plotted the variation of $d\langle D_{rot}\rangle/dE$ as a function of agarose concentration for different compositions of the binary solvent. The double logarithmic scale highlights power law variations ($d\langle D_{rot}\rangle/dE \sim C^{-\nu}$) whose exponents, ν, are dependent upon the binary solvent composition. The variation of ν as a function of the solvent composition (DMSO molar fraction) is shown in Fig. 7. As is apparent from this figure, ν first increases with increasing the molar fraction of DMSO, and then tends to level off above $f_{DMSO} \geq 0.2$. That ν differs from 0 implies that the higher the agarose concentration, the weaker the effect of the electric field magnitude on $\langle D_{rot}\rangle$. Similarly, as ν is markedly dependent upon solvent composition, aggregates prepared from DMSO-rich binary solvents are more affected by the electric field at low agarose concentrations than those prepared from water-rich mixtures while the situation differs at higher agarose concentrations. A plot of $d\langle D_{rot}\rangle/dE$ vs composition is shown in Fig. 8 for two agarose concentrations ($C = 0.05$ g/l and $C = 0.1$ g/l). Interestingly, this parameter goes through a maximum at a DMSO mole fraction of $f_{DMSO} = 0.2$ for $C = 0.05$ g/l. Note that the same effect is observed when considering those values of $\langle D_{rot}\rangle$ at low field.

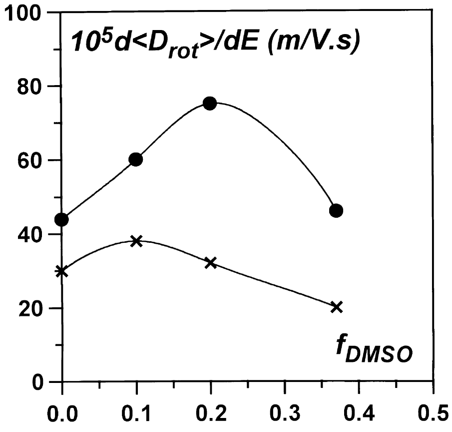

Fig. 8. Variation of dD_{rot}/dE as a function of the DMSO molar fraction for $C_{agarose} = 0.05$ g/l (●); $C = 0.1$ g/l (×). Solid line is a guide for the eye.

It is worth emphasizing that Dormoy and Candau [10] derived a power law ($\langle D_{rot}\rangle \sim C^{0.99}$) from values obtained at low electric fields for aggregates prepared from aqueous solutions heated at 130°C. In view of the electric field effect reported here, one may wonder whether the exponent 0.99 found by these authors convey any meaning as to the molecular structure.

Finally, it is worth discussing the electric field effect in the light of the thermodynamic properties, i.e. the melting behaviour of agarose gels in water/DMSO mixtures, as studied by Watase and Nishinari [12] and Ramzi et al. [6] by means of differential scanning calorimetry (DSC). The typical temperature–composition phase diagram together with the corresponding Tamman's diagram obtained by Ramzi et al. [6] are shown in Fig. 9. As can be seen, the gel formation temperatures (T_{gel}) and the gel melting temperatures ($T_{melting}$) go through a maximum at a DMSO molar fraction of about $f_{DMSO} = 0.17$. So do the corresponding gelation/melting enthalpies (Tamman's diagram). Note that the melting temperatures of agarose gels are virtually invariant in a broad range of agarose concentrations at fixed composition [6,18].

The temperature–composition phase diagram drawn in Fig. 9 has been interpreted by Ramzi et al. [6] in terms of a ternary complex, agarose/water/DMSO (as discussed by these authors, the phase diagram also implies the existence of a binary complex agarose/water). This means that, in the organized parts of the gel fibres, water and DMSO "cocrystallize" together with agarose so as to form a compound (in some instances the term "crystallosolvate" is also employed). Regrettably, the paucity of reflections on diffraction patterns obtained from agarose gels does not allow one to confirm or invalidate the existence of a ternary compound [4]. As a rule, in compound systems the highest melting temperature together with the maximum in gelation/melting enthalpies yields the stoichiometry of the compound. In the present case, since a ternary compound is involved, only the stoichiometry of the binary solvent within the compound can be determined, namely 5 H_2O/1 DMSO. Note that a complex differs from preferential adsorption which would not give the kind of diagram in Fig. 9 as discussed in Ref. [6].

The accelerated dynamics which occurs on increasing field strength eventually suggests gradual disruption of the aggregates unless some unexpected artifacts were missed. A suggestive correlation is found between thermodynamics and TEB experiments, particularly the maximum observed for $d\langle D_{rot}\rangle/dE$ as a function of solvent composition for a given agarose concentration. The variation of $d\langle D_{rot}\rangle/dE$ for low agarose concentrations may seem, however, in

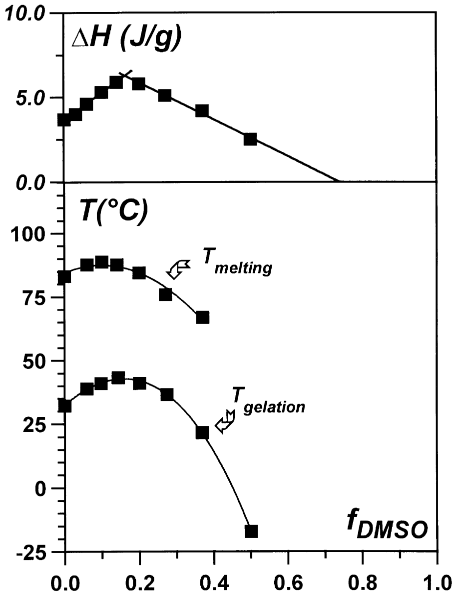

Fig. 9. Temperature–DMSO molar fraction phase diagram (below) and the corresponding Tamman's diagram (above).

apparent conflict with the thermodynamic data. The water/DMSO composition at which the agarose aggregates are the most fragile from the TEB viewpoint (maximum of Fig. 7) corresponds to the system with the highest melting point (maxima in Fig. 9), supposedly less fragile from the viewpoint of thermodynamics. Incidentally, this ascertains that the gradient d$\langle D_{\mathrm{rot}}\rangle$/d$E$ does not result from a partial melting of the aggregates caused by a burst of heat while switching on the electric field.

A possible origin of the electric field effect, which would remove the thermodynamics paradox and account for the discrepancy with PVC/DEO aggregates, may be found in the nature of the physical links. In PVC/DEO aggregates, microcrystallites devoid of solvent molecules are known to ensure the stability of the PVC fibrillar aggregates [19], whereas the stability for a complex, especially when hydrogen bondings are involved between agarose, water and DMSO, depends strongly upon the subtle organisation between the three components. It is a possibility to explore that the electric field perturbes this molecular organization between solvent and polymer within the physical junctions (by disorienting solvent molecules for instance), resulting in destabilizing and destroying those of lower stability.

4. Concluding remarks

Agarose aggregates formed below the critical gelation concentration in water/DMSO mixtures seem to undergo disruption when submitted to an electric field pulse as suggested by the strong, linear-like dependence of the rotational difffusion coefficient, $\langle D_{\mathrm{rot}}\rangle$, as a function of the field strength. The magnitude of the effect is seen to be dependent upon both the agarose concentration and the binary solvent composition. The quantity d$\langle D_{\mathrm{rot}}\rangle$/d$E$ appears to characterize unambiguously the aggregates and appears to be correlated with the occurrence of a ternary agarose/water/DMSO complex. The disruption of the aggregates is thought to arise from the disorganization of the complex structure. Further studies of the phenomenon reported here should combine techniques capable of studying the behavior of the different components of the complex so as to confirm or invalidate the notion of aggregate disruption under an electric field.

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