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Experimental Studies of the Rheological Behavior of a Demixing Biopolymeric Sol[1]

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Experimental data are presented concerning a large transient viscosity surge occurring in the course of spinodal demixing of agarose aqueous solutions. The study includes the effects of water perturbation by minor proportions of compatible cosolutes. Three observations are noteworthy. One concerns an upward or downward shift of the spinodal temperature, caused by cosolutes, which agrees with their expected modulation of solvent-induced forces. The second concerns the time of appearance of the viscosity surge. This is observed to follow a critical law, with an exponent independent of polymer concentration and solvent perturbation. The third concerns the inverse-power-law dependence of the viscosity peak value on shear. When the shear is scaled with an appropriate relaxation time, related to the overall interdomain structure generated by spinodal demixing, all data fall on a master curve, independent of polymer concentration, quenching depth, and solvent perturbation. This allows assigning the observed viscosity behavior to distortion and rupture of the overall interdomain structure generated by spinodal demixing.

KEY WORDS: scaling behavior; shear viscosity; solvent-induced forces; solvent perturbation; spinodal demixing; water structure.

1. INTRODUCTION

The rheological behavior of binary solutions during spinodal demixing, and conversely the effect of shear on demixing, have attracted considerable interest particularly with reference to scaling and to criticality and universality [1–9]. Here we present experimental studies of the rheology of

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agarose solutions in pure and in perturbed aqueous solvent in the course of spinodal demixing.

Agarose–water sols form thermoreversible biostructural gels upon cooling. Cross-linking implies a conformational (two coils) → (one double helix) change [10, 11]. As for many biopolymeric systems, the topological phase transition of gelation occurs even well below the concentration threshold for random cross-link percolation. Solute–solute correlations promoting nonrandom cross-linking are expected in this case as necessary initial symmetry breaking. In low-concentration agarose sols this step occurs at the mesoscopic level and corresponds to the thermodynamic phase transition of spinodal demixing. Much later in time, the topological (percolative) phase transition of gelation occurs through the canvas of polymer-rich regions resulting from demixing [12]. The two transitions are unmistakably separated in time and in no way simultaneous or even partially overlapping [12–18].

Studies at our laboratory [12, 17] have allowed determining the quantitative and complete phase diagram shown in Fig. 1, where it must be

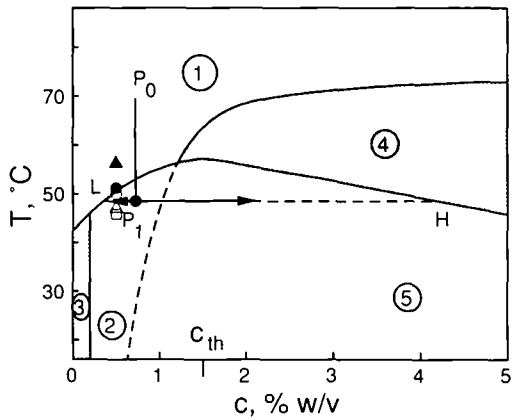

Fig. 1. Quantitative phase diagram of agarose–water systems. (1) Thermodynamically stable sol. (2) Quenching from P_0 to P_1 causes spinodal demixing followed by macroscopic gelation. The latter occurs in the percolating canvas of polymer-rich regions such as H. (3) As in region 2, but polymer-rich regions are disconnected. Mesoscopic, freely drifting, gelated regions are formed. (4) Direct gelation. (5) Demixing-mediated and direct gelation may compete kinetically, depending upon quenching. Points at $c = 0.5\%$ indicate the shifts caused by cosolutes (cosolute concentrations in molar fraction): □, 6.5% EtOH; △, 2% TBOH; ▽, 0.36% TBOH; ●, 0.36% TMAO; ▲, 2% TMAO.

understood that quenchings in region 2 cause spinodal demixing first, and (much later) gelation [11–18]. Here we study the effects on transport properties of agarose sols (with and without cosolutes) due to quenching in region 2 of Fig. 1. The observed complete separation in time of spinodal demixing and gelation and the considerably slow demixing kinetics have allowed the present studies. Specific questions addressed are the time-resolved behavior of shear viscosity during spinodal demixing and its dependence upon the rate of shear, quenching depth, and polymer concentration. Further, considering that the thermodynamic instability of the homogeneous solution is dictated by the balance of solute–solute and solute–solvent interactions [19, 20], interesting effects can be expected from solvent perturbations [21–24]. Compatible cosolutes are expected to alter solvent-induced solute–solute interactions [21–24] and to change the location and shape of the spinodal line. Also, the entire dynamical response of the systems can be affected, in ways which remain to be investigated. In view of this interest we report on the effects of small amounts of compatible cosolutes: ethanol (EtOH), *tert*-butanol (TBOH), and trimethyl amine *N*-oxide (TMAO). Alcohols and TMAO are known to alter in opposite ways solvent-induced interactions [21–23, 25] and to cause opposite shifts of the instability line of agarose–water systems [22]. This is of particular interest in comparing the TBOH and TMAO cases, since the structural difference between these two molecules is a mere substitution of a C–OH with an N–O group [22, 23].

2. EXPERIMENTS

Viscosity at a constant shear rate in the 1.6×10^{-2}–$1.2 \times 10^{2}\ s^{-1}$ range was measured using a computer-interfaced Couette-type Contraves Low-Shear 30 Viscometer. Light-scattering apparatus for ELS and DLS are described elsewhere [12, 18]. Agarose [Seakem HGT(P) from FMC Bioproducts] was dissolved at 100°C for 20 min, filtered through 0.22-μm filters, and directly transferred in prethermostatted cells. Measurements of viscosity and other quantities of interest started immediately after quenching and were performed as a function of time. Temperature control was within ± 0.2°C. Quenching depths (region 2 of Fig. 1) not exceeding 5.5°C allowed sufficiently slow kinetics for time-resolved measurements.

Viscosity and light-scattering experiments on different aliquots of the same solution quenched at the same temperature were performed in parallel experiments for various concentrations and quenching depths ε. The growth of scattered light intensity, recorded at different angles, showed unmistakable signatures of spinodal demixing [4, 12, 13, 17] with the formation of a pattern of polymer-rich and polymer-poor domains. The rate of growth

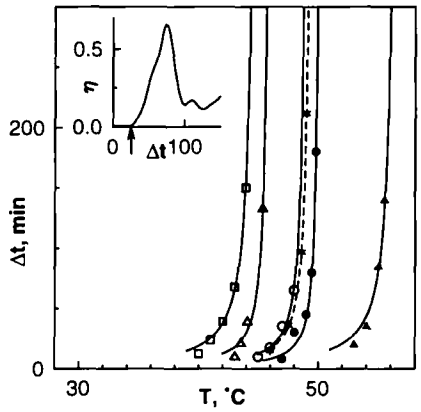

Fig. 2. Delay time of appearance of the viscosity peak, at different quenching depths, for the 0.5% w/v agarose concentration: *, pure H_2O. Other symbols as in Fig. 1. Inset: a typical example of the viscosity peak. $T = 41$°C, $c = 0.5\%$ w/v + 6.5% EtOH, η in Pa · s.

and the time-dependent structure function $S(q)$ exhibited the predicted [26], rather broad maximum, around a value q_M of the wave vector, determined by concentration and quenching depth. This allowed evaluating for each experimental condition a characteristic length $L_M = 2\pi/q_M$ corresponding to the maximum of the distribution of interdomain distances. In our conditions, L_M was of the order of a few microns. The mean size of the polymer-rich regions was shown to be about one order of magnitude smaller [4, 12, 16–18]. A large and well-reproducible surge of viscosity (two or three orders of magnitude) was observed after a delay time depending on concentration and quenching depth [4]. It appeared when the initial (linear) stage of demixing was completed and a stable low-angle light-scattering ring was observed. At decreasing quenching depth, the intensity of the surge decreased and its time of appearance increased. Effects of cosolutes are evident in Fig. 2. Fittings of the same data to a critical power law gave a common exponent $\gamma = -1.3$ and different spinodal temperatures, lower in the presence of EtOH or TBOH and higher in the presence of TMAO.

In experiments performed at different values of shear rate S, the viscosity peak showed an inverse power-law dependence upon S, with exponent $p = 1.86 \pm 0.06$, in the whole range $0.016 < S < 0.2\ s^{-1}$. This remarkable shear dependence suggested that at least one of the relaxation times of the system τ is such that $S\tau > 1$ in all our experimental conditions

[1, 2, 4]. This relaxation time should be associated with an appropriate scale length l by the relation $\tau = (k_B T)^{-1} \cdot 6\pi\eta l^3$ [27]. Among the relevant scale lengths, the molecular size (relative to either the coil or double helix conformation), the correlation length $\xi = \xi_0 \varepsilon^{-\nu}$, and the size of domains would all correspond to $S\tau \ll 1$. Therefore, the shear is expected to cause negligible deformation of polymer conformation, of thermal fluctuations, and of polymer-rich domains generated by spinodal demixing. The remaining relevant length is the mean interdomain distance L_M. The associated relaxation time $\tau_M = (k_B T)^{-1} \cdot 6\pi\eta L_M^3$ is of the order of 100 s and gives $S\tau_M > 1$ in all our experimental conditions. It is therefore of interest to plot all our $\Delta\eta/\eta$ data vs. $S\tau_M$, which is a measure of the deformation experienced by the whole distribution of domains throughout the sample. This plot is shown in log-log form in Fig. 3. For the pure water solvent, all data corresponding to different shear rates, polymer concentrations, and quenching depths fall on one straight master line (a in Fig. 3), corresponding to $\Delta\eta/\eta = (\text{const})\, S^{1.97}$. This is evidence that the observed viscosity surge is related to a shear deformation of the overall pattern of polymer-rich and polymer-poor domains generated by spinodal demixing on the interdomain scale length.

Similar experiments in perturbed solvent showed the same power-law dependence upon S, with the same exponent. When data are plotted

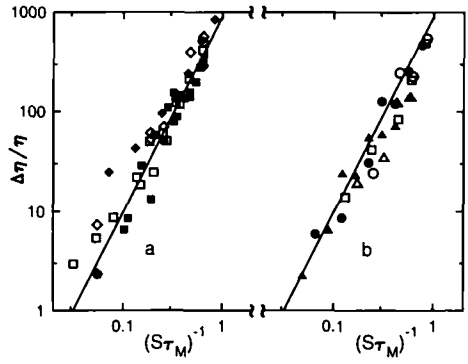

Fig. 3. Log-log plots of viscosity peak values $\Delta\eta/\eta$ vs. $(S\tau_M)^{-1}$. (a) Data points: different agarose concentrations (◇, $c = 0.05\%$; ■, $c = 0.25\%$; □, $c = 0.5\%$), and at least two different quenching depths at each concentration. Solvent is pure water. The straight line is the best fit of data points. (b) Perturbed solvent. Symbols as in Fig. 1. Agarose concentration $c = 0.5\%$ w/v. The straight line is the one in part a.

vs. $S\tau_M$ (b in Fig. 3), data points relative to all cases of solvent perturbation again lie on the same straight line as those obtained in pure water solvent.

3. CONCLUSIONS

We have presented the viscous behavior in the course of spinodal demixing of 0.5% w/v agarose pure aqueous and cosolute-perturbed sols. A surge and decay of viscosity are observed as soon as the pattern of polymer-rich and polymer-poor domains generated by demixing becomes stable. Compelling evidence shows that stabilization occurs long before detectability of the subsequent and known (two coils) → (one double helix) transition associated to cross-linking and gelation [12, 16, 17]. Points of interest of our results are the delay time and size of the η peak value (much larger than η_{sol} and yet much smaller than η_{gel}) and the scale invariance of the shear dependence. The appropriate scaling factor of the shear rate is the relaxation time τ_M characterizing the dynamic response of the overall interdomain structure generated by spinodal demixing. On these grounds, the large viscous dissipation peak is ascribed to deformation and rupture of sizable interdomain links responsible for the stability of the overall distribution of domains throughout the sample. These links cannot be associated to a very early stage of the gelation process, such as to keep below detectability the optical rotation signal monitoring the (two coils) → (one double helix) transition. Indeed, the existence of a small number of double helices and related cross-links across the polymer-poor regions would imply a much larger (and therefore detectable) number of them in polymer-rich regions, against strong existing evidence (17). It follows that, while the involvement of the interdomain structure is clearly evidenced by the scaling behavior of viscosity, the problem of interdomain forces remains open. A (small) number of polymer chains may be involved in them, through mechanisms probably different from those of gelation, and perhaps involving solvent-induced forces (21–24).

Results on perturbed-solvent solutions confirm this view. In agreement with previous data and predictions [21–23, 25], cosolutes cause upward or downward shifts of the spinodal line, corresponding to opposite modulations of solvent-induced forces becoming weaker in the presence of alcohols, stronger in the case of TMAO. In the cases of 6.5% ETOH and 2% TBOH or TMAO the observed shifts are quite relevant (-4.6, -3.5, and $+7.1$°C, respectively), $\Delta\eta/\eta$ changes, and L_M also changes. However, the scaling behaviors and in particular the scale invariance of the shear dependence remain valid even when the solvent is subject to rather severe perturbation. This further confirms that the dynamic response of the system

is characterized by the relaxation time τ_M associated with the overall interdomain structure generated by spinodal demixing. Further support to the present interpretation also comes from similar studies of the viscoelastic response during spinodal demixing [5, 28].

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