Mesoscopic Gels at Low Agarose Concentration: Perturbation Effects of Ethanol

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ABSTRACT Aqueous agarose solutions at low concentrations (0.5 g/liter) were temperature quenched below the spinodal line to form mutually disconnected mesoscopic gels. In the presence of 6% ethanol, these solutions, obtained by quenching at the same temperature depth as in pure water, appear much more fluid, as determined by probe diffusion experiments. We show by static and dynamic light scattering that this can be explained by the solvent-mediated effects of ethanol, leading to a globular shape of mesoscopic agarose gels, rather than to an extended rodlike structure observed in pure water. Our findings show the significant effects of solvent perturbations on particle condensation and, therefore, may be useful in understanding the role of the solvent in the folding of biomolecules.

INTRODUCTION

Biopolymeric gelation is a field of great theoretical, practical, and biological interest (Burchard and Ross-Murphy, 1988; Clark, 1996b; de Gennes, 1979; Kolb and Axelos, 1990; Martin and Adolf, 1991; Mitchell and Ledward, 1985; Stauffer and Aharony, 1991). In the percolative description, gelation corresponds to random cross-link percolation (Essam, 1980; Stauffer, 1979, 1981) that occurs when a threshold value of polymer concentration that depends on temperature and bond energy is reached. Spontaneous concentration fluctuations within, or close to, the instability region (which is the region encompassed by the spinodal line in the temperature (T)-concentration (c) plane) can play an important role in the kinetics of the ordering process (Nose, 1987; Hashimoto, 1988). Much theoretical work has been done on systems in which demixing and aggregation are competing processes (Coniglio et al., 1979; Tanaka, 1990; Tanaka and Stockmayer, 1994). Experiments and computer simulations have shown that the structural properties of gels, formed under conditions of competition between gelation and spinodal decomposition, are strongly determined by the relative time scales of the two processes (Bansil et al., 1992; Glotzer et al., 1994; Hayward et al., 1987; Liu and Pandey, 1996). Domains of high-concentration regions generated by spinodal demixing are seen to be connected in an extended structure superimposed on the more homogeneous one generated by gelation (Bansil et al., 1992; Clark, 1996b). The existence of such inhomogeneous microstructure provides both increased stability as well as flexibility.

Previous work in our laboratory concerned time-resolved studies of self-assembly in different biomolecular systems at concentrations well below the threshold for random cross-link percolation (Emanuele et al., 1991; San Biagio et al., 1986, 1991, 1992, 1996a,b; Sciortino et al., 1993). Under these conditions, solute-solute correlations are necessary to provide the channeling needed to form nonrandom cross-linking and correlated percolation that leads to network formation. It has indeed been shown that, at low to moderate concentrations, percolation is preceded and promoted by a preliminary break in symmetry that occurs as a result of spinodal demixing of the sol (San Biagio et al., 1991, 1996a,b; Sciortino et al., 1993). Quenching of a polymer solution near or inside its instability region causes demixing into lower and higher than average concentration regions. As denser regions form, the threshold value for gelation can be reached locally, even when the average concentration is much lower. Under these conditions, competition between demixing and gelation is excluded, and the final gel structure is dictated by the earliest demixing process (Leone et al., 1987; San Biagio et al., 1986).

Further insight into the gelation process has been provided by studies on aqueous solutions of agarose at very low concentration (0.01–0.05%), reported as nongelling concentrations (Norton et al., 1986). It has been shown that mesoscopic gel-like regions form throughout the sample at steady state after a temperature quench (Bulone and San Biagio, 1991). At the end of the gelation process, elongated agarose aggregates are observed to diffuse freely in a sample that remains macroscopically a liquid (Bulone and San Biagio, 1991, 1995). Samples can be restored to the sol state by heating, with exactly the same thermal hysteresis of macroscopic gels. These results revealed novel aspects of the notion of nongelling concentrations in terms of cross-link percolation. At very low polymer concentrations, gelation actually occurs in mutually disconnected, high-concentration regions generated by spinodal demixing. However, such regions do not percolate throughout the sample, and gelation occurs only on a mesoscopic scale (Bulone and San Biagio, 1991, 1995; San Biagio et al., 1996b). Formation of

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large aggregates and subsequent percolation have been observed in other biopolymeric systems (Clark, 1996a,b); however, at nongelling concentrations, the aggregates are found clustered into phase-separated regions (Clark, 1996a). On the basis of these findings, Clark has pointed out that the notion of a critical gel concentration can be viewed in terms of percolation of phase-separated regions (Clark, 1996b). This suggests that studies of the dependence of microgel aggregates on experimental conditions (temperature quenching depth, solvent composition, or concentration) can be useful in predicting how the structural properties of macroscopic gels can be controlled. At the same time, such studies allow one to characterize the microgel structure using experimental techniques available for liquids.

In the present work we study the effects of cosolute addition (such as occurs in vivo by biosynthesis) on the start of the self-assembly process and on the structural properties of the microgel aggregates in agarose solutions. It has been shown that the presence of cosolutes can profoundly affect the large-scale structure and final stability of a biologically significant supramolecular structure (Emanuele and Palma-Vittorelli, 1995; San Biagio et al., 1989; San Biagio and Palma, 1992). These effects are due to modulation by cosolutes of the solute-solute interactions through the observed perturbation of the solvent configurations (Bulone et al., 1993a,b; Palma et al., 1994). Previous work has also shown the effects of ethanol during spinodal demixing on the approach to the final structure of the macroscopic gel (San Biagio et al., 1989). It was found that ethanol inhibits the long-range ordering process. Here we report on the effects of ethanol on the same agarose solutions under nongelling concentrations. In the presence of ethanol, the agarose aggregates appear to collapse to less asymmetrical structures than in pure water, resulting in a still liquid sample, and allowing increased mobility of probe particles. Our studies allow a detailed picture of the system after complete gelation on a mesoscopic scale and confirm the expected qualitative effects of ethanol on the thermodynamic stability of the solution while revealing interesting features of the final mesoscopic gel regions.

MATERIALS AND METHODS

Agarose was prepared and filtered for light scattering following previously published procedures (Bulone and San Biagio, 1995). In preparing agarose samples with 6% (molar fraction) ethanol, we initially added an appropriate volume of ethanol to the dissolved agarose at 80°C and filtered the solutions at the same temperature, but found that the ethanol was dissolving material from the filters (Sartorius, 0.22 μm, advertised as compatible with ethanol). We modified this procedure by first filtering cold ethanol, then heating to 80°C and adding appropriate volumes to the 80°C filtered agarose solutions in water. When necessary, a small quantity of a prefiltered solution of polystyrene latex spheres (PLS) of 60 nm diameter (from Polyscience, Warrington, PA) was added to an agarose solution at high temperature. Under these conditions, the light scattered by the PLS was at least 20 times greater than that from the agarose aggregates (Bulone and San Biagio, 1995). Sticking of agarose molecules to PLS can be ruled out from previous work at even higher agarose concentrations (San Biagio et al., 1986). After quenching samples to a final temperature, dynamic and static light scattering experiments were used to monitor the samples until they reached a steady state, usually after about 3 days, before recording our results.

In the initial monitoring of the dynamics of the spinodal decomposition of agarose solutions to determine the spinodal temperature, we analyzed the intensity record, as reported previously, to find the characteristic times to reach a slower intensity growth phase (Bulone and San Biagio, 1991).

Polarized dynamic light scattering experiments were performed as previously reported, except that a 30-mW He-Ne Spectra Physics laser was used, with a wavelength of 632.8 nm. Measurements were recorded over the angular range of 13° to 160°, resulting in a range of K where

$$K = \left(\frac{4\pi n}{\lambda}\right)\sin\left(\frac{\theta}{2}\right), \quad (1)$$

of 0.026 to 0.003 nm^{-1}. Time autocorrelation functions were analyzed as previously reported (Bulone and San Biagio, 1995) for diffusion coefficients, mean scattered intensities, and the mobile fraction of scatterers. We also used the constrained regularization analysis program CONTIN to obtain distributions of diffusion coefficients (Provencher, 1982a,b).

RESULTS

Agarose sols at 0.5 g/liter prepared as previously reported and stored briefly at 80°C were quenched to various temperatures in the range of 32–44°C. For a point of reference, at 5 g/liter rapid macroscopic gelation occurs when a sample is quenched to 30°C. The scattered intensity at 90° was monitored as a function of time, resulting in an initial exponential increase phase (Cahn and Hilliard, 1958) followed by a slower growth phase, expected for an intermediate stage of spinodal demixing (Hashimoto, 1988). A logarithmic plot of the intensity versus time revealed the characteristic time at which the intensity growth slowed, shown in Fig. 1 as a function of the quench temperature. The observed divergence of this characteristic time occurs at a temperature that can be considered the spinodal temperature for 0.5 g/liter agarose samples. Fig. 1 shows that the data in the presence of 6% ethanol are displaced toward a lower temperature by a constant 6.3°C. The smooth curves drawn in the figure are best fits to the expression

$$t = A\left(\frac{T_0 - T}{T_0}\right)^{-\gamma}, \quad (2)$$

where T_0 is the spinodal temperature and γ is a critical exponent. We find the same values for A ($A = 0.48$) and γ ($\gamma = 1.3$) for the data for agarose in water and in 6% ethanol. Shifting the data in ethanol by a constant ΔT_0 of 6.3°C, we rescale those data and plot them together in the insert, with the smooth curve showing the common fit to Eq. 1.

To facilitate a comparison between agarose samples in pure water and in 6% ethanol in the experiments discussed below, we quenched samples in ethanol to a temperature 6.3°C lower than samples in water, keeping all other parameters equal. In this way we maintain the same depth of quench below the spinodal line for both samples. We reiterate here that the data reported in Figs. 2 to 4 were obtained

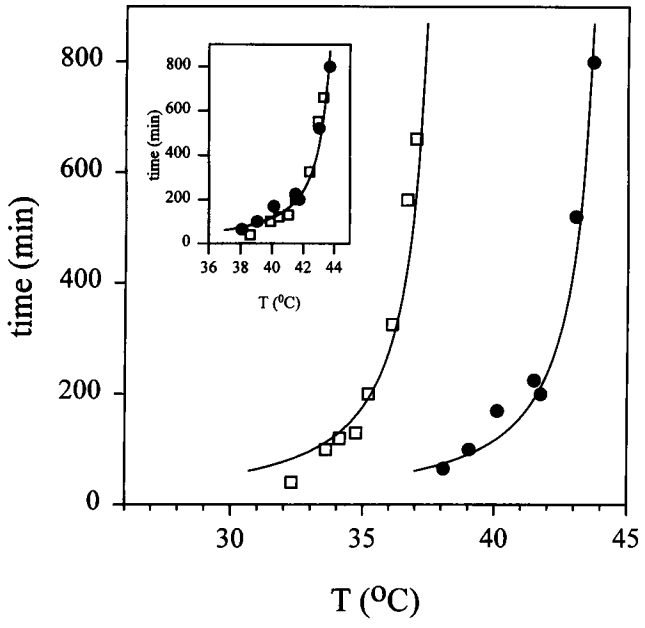

FIGURE 1 Characteristic times for the onset of slower nonexponential light scattering intensity growth as a function of quench temperature for 0.5 g/liter aqueous agarose solutions (●) or 6% ethanol-aqueous agarose solutions (□), in the absence of probe spheres. The continuous lines are the best fits to Eq. 2 that yield the same values for both A and γ. Temperatures at the divergence points correspond to spinodal temperatures under these conditions of 44.7°C and 38.4°C. The inset shows the same data with those for 6% ethanol shifted by 6.3°C, and the common fit, using the same values for A and γ.

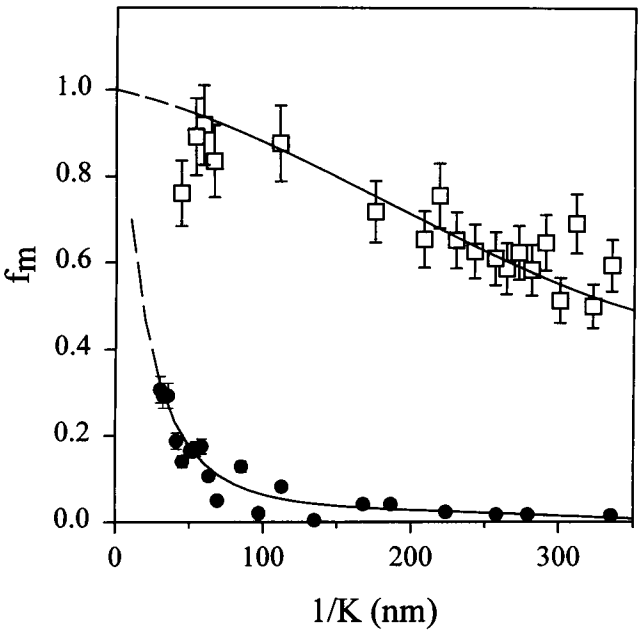

FIGURE 2 Mobile fractions of 55-nm polystyrene latex sphere probes diffusing in solutions of mesoscopic gels as a function of probing distance, $1/K$. Symbols are the same as in Fig. 1, and the lines are drawn simply to guide the eye.

after waiting for the steady state, which typically occurred after about 3 days.

We had previously investigated the mobility of 55-nm-diameter polystyrene latex sphere diffusion probes in 0.5 g/liter agarose solutions in water quenched to 20°C (Bulone and San Biagio, 1995). The amplitude of the time autocorrelation function can be related to the fraction of the probes that are mobile (Madonia et al., 1983; Newman et al., 1991). In Fig. 2 we compare the previously reported data for the mobile fraction of probes as a function of the probing distance, $1/K$, in aqueous agarose solutions quenched to 20°C with data for the same probes in agarose solutions with 6% ethanol, quenched to 13.5°C. There is a marked increase in the fraction of probes that are mobile at all probing distances, with nearly all probes fully mobile over the shorter distances. Fully 70% of the probes are mobile over probing distances of 200 nm. Moreover, as previously reported (Bulone and San Biagio, 1995), analysis of data collected from different regions of the same sample or different samples at the same agarose and PLS concentration consistently gave the same fractions of trapped PLS. This is a result of the mobility and flexibility of the microgel particles, and because of this it was not necessary to apply the data analysis of the Pusey-van Megen theory (Pusey and van Megen, 1989) for nonergodic media.

Dynamic light scattering experiments directly measuring the intensity autocorrelation functions and mean scattered intensities from samples of 0.5 g/liter agarose in water and in 6% ethanol, in the absence of diffusion probes, were performed over a wide range of scattering angles for quenches to 20° and 13.5°C, respectively. Fig. 3 shows a

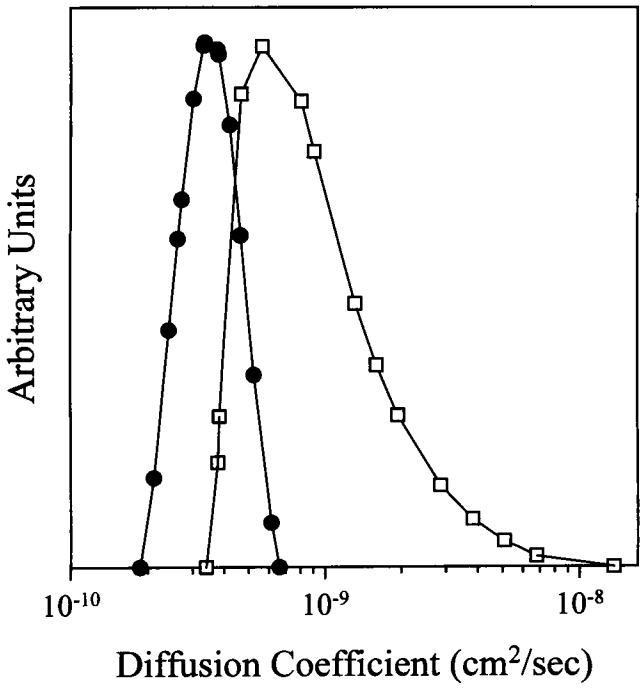

FIGURE 3 Distribution of diffusion coefficients of mesoscopic agarose gels obtained from CONTIN. The symbols are the same as in Fig. 1.

typical result from an analysis of the autocorrelation functions using CONTIN. Diffusion coefficients for the diffusing species in water are symmetrically spread about a peak of $D = 3.4 \times 10^{-10}$ cm^2/s when displayed on a logarithmic scale. Corresponding values for the diffusing species in 6% ethanol are significantly larger with a peak value of 5.6×10^{-10} cm^2/s, but with an asymmetrical distribution extending up to about 1×10^{-8} cm^2/s. For comparison, the diffusion coefficient of agarose coils in the sol state is about 5×10^{-6} cm^2/s at 80°C. Assuming both species are spherical, the corresponding peak diameters are about 1200 nm in water and 350 nm in 6% ethanol, after making a viscosity correction. It should be noted that in the case of water solutions we are dealing with a semidilute system (Bulone and San Biagio, 1995). As a consequence, the size value of 1200 nm reported above is probably overestimated. Nevertheless, the overall interpretation of our results is not affected. It is useful to remark that at this low agarose concentration the sample appears to be macroscopically liquid, with agarose microgels freely diffusing in the sample. Thus we can use QELS to determine the mean size and polydispersity of these agarose microgels.

From the same experiments we also obtain the mean scattered intensity as a function of scattering angle for both samples. After a small correction for background scattered light and after normalizing the data to the extrapolated intensity at forward scattering, the data are shown as a function of scattering angle in Fig. 4 *a*. The abscissa in Fig. 4 *a* is scaled to the dimensionless parameter KL, where L is a maximum linear dimension for the scatterer discussed below. To perform this kind of analysis we used, as discussed below, L and the shape of aggregates as fit parameters. Data for the two different samples are quite distinct.

In an attempt to fit these data to a model, we recall first that 0.5 g/liter agarose solutions in water produce sufficient depolarized scattering to measure diffusion coefficients. In the presence of 6% ethanol there is no measurable depolarized scattered light, suggesting that the scatterers are optically isotropic. Based on this finding, we first used a value of $L = 2R = 400$ nm (similar to the value obtained from the above diffusion coefficient) to scale the agarose in 6% ethanol data in Fig. 4 and then fit those data to the particle scattering form factor for a 400-nm-diameter sphere. The solid curve drawn through those data represents that theoretical form factor, which is seen to fit the data quite well. Attempts to fit these data to a form factor for rods were not successful.

The intensity data for agarose in water cannot be well fit to a form factor for spheres. Depolarized dynamic light scattering data on these samples have been analyzed and interpreted in terms of a flexibly jointed chain with a persistence length of 1200 nm (Bulone and San Biagio, 1995). We therefore first used a value of $L = 1200$ nm (based on the previously measured persistence length obtained from depolarized scattering; Bulone and San Biagio, 1995)) to scale the data and obtained a reasonable fit to the data for $KL < 5$, using a rod particle scattering form factor, but with

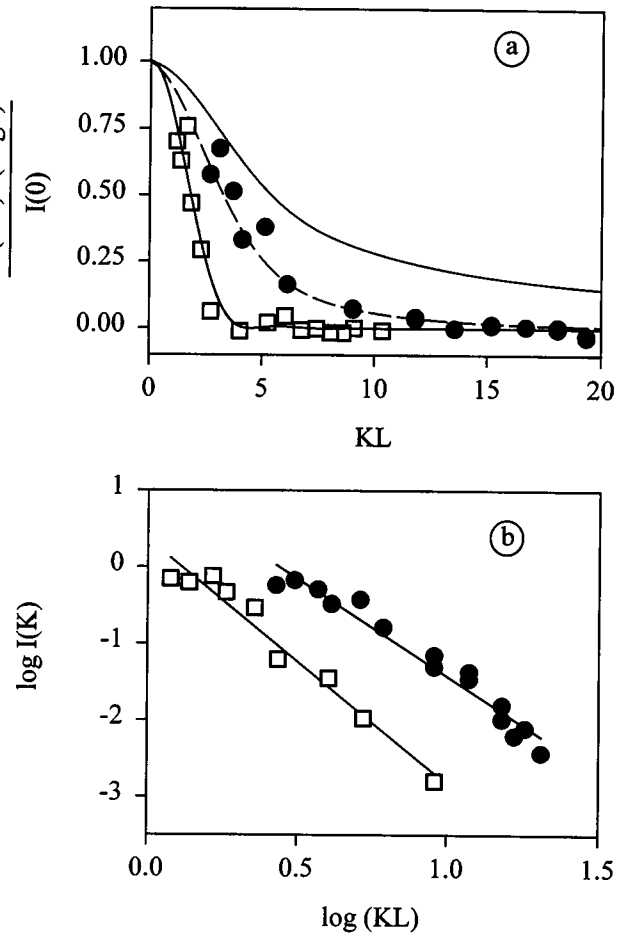

FIGURE 4 (*a*) Normalized mean scattered light intensities as a function of the dimensionless parameter KL. Symbols are the same as in Fig. 1. The continuous line through the squares is the form factor for noninteracting spheres, where the squares are plotted using the best fit value of diameter $L = 400$ nm. The top curve is the form factor for noninteracting rigid rods. The circle data are plotted using a value of $L = 900$ nm, which was obtained as the best value from a fit of the data to Eq. 3 for weakly interacting rods. The only other parameter of the fit was determined to be $\beta = 10$. (*b*) Log of scattered light intensity versus log KL. The solid lines represent the best linear fitting of data. Symbols are the same as in Fig. 1.

significant deviations of the data below the theory at larger values of KL (not shown). From the data in Fig. 2 for probe diffusion in aqueous agarose solutions, it is clear that the agarose must interact and cannot act as independent scatterers. The deviations of the light scattering data below the rod model for $kL > 5$ are due to interparticle interactions. In an attempt to account for these interactions, we fit the intensity data to the form

$$\frac{I(KL) - I(bkgd)}{I(0)} = P(KL)\frac{[1 - \beta P(KL)]}{[1 - \beta]}, \qquad (3)$$

where $P(KL)$ is the form factor for a rod and β is a dimensionless parameter proportional to the second virial coefficient (Schmitz, 1990). We obtained a good fit over the entire range of KL, with a choice of $L = 900$ nm and a single parameter fit of $\beta = 10$ (for $\beta > 10$ there is little sensitivity

of the fit; $\beta = 8$ for hard spheres). Fig. 4 *a* shows the theoretical fits to the agarose in water data for both the noninteracting and the interacting rod model (Eq. 2) for rods of 900 nm length.

The same intensity data can be analyzed in a different way to determine the fractal dimension of microgel structures formed in pure water or 6% EtOH. It is well known, in fact, that when a polyfunctional monomer bonds at random (that is, in our case, when single agarose molecules start to form aggregates after the spinodal demixing), it is common to form fractal structures. The fractal dimension (d_f) of these structures (the microgels in our case) can be determined by looking for the linear relationship between log (I) and log (KL) (Brinker and Scherer, 1990). If a linear relationship is found in the range of measured K, the slope of the linear best fit to the data gives the fractal dimension of the structures. Fig. 4 *b* shows the existence in pure water as well as in 6% EtOH of a linear relation between log $I(K)$ versus log (KL) in the range of K here studied. The slopes of linear fitting of the data are $d_f = -2.5 \pm 0.1$ and $d_f = -3.2 \pm 0.3$ for agarose solutions in water and 6% EtOH, respectively.

DISCUSSION

Extensive time-resolved studies of the self-assembly of agarose gels have allowed a thorough determination of the phase diagram of the aqueous agarose system and an elucidation of the gelation mechanism (Emanuele et al., 1991, 1992; San Biagio et al., 1986, 1989, 1996b). Features of the phase diagram of agarose water systems, as experimentally determined, are schematically shown in Fig. 5. The overall process leading to gelation is seen to occur through the following sequence: 1) an initial breaking of symmetry (homogeneity) in the sol upon quenching from the stability region to the instability region ($P_0 \rightarrow P_1$ path in Fig. 5), that is, spinodal demixing causing the spontaneous generation of polymer-rich and solvent-rich regions (R_P and R_S regions); 2) the start of polymer cross-linking within polymer-rich regions; 3) percolation of polymer-rich regions through the sample, still in the sol state; and 4) the growth of cross-linked percolation, channeled along the pathways of polymer-rich regions, leading to a macroscopic gel.

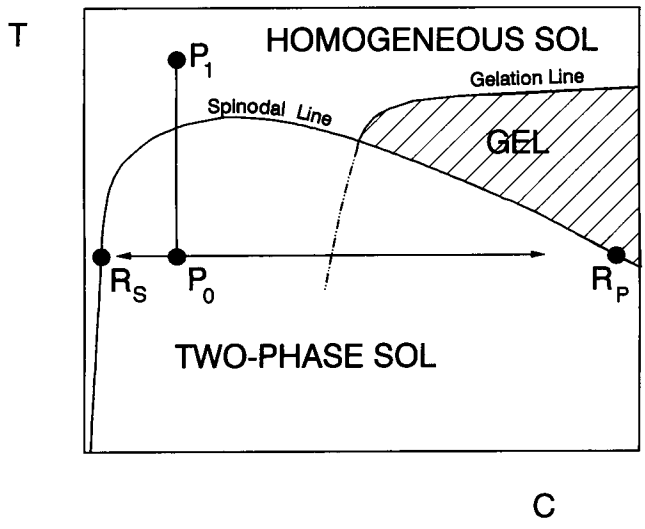

FIGURE 5 Schematic phase diagram of a generic polymer system capable of gelation. When a sol is brought into the region of thermodynamic instability (for example, by a temperature quench to P_o), polymer-rich (R_P) and solvent-rich (R_S) regions are formed. Through the $P_1 \rightarrow P_o \rightarrow R_P$ path, concentration values above the gelation threshold can be reached locally.

At very low concentrations (below 1 g/liter), macroscopic gelation is not observed, but gelation still occurs in mutually disconnected high-concentration regions generated by spinodal demixing (Bulone and San Biagio, 1991). Objects having a diffusion coefficient 2 orders of magnitude smaller than that of the free agarose polymer coils are observed in the final state, but the sample remains liquid and free flowing. Samples containing these objects can be restored to the sol state (with a corresponding larger diffusion coefficient) by heating, and exactly the same thermal hysteresis is observed as in the case of macroscopic gels (Bulone and San Biagio, 1991; Vento et al., 1979). These objects are found to behave as large (in fact, mesoscopic) polymer fibers, entangled in a continuously rearranging mesh of partially flexible, neutral chains (Bulone and San Biagio, 1995).

The presence of cosolutes is known to affect the thermodynamic stability of biomolecular solutions (Bulone et al., 1993a,b; Emanuele and Palma-Vittorelli, 1995; Palma et al., 1994; San Biagio et al., 1989; San Biagio and Palma, 1992). These effects are reflected in changes of the Flory-Huggins P parameter (de Gennes, 1979; Kurata, 1982), expressing the difference between free energies of interactions between like and unlike species. One contribution to P comes from the solute-solvent interaction. The latter can be modulated by the presence of a cosolute imposing nonadditive constraints to the H-bond network of the solvent and so changing the thermodynamic cost of keeping solutes exposed to the solvent (Bulone et al., 1993b; Palma et al., 1994). As it is known that alcohols reduce the thermodynamic cost of hydrophobic hydration (Bulone et al., 1991; Palma et al., 1994), we expect that in their presence the thermodynamic instability region of aqueous agarose solutions would be shifted to lower temperatures. This is indeed observed in the data of Fig. 1. The addition of a small quantity of ethanol shifts the spinodal temperature downward by about 6.3°C. In the inset of the same figure it is shown that the same divergence law with an identical critical exponent can be used for the characteristic times of the occurrence of the spinodal demixing in both pure water and in the presence of ethanol. Choosing the same quenching depth inside the instability region for both solutions will produce the same wavelength of concentration fluctuations (Hashimoto, 1988). Nevertheless, the final states at the end of the gelation process are not necessarily expected to be identical. The diffusion properties and kinetic processes of the growing aggregates are paramount in determining the final state of

the gel, and they are obviously dependent on the properties of the solvent.

Properties of mesoscopic agarose gels formed in pure water have been studied previously by PLS probe diffusion experiments (Bulone and San Biagio, 1995). Mean pore sizes in the 0.5 g/liter aqueous agarose mesoscopic gels were found to be about 100 nm. A major result of this study is the finding presented in Fig. 2 that, in the presence of 6% ethanol, the mean pore size accessible to the 55-nm PLS probe is much larger than that in water, with a much larger fraction of the probes mobile at any probing distance studied.

Three possible explanations for this large difference come immediately to mind. In one scenario, the PLS adhere to agarose particles in water, becoming immobilized, but detach in the presence of ethanol. Earlier controls (San Biagio et al., 1986) ruled out the binding of PLS to agarose in water through a number of independent experiments, and so we do not consider this explanation possible. A second scenario is that the mesoscopic regions formed in the presence of ethanol aggregate, creating larger regions of pure solvent and thus more accessible volume for the probes. This will be discussed shortly in conjunction with a third scenario, in which the mesoscopic regions themselves collapse, so that there is more accessible solvent volume.

The experiments reported in Figs. 3 and 4 on agarose solutions without PLS probes can be used to distinguish between the latter two possible explanations for the probe results just discussed. Measured diffusion coefficients for the agarose species indicate that those in ethanol are clearly smaller in size than those in water. The diffusion coefficient measured for agarose in water is in good agreement with a previous value obtained from depolarized dynamic light scattering (Bulone and San Biagio, 1995). If the diffusing species detected in the dynamic light scattering in fact correspond to the mesoscopic domains hydrodynamically interacting with the PLS, then these data clearly rule out the aggregation of domains in ethanol and favor a collapse in domain size. This interpretation is further supported by the static intensity light scattering results of Fig. 4 *a*. The data from ethanol are best fit by a 400-nm sphere model, whereas the data from water are best represented by an interacting rod model with a length of about 1 μm. Qualitatively, the same conclusion is suggested by the observed fractal dimension of agarose microgels as reported in Fig. 4 *b*. A comparison between the fractal dimension in pure water ($d_f = -2.5$) and 6% EtOH ($d_f = -3.2$) suggests that the presence of ethanol causes the formation of less fractal (i.e., more dense) objects. All of the data presented are thus consistent with the last of the three scenarios, in which ethanol causes the collapse of agarose domains into globular particles instead of more extended, rodlike structures in the final mesoscopic gel state.

A simple entropy argument can help to explain the collapse of mesoscopic agarose gel regions in the presence of ethanol. There are two major competitive contributions to the entropy in regions of higher agarose concentration: the entropy due to ordering of water at the exposed surface layer of biopolymer, and the configurational entropy due to the overall conformation of agarose within these regions. If the entropic cost of keeping hydrophobic groups exposed to the solvent is too large, there may be a driving force toward "bundling" or the formation of rodlike organized structures. On the other hand, if the interaction energy between the solvent and the hydrophobic groups is reduced, as is the case with a solvent perturber such as ethanol (Bulone et al., 1991), then the entropic cost is reduced and a smaller driving force will lead to the formation of a less well organized structure. In the agarose-water system, the decrease in entropy caused by the extended rodlike conformation is balanced by an increase in entropy from the local random structure of buried hydrophobic groups relative to a more ordered state of water if the hydrophobic groups were sitting at the surface. When ethanol is present, because of the reduced entropy cost to order the surface water, the driving force needed to order agarose into rodlike structures to bury hydrophobic groups is not sufficient, and the agarose region collapses to a less asymmetrical and more compact structure.

CONCLUSIONS

Under dilute conditions, at which a macroscopic agarose gel does not form, we have shown that with the addition of 6% ethanol, PLS probe spheres become quite mobile as compared to their nearly total trapping in agarose-water mesoscopic gels. In an attempt to understand this phenomenon, we have shown that although the mesoscopic regions in water are well represented by roughly 1 μm interacting rodlike structures, the presence of 6% ethanol causes these to collapse to spheres of roughly 400 nm diameter. The agarose system can be viewed as a model system for studying the gelation process in uncharged macromolecules and the effects of cosolutes on the final gel structures formed. Entropy arguments can qualitatively explain our findings. Moreover, recent experiments point out the role of kinetics as well as thermodynamics on the size and morphology of condensed DNA particles (see Bloomfield, 1996, and references therein). In this framework, the present results illustrate how cosolutes can also affect particle condensation and, therefore, be important in the folding of other biologically relevant macromolecules.

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