Rheology of a Simultaneously Phase-Separating and Gelling Biopolymer Mixture

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ABSTRACT: The dynamic shear modulus of aqueous solutions of the biopolymers gelatin and dextran was found to be highly sensitive to thermal history even for samples of identical composition. Rheological differences could be correlated with differences in morphology, visible by optical microscopy, arising from the interaction of gelation with phase separation. Quenching a single-phased morphology to a temperature at which phase separation and gelation occurred simultaneously, gave a relatively high shear modulus—reflecting the maintenance of a connected gelatin rich phase. A sample which before its final quench was annealed at an intermediate temperature, in the two-phase region but above the gelation temperature, developed a much lower modulus. In both cases, the mechanism of phase separation at early stages was spinodal decomposition, but in the latter case the much lower modulus reflects a transition to a morphology of disconnected gelatin rich droplets during the late stage of phase separation.

1. Introduction

The study of phase separation in polymer mixtures has recently led to considerable progress toward the goal of understanding the kinetics of development of morphology in terms of mechanisms such as spinodal decomposition.[1−3] Substantially greater richness and complexity are introduced to the problem when there is competition between phase separation and other processes such as vitrification or gelation;[4] here one can obtain stable but nonequilibrium morphologies whose characteristics may be strongly dependent on the history of the sample. Examples from the world of synthetic polymers include semi-interpenetrating networks[5] and blends of thermoplastic and thermosetting resins;[6,7] in solutions of synthetic polymers vitrification of a polymer rich phase may lead to thermoreversible gelation phenomena,[8,9] while in the case of ternary solutions containing two polymers, one of which cross-links, so-called "spinodal gels" may be formed,[10] which may have interesting separation properties. Important applications of these concepts are found for mixtures of biopolymers in aqueous solution, which are widespread in the food and pharmaceutical industries.[11] Here the aim is often to design systems with specified rheological properties to produce some desired effect in the final product.

In this paper we focus on the relation between the shear modulus of a phase-separated biopolymer mixture, the morphology of that mixture and the way that morphology may be controlled. The vital factor in controlling the modulus is the connectivity of the gelling phase, and a number of authors have made a simple connection between connectivity and the mechanism of phase separation, stating that phase separation by nucleation and growth will lead to a nonconnected structure, while phase separation by spinodal decomposition will lead to a connected structure (see, for example, ref 12). We believe this simple correlation to be an oversimplification for at least two reasons. First, even at the very earliest stages in phase separation there is no need for a structure evolving by spinodal decomposition to remain connected; there will be a line on the phase diagram separating percolating from nonpercolating structures, but there is no reason to suppose that this corresponds to the spinodal line.[1] Second, even if phase separation leads at very early times to a structure that is connected, during the complicated and ill-understood development of the structure in the so-called intermediate and late stages of phase separation, a morphological transition may take place to an unconnected structure.

It is the possibility of manipulating the rheological properties of a mixture by exploiting such morphological transitions in time that we discuss here. What we find is that quite small changes in the history of a sample lead to qualitative changes in the morphology, which in turn lead to large changes in the rheological properties of the mixture. In particular, we find that in our system consisting of an aqueous solution of a protein and a polysaccharide, one of which gels, we can control the connectivity of the phase rich in the gelling component by adjusting the length of time that the system spends under conditions in which the system can phase separate without a significant degree of simultaneous gelation. In this way, we can take samples with identical compositions, preparing the mixtures at identical starting temperatures, and quench them to the same final temperature, but merely by adjusting the length of time spent at an intermediate temperature, we can obtain samples with shear moduli differing by as much as 1 order of magnitude. In particular, our findings stress that the connectivity of a phase-separated gel is not necessarily controlled by whether the mechanism of phase separation is by spinodal decomposition or nucleation and growth, as is often stated in the literature; even in a situation in which the

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early stage of phase separation is spinodal decomposition, a transition to an nonconnected structure may take place in the later stages of phase separation.

2. Materials and Methods

The protein derivative used was acid-cured porcine skin gelatin (bloom number 175) obtained from Sigma Chemical Co. This biopolymer gelled at temperatures below approximately 25 °C. The polysaccharide used was dextran (from *Leuconostoc mesenteroides* strain no. B-512), also from Sigma. This component showed no tendency to gel. Both polymers were dissolved in an aqueous solution of 0.5 *m* NaCl, with a weight fraction of 4.2% gelatin and 4.2% dextran, which is a near critical composition.

Optical micrographs were obtained using a Linkam hot-stage mounted in a Zeiss Axioplan phase-contrast microscope. The rheological measurements were carried out using a Rheometrics dynamic stress rheometer (DSR) operating with a 50 mm diameter cone and plate geometry. The storage and loss moduli G' and G'' were obtained at frequencies between 0.3 and 10 rad/s, as a function of aging time. Samples were held previous to measurement at 45 °C in a bottle placed in a temperature-controlled water bath. Samples were then pipeted onto the rheometer plate either at 21 or 30 °C.

3. Results and Discussion

Both the equilbrium phase diagram and the kinetics of phase separation and gelation in this mixture have already been studied using light microscopy, small-angle light scattering (SALS),[13] and infrared measurements.[14] At the composition chosen there is an upper critical solution temperature (UCST) at 38 °C, well above the temperature at which the gelatin solution would gel, which is about 25 °C. Thus a one-phase mixture can be prepared at 50 °C and can be quenched either to temperatures above the gelation temperature, but below the UCST, in which case phase separation can take place without the interference of gelation of the gelatin, or to a temperature below both the UCST and the gelation temperature, in which case phase separation and gelation occur simultaneously. For phase separation at temperatures both above and below the gelation temperature, the mechanism of phase separation was found to be spinodal decomposition; a peak appears in the light scattering intensity whose growth may be analyzed by using the classical Cahn−Hilliard theory.[1] Light microscopy suggests that in both cases the morphology at early times is interconnected. Thus the early stage of phase separation is not strongly influenced by whether gelation is simultaneously occurring. However, in the intermediate and late stages of phase separation, when the characteristic size of the phase-separated structure is growing, there are qualitative and quantitative differences in the behavior of the system in the presence or absence of gelation. In particular, in the absence of gelation, as the system coarsens, there is a transition from an interconnected structure to a droplet morphology, in which spherical domains of the gelatin rich phase are present in a dextran rich matrix. In the presence of gelation, the coarsening process is slowed, and no transition to a droplet morphology takes place. Thus the morphology always remains interconnected.

Quenching a single-phased solution from 45 °C, through the UCST, to 21 °C induced phase separation by spinodal decomposition with simultaneous gelation. Before and immediately after the quench there was no contrast in the light microscope, but over a period of several minutes a phase-separated structure gradually appeared on a scale that was barely resolvable. The structure then appeared to coarsen with time and finally became frozen in as a bicontinuous interpenetrating network structure (Figure 1a).

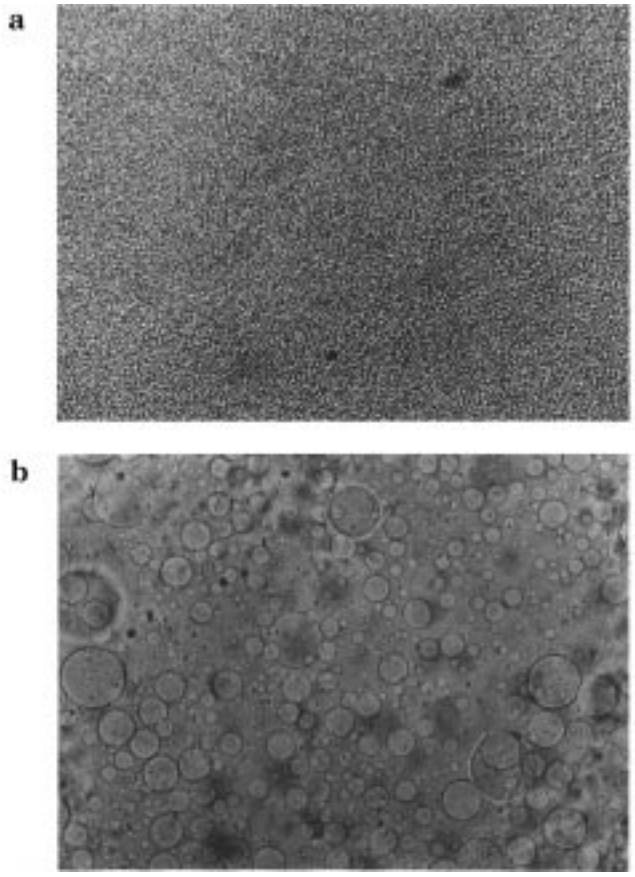

Figure 1. Phase-contrast microscope images for gelatin/dextran blend quenched to (a) below the gelation temperature and (b) above the gelation temperature. The shorter dimension of each photograph corresponds to 970 μm.

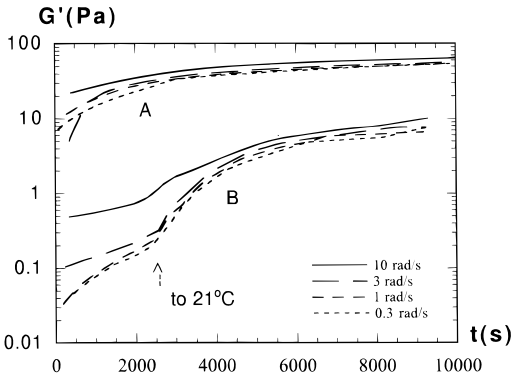

Figure 2. Dynamic shear modulus G' for gelatin/dextran blend at 10, 3, 1, and 0.3 rad/s: (A) quenched to 21 °C; (B) quenched to 30 °C then cooled to 21 °C after 40 min.

Figures 2 and 3 (curve sets A) show the corresponding real (G') and imaginary (G'') parts of the dynamic shear modulus plotted logarithmically as a function of time at four frequencies. The shear moduli rose rapidly, with gelation occurring after approximately 200 s. G' was almost independent of frequency in the gelled state, whereas the imaginary part showed a power law dependence with frequency, given by $G'' \propto \omega^{0.2}$.

Quenching from 45 to 30 °C induced phase separation without gelation. The bicontinuous structure that formed as a result of spinodal decomposition was

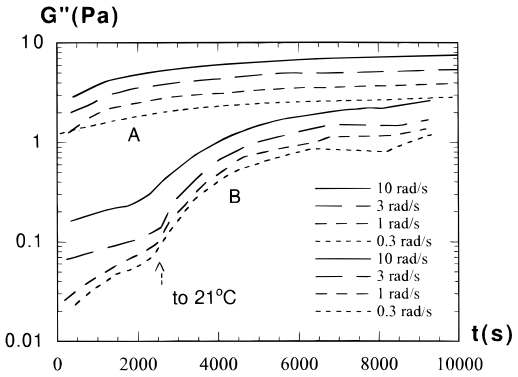

Figure 3. Dynamic shear modulus G'' for gelatin/dextran blend at 10, 3, 1, and 0.3 rad/s: (A) quenched to 21 °C; (B) quenched to 30 °C then cooled to 21 °C after 40 min.

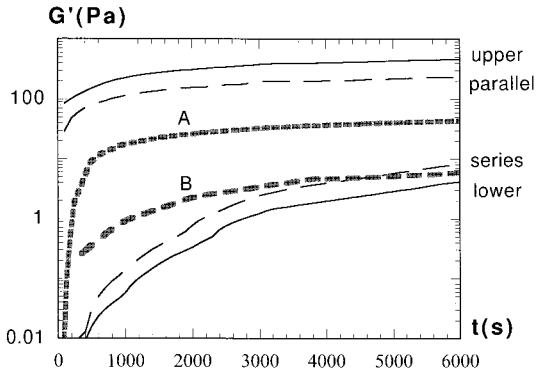

Figure 4. Storage modulus at 0.3 rad/s for gelatin/dextran samples: quenched to 21 °C (curve A); quenched to 30 °C and cooled to 21 °C after 40 min (curve B); upper and lower layers after 24 h at 30 °C and calculated parallel and series bounds for the 50/50 blend.

observed in the microscope to break up into a droplet morphology after approximately 700−1000 s. Light scattering measurements[13] show Porod law behavior at high values of wavevector, indicating that the interfaces between domains are sharp on corresponding length scales. We infer that this morphology comprised unconnected gelatin-rich liquid droplets suspended in a dextran-rich liquid matrix (Figure 1b).

For the purposes of the present rheological work, samples were then cooled to room temperature (21 °C) after various aging times at 30 °C, to compare the behavior with that for the samples directly quenched to 21 °C. Figures 2 and 3 (curves B) show G' and G'' for a sample cooled to 21 °C after aging at 30 °C for ca. 40 min; i.e., from 0 to 2400 s the sample is held at 30 °C. In this region G' and G'' display strong frequency dependence, which is consistent with the behavior expected for a simple viscoelastic fluid at low frequencies ($G' \propto \omega^2$; $G'' \propto \omega^1$)(see, e.g., ref 15). Under these conditions our morphological measurements indicated that we should expect a phase separation to a droplet morphology; however, since no simultaneous gelation takes place this phase separation does not have a dramatic effect on the rheological properties (although both G' and G'' increase somewhat). When the temperature was then lowered to 21 °C in the rheometer, the moduli increased due to gelation, but no dramatic rise occurred.

The G' results for curves B are much lower than those for curves A (after an equivalent time at 21 °C). Figure 4 shows comparative G' results at a frequency of 0.3 rad/s for a sample quenched directly to 21 °C (curve A, bicontinuous morphology) and for a sample cooled to 21 °C after an aging time of 40 min at 30 °C (curve B, droplet morphology).

The graph also shows data for the shear modulus as a function of time for the upper layer (gelatin-rich phase) and lower layer (dextran-rich phase) of a sample that had first been left to phase separate in a sealed bottle for 24 h above the gelation temperature, and then quenched to 21 °C. Here, the phase separation has proceeded to completion, resulting ultimately in the formation of two horizontally separated layers representing the equilibrium compositions in the blend. These data can be used to calculate estimates of the upper and lower bounds on the time-dependent shear modulus of the phase separating blend, using the well-known parallel and series models.[16] (We assumed here that water partitions equally between gelatin and dextran; i.e., we assumed a 50/50 volume % of gelatin-rich and dextran-rich phases in the blend.) This calculation shows that for the bicontinuous structure (curve A) the G' modulus lies intermediately between the parallel and series bounds, whereas for the droplet morphology (curve B) the modulus is much closer to the estimated series or lower bound. It should be mentioned that the calculation is not expected to be appropriate at short times, since the equilibrium compositions in the blend have not yet been reached.

4. Conclusions

In conclusion, we have shown that samples of phase-separated biopolymer mixtures of identical compositions, both prepared at the same starting temperature and taken to the same final temperature, may show very different ultimate shear modulus behavior. The final shear modulus depends on the connectivity of the gelatin rich phase, which in turn depends on the temperature history. A sample quenched directly to a temperature at which both phase separation and gelation occur simultaneously maintains the inteconnected, bicontinuous morphology that arises from the process of spinodal decomposition. An identical sample quenched first to an intermediate temperature, where phase separation occurs without gelation, and then later quenched to a lower temperature, also phase separates by spinodal decomposition. However, in the later stages of phase separation a transition occurs from an interconnected morphology to a morphology in which the gelatin rich phase occurs in isolated droplets. The shear modulus values are higher for the bicontinuous morphology compared to the droplet morphology, due to better stress transfer (or coupling) of the stiffer gelatin-rich component in the bicontinuous structure. The present findings for this model biopolymer blend may be of wider relevance to many mixed polymer systems where phase separation and gelation interfere with each other.

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