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The influence of gelation on the mechanism of phase separation of a biopolymer mixture

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Abstract

The influence of the gelation of one component (gelatin) on the phase separation and morphology of an aqueous mixture of gelatin and dextran has been investigated. Small angle light scattering and confocal microscopy experiments show that the mechanism of phase separation is similar to spinodal decomposition, even in the presence of a rapid gelation. At temperatures well below the gelation temperature the phase separation kinetics are halted by the gelation, resulting in an immobile, interconnected morphology. This effect is seen as a pinning of the peak in the light scattering data. We also show that the gelation affects the position of the scattering peak, effectively deepening the quench as the gelation proceeds, through an apparent increase in molecular weight.

Keywords: Phase separation; Gelation; Spinodal decomposition

1. Introduction

Recently much experimental and theoretical work has concentrated on phase separation of polymers in the presence of other ordering phenomena such as crystallisation or glass transitions. Physical gelation of one of the separating components is another similar effect influencing the phase ordering process. A system in which phase separation occurs alongside gelation can exhibit a wide range of morphologies. The rate and onset of the different ordering mechanisms and the effect of the competing processes (a glass transition would tend to oppose phase separation, for example), dictate the morphology of the material produced. For instance, by halting the phase separation at an early stage a percolating network can be formed. The use of phase separation to produce such structures is of great importance [1], say, to alter the mechanical properties of materials. Novel electronic and optical properties can also be produced. Photodiodes are an illustration of this: these can be made from two different polymers that are in close proximity — hence, an interpenetrating network of two phases, each containing different polymers is the ideal form since there is a large area where the two polymers are in close proximity [2]. Physical properties of structures produced depend sensitively on the interactions of the different mechanisms involved; understanding the relationships between the various phenomena will allow control over the properties of the material produced. Thus the interplay and effects of the various competing mechanisms affecting the conventional phase separation of polymer mixtures have attracted attention of late.

There are several well-established theories describing phase separation and the subsequent coarsening of the structure (see, for example the reviews by Binder [3] and Gunton [4]). In many cases, including those we examine here, the initial phase separation proceeds via spinodal decomposition, where a characteristic length scale is seen throughout the sample. This leads to, if each phase is present in sufficient quantities, an interconnected structure spanning the sample. At this point the compositions of the two phases are (theoretically) almost at equilibrium. There are various mechanisms of subsequent coarsening whereby this structure can evolve, forming larger regions of each phase. Such mechanisms are distinct by different rates of change of the dominant length scale in the sample, r. For example, if the coarsening mechanism is that of evaporation–condensation, whereby material from small droplets of one phase diffuses through the sample to larger droplets, then a growth rate of $r \sim t^{1/3}$ would be expected, similarly for the case where droplets can move around and coalesce. If, on the other

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hand the coarsening is via a hydrodynamic mechanism then one expects a growth rate of $r \sim t$.

In addition, there has recently been interest in the effect of viscoelasticity on phase separation [5–10]. Work done by Keller et al. [11] on systems close to the glass transition of one of the pure components indicated how the phase separation process could be halted by a change in mobility of one component. Several authors have investigated the effect of a glass transition on spinodal decomposition. Sappelt and Jackle [7] modelled the problem by using a mobility that decreased rapidly with increasing concentration of the glass-forming component. Barton et al. [12] also included a mobility dependent on the state of the phase, and found that spinodal decomposition proceeds until the glass composition is reached, after which the growth proceeds much more slowly such that it appears arrested on experimental timescales. The images obtained by Isayeva et al. [13] show this in a handsome manner — they examined a system in which simultaneous crystallisation and phase separation occurred, both were arrested by an interjecting glass transition, resulting in very different morphologies depending on the initial composition and quench temperatures. The effect of the glass transition on phase separation was also examined by de Graaf et al. [14], who studied mixtures of polystyrene and methacrylate, which separated upon cooling, and reported a physical arrest of the phase separation as the polystyrene rich phase underwent a glass transition.

More recently, work has concentrated on systems in which an asymmetry in the bulk and shear moduli of the phases leads to changes in the separation dynamics. Onuki and Taniguchi [9,15] have done a number of computer simulations of spinodal decomposition in polymer solutions. They include an elastic energy term in the free energy to model the effect of a mechanical imbalance. Following an incubation time, they find that 'holes' of the solvent appear, then the polymer-rich regions become thin, forming a 'sponge-like' network. This structure coarsens with time, eventually breaking up into bulk polymer rich domains. These simulation findings correspond well with experimental results of Tanaka et al. [16] who studied the system of polystyrene and poly(vinyl methyl ether). This system separates on increasing the temperature, the poly(vinyl methyl ether) rich phase being less viscoelastic than the polystyrene rich phase. This asymmetry was found to result in the phase separation pattern of such a mixture, when close to its critical composition, being characterised by a sponge-like continuous structure of the more viscoelastic phase. Tanaka et al. suggest that the system is initially dominated by elastic energy, behaving as an elastic gel. During the later stages of separation the rate of deformation slows down, allowing the stresses to relax, thus the domain shapes can transform to the shape of lowest interfacial energy. Tanaka has suggested that an asymmetry in the moduli of the two components would generally be expected to produce such viscoelastic phase separation [8,16].

The majority of the examples so far have examined synthetic polymer systems. Bansil et al. [17,18] used a system of gelatin, water and methanol to look at the effect of gelation on phase separation. When the quench depth was such that the sample was below the gel temperature for pure gelatin, T_{gel}, the gelation of the gelatin was found to halt the phase separation process. For quenches to higher temperatures the phase separation was found to be similar to classical, non-gelling mixtures.

The biopolymer system of gelatin and dextran in a solution of sodium chloride has been studied previously [19–21]. Both gelatin and dextran are soluble in water and the addition of sodium chloride screens the weakly charged gelatin and adjusts the upper critical phase separation temperature, T_c, and gelation temperature to within a range suitable for experimentation [22]. The dependence of the phase boundary on the salt concentration was not explicitly studied, however previous work [19] found that 0.5 M sodium chloride brought the system into a regime where phase separation and gelation occurred at rates and temperatures which were convenient to study. The simplest model of the gelation of gelatin is that in solution at high temperatures the conformation of the gelatin chain is a random coil and, as the solution cools, the gelatin undergoes a coil to helix transition — this is sometimes referred to as the 'frustrated renaturation' of the collagen triple helix. The helices associate and a gel is formed with a structure of connected triple helices. When the temperature is lowered below the temperature of the coil–helix transition a gel will be formed provided the concentration is above a certain level. Below this concentration the coil–helix transition will still occur, but a percolating network (and hence elastic gel) will not be formed. In order to study the gelatin/dextran/water system as a pseudo-binary system the total polymer concentration in solution is fixed and only the ratio of gelatin and dextran is altered. Tromp et al. [19] examined such a mixture above and below the gelation temperature for a concentration of 4.2% gelatin, 4.2% dextran using small angle light scattering and optical microscopy. They found that the early stages were similar for both gelling and non-gelling situations (cooling to just above and just below T_{gel}). Classically, a quench of a mixture of such a ratio of gelatin to dextran is expected to phase separate via the mechanism of spinodal decomposition, as predicted by the linear Cahn–Hilliard theory. Microscopy experiments revealed a composition variation of a characteristic wavelength throughout the sample, as would be expected for spinodal decomposition, and a peak in the light scattering data was observed. However, in contrast with the predictions of the Cahn–Hilliard theory, the peak position in reciprocal space was not stationary and moved to lower values of the wavevector, $\mathbf{q}$, at the earliest experimentally accessible times. The Cahn–Hilliard plot, $R(q)$ vs. q^2, was non-linear in all cases. The morphology as observed by optical microscopy matches the light scattering data quite well: the change in the power-law exponent for the growth of the dominant length scale occurs at the same time as the interconnected

sample appears to break up into isolated domains of gelatin-rich droplets, which go on to coalesce. This transition to droplets does not occur for the quench to low temperatures and the gelation 'freezes in' the interconnected structure. The rheology of the system has also been studied [21]. The dynamic shear modulus of a phase separating solution was followed as a function of time. It was found that samples quenched to a low temperature, where optical microscopy showed that a spinodal structure was frozen in by the gelation, had a high shear modulus, reflecting the connectivity of the gelatin rich phase.

These studies demonstrate the variety of structures that can be obtained simply by adjusting the rates and onsets of various mechanisms. How these mechanisms interact with and affect each other is, however, less clearly understood. It is likely that, as with the glass transition case mentioned earlier, the changing mobility of one component will affect the separation kinetics. Here we investigate the phase separation of gelatin and dextran further, essentially considering the phase behaviour of a two-component mixture of polymers, with the fixed-concentration water playing the role of a suspending matrix only. The effects are likely to depend on the onset and the rate of the gelation process compared to that of phase separation. It is possible that a slightly faster gelling rate would produce a more enhanced effect on the separation mechanism; indeed it is not obvious that the classical mechanisms of phase separation (spinodal decomposition, for example) will remain under such circumstances. If the mixture is quenched below T_c but above T_{gel} one might expect classical phase separation behaviour. Below T_{gel}, the formed elastic network would have a profound effect on the kinetics and morphology.

The compositions under study are in the range of 3.8–5.0% gelatin, with a fixed total polymer concentration of 8.4%. For such a high degree of dilution it is assumed the system is pseudo-binary, that is, the water partitions equally between gelatin rich and dextran rich phases. The values of T_c and T_{gel} for the range of mixtures used are shown in Fig. 1. The ratios examined here are indicated. The cloud point temperature, T_c, is determined by measuring the temperature at which a mixture, cooled slowly from the homogeneous state, becomes an opaque, scattering liquid. The cloud point curve essentially determines the equilibrium compositions to which the mixture will separate, the volumes of the phases are governed by the initial composition. The gel curve shows the temperatures, T_{gel}, below which a pure gelatin solution, at given concentrations, would form a gel. These two temperature curves define the region relevant for our present findings.

The temperature at which the coil–helix transition occurs is, for gelatin, in the region of 30°C. The cloud point temperatures are higher than this, thus it is likely that the phase separation (seen at the cloud point) is due to the unfavourable interaction between the gelatin coil (the high temperature state) and the dextran coil. The gel line measured is lower than the coil–helix transition because

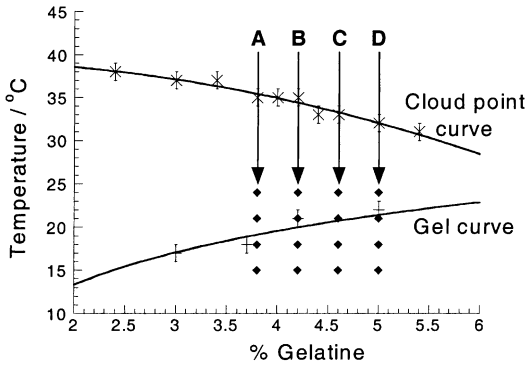

Fig. 1. Phase diagram for a mixture of gelatin and dextran in 0.5 M NaCl. The total polymer concentration is 8.4%. Below the cloud point curve the mixture will separate into gelatin-rich and dextran-rich phases. The gel curve shows those temperatures below which a solution of gelatin in 0.5 M NaCl will form a gel. Lines are drawn merely to guide the eye. Note that if polymers of higher molecular weight were used the cloud point curve would be shifted upwards, similarly if for different gelatins, or salt concentrations would also affect both the cloud point curve and gel line. The diamonds indicate the mixtures A, B, C and D, and the temperatures to which samples of the mixtures were quenched.

the gel takes time to form, thus even though the coil–helix transition may occur quite quickly, the slower gel formation means that within the experimental time scale a gel does not appear to form at higher temperatures, even though the gelatin may be in the helix form. Thus the gel line should be taken as an indication of gelation occurring within the experimental timescale. The quench depth, that is, how far below T_c the mixture is cooled, affects the driving force for phase separation. Similarly the concentration of gelatin affects if and how quickly the mixture gels. Note that the mixtures are all quenched to temperatures below the coil–helix transition, thus we might expect a change in the phase separation character due to this effect, that is, there was an initial unfavourable interaction between the gelatin coil and the dextran coil, but below the coil–helix transition the interaction between gelatin helix and dextran coil must be considered. Similarly, when the gelatin helices associate to form a gel the interaction between the associated helices and the dextran coil has to be taken into account.

Various mechanisms by which phase separation can take place have been detailed in some classical reviews [3,4]. These theories offer a framework with which the light scattering data can be analysed. The classical Cahn–Hilliard theory predicts that the composition difference, $\delta\phi$, evolves as:

$$\delta\phi(q,t) = \delta\phi_0 \exp\left(-\frac{R(q)}{2}t\right) \text{ where } R(q) = 2Mq^2[|f''| + gq^2] \quad (1)$$

where $\mathbf{q}$ is the wavevector, $\delta\phi_0$ is the composition difference at time $t = 0$ *s*. M is the mobility constant, ϕ is the composition and g is an elastic constant. The equilibrium

free energy density, f, is assumed to depend on composition, ϕ, with the gradient term, gq^2, penalising the variation of ϕ on interfaces; f'' denotes a second derivative with respect to ϕ. Eq. (1) predicts that a characteristic lengthscale appears in the sample, the composition difference between the two phases growing with time. That the composition evolution is written in terms of wavevector is convenient since data from small angle light scattering can be analysed easily in terms of this theory. This characteristic length scale (or the equivalent wavevector, $\mathbf{q}$) is determined by the driving force to phase separate and the energy penalty caused by having a composition gradient in the sample. This characteristic wavevector, $\mathbf{q}_{\max}$, is given by:

$$\mathbf{q}_{\max} = \sqrt{\frac{|f''|_{T,\phi_0}}{2g}} \tag{2}$$

A critical wavevector above which no composition variations grow, can also be defined as

$$\mathbf{q}_{\text{crit}} = \sqrt{\frac{|f''|_{T,\phi_0}}{g}} \tag{3}$$

This corresponds to the critical wavelength below which the energy penalty due to the composition gradient is so great that the composition variation is unstable and is suppressed. Thus, Eq. (1) predicts a peak in the scattered light at a value $q = q_{\max}$, the intensity, $I(q)$, at each wavevector increasing exponentially with the exponent $R(q)$, and a critical wavevector, $\mathbf{q}_{\text{crit}}$, above which no increase in intensity is expected. As the composition difference between the phases increases, the linear theory breaks down and the sample starts to coarsen, the characteristic wavelength increases and the two phases become more different.

Although complications such as the presence of a gelling component indicate that the classic, linear Cahn–Hilliard theory is unlikely to be completely successful, it is nonetheless worthwhile to see how well the initial sample evolution can be described by this theory. The first and most obvious complication is the formation of the elastic network. This itself has its complexities. The gelatin molecules first undergo a coil–helix transition and then link up with other helices to form a network. This initial configuration change of the molecules may alter the interaction strength or range and will introduce a dynamic asymmetry in the elasticity of the separating components, thus altering the dynamics of subsequent separation. This transformation takes place quickly and is followed by a slower change, thought to be a refinement of the structure [23]. Also to be considered are the approximations inherent in the classical Cahn–Hilliard theory. We use the simplest linear Cahn–Hilliard theory, ignoring, for example, the compositional dependence of the parameter g describing the gradient term in Eq. (1), and indeed other effects frequently found to lead to, for example, lower critical temperature behaviour. This approach allows us to examine how far the linear Cahn–Hilliard theory can be stretched to predict phase separation behaviour of low-concentration polymer mixtures having an additional elastic degree of freedom.

We show that although gelation is present from the initial stages and strongly influences the final morphology of the sample the phase separation process retains many of the characteristics of spinodal decomposition, being affected only by the apparent increase in molecular weight of the gelatin chains and the final gelling of the structures.

2. Experimental

The gelatin and dextran samples used in this work were obtained from Aldrich. The gelatin was Type A, extracted from porcine skin. It had a bloom number of 175 and a molecular weight of 200 000. The dextran was produced by *Leuconostoc mesenteroides* with an average molecular weight of 167 000. Both polymers are expected to have a high polydispersity. In all cases, samples were made by mixing the appropriate masses of polymers and salt solution in a vial. The polymers were allowed to swell overnight, then heated to 60°C, well above both T_{gel} and T_c, and stirred for at least 30 min to produce a homogeneous solution. To prevent microbial attack, a small amount of NaN_3 was added (approximately 0.1% of the salt solution). The samples were then filtered to remove any small dust particles.

The sample quench consisted of cooling an homogeneous mixture from 60°C, where it had equilibrated, to the quench temperature, at a rate of 90° per minute. All times after quenching that are quoted are such that the sample reaches the quench temperature at $t = 0$ s. The subsequent phase separation process was followed by light scattering, microscopy and rheology.

Small angle light scattering is an appropriate tool to examine the phase separation in polymers, since the length and time scales are often in the accessible region. Such experiments have been carried out on several samples quenched to various temperatures in order to examine the effect of gelation on phase separation. The apparatus used for small angle light scattering has been described previously by Tromp et al. [19,20]. The sample was mounted on a Linkam hot stage, thus the temperature of the sample could be adjusted in the scattering apparatus itself. Sapphire crucibles with glass coverslips, which hold approximately 0.02 ml, obtained from Mettler Talbot, were used to contain the sample; the small volume and high thermal conductivity of sapphire allows rapid temperature equilibration.

The microscope used in this work is a Laser Scanning Confocal Microscope (LSCM 510) produced by Zeiss. The sample was also mounted on a Linkam hot stage. Fluorescent rhodamine was used to label the components (this partitions into the gelatin-rich phase). Samples were made up with approximately 0.5% rhodamine and filtered to remove any undissolved rhodamine. The major advantages of the

confocal microscope are that it can produce a three-dimensional image of the sample, thus the interconnectivity (or the absence of one) of the samples can be established, and the fluorescent labelling makes identification of the phases easier.

Rheology measurements were done using a Rheometrics Dynamic Stress Rheometer with the parallel plate geometry. The cooling rate of the sample in the rheometer was slower than in the hot stage and in order that the gelation could be followed over a similar time scale as in the optical study, special tools that allowed the rapid cooling of the sample, were designed. This adjustment gave a cooling rate of 30°C per minute. Measurements of the real and imaginary parts of the shear modulus, G' and G'', were made at constant stress and constant frequency.

3. Results

The samples examined contain different ratios of gelatin to dextran and are labelled A (gelatin poor sample) through to D (a gelatin rich sample). The total polymer concentration in each sample is 8.4%. For convenience we summarise the compositions of the samples below; the corresponding ratios of gelatin to dextran are given in brackets:

A:3.8% gelatin:5.0% dextran (0.45:0.59);
B:4.2% gelatin:4.2% dextran (0.50:0.50);
C:4.6% gelatin:3.8% dextran (0.55:0.45);
D:5.0% gelatin:3.4% dextran (0.60:0.40).

In all the samples studied, (indicated in Fig. 1), the initial phase separation produces the structure of two percolating phases shown in Fig. 2a. On the left Fig. 2a shows a sample (C in Fig. 1) quenched to a high temperature, in the initial stages of phase separation. This morphology, where phases of a characteristic lengthscale appear throughout the sample, the composition of the phases continuing to evolve, is as expected for spinodal decomposition. Subsequent coarsening in this sample produces gelled gelatin-rich droplets in a dextran-rich matrix, this is shown in Fig. 2b. On the right of Fig. 2a is a similar image of a sample, quenched to a low temperature (A in Fig. 1) several minutes after the quench. The similar, interconnected, phase separated structure is easily seen, however, this structure does not evolve further and has been frozen in by the gelation of the gelatin rich phase. The remarkable resemblance of the morphology of the two samples indicates that the early phase separation mechanism is similar, but that gelation creates profound differences in the final system; in one case, freezing in the percolating structure, in the other case allowing the phase separation and coarsening to proceed further. It is the effect that the gelation has on the resulting morphology and the mechanisms, by which the sample evolution is influenced that we investigate here.

At the later stages, the structures observed are different.

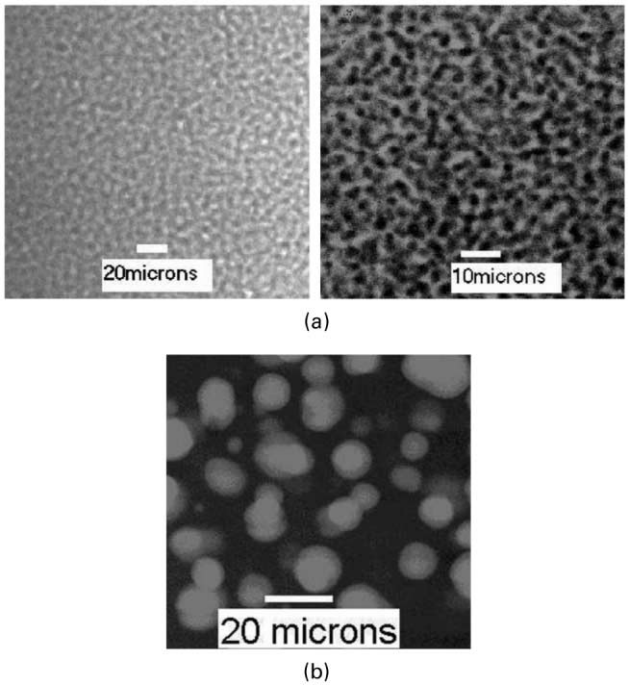

Fig. 2. Laser scanning confocal microscopy images of (a) (left) sample C quenched to 25°C during the initial stages of phase separation and (right) sample A quenched to 15°C several minutes after phase separation where the structure evolution has been halted by gelation; (b) sample C quenched to 25°C after coarsening to a droplet structure has taken place. The light areas show the gelatin rich phases.

The samples quenched to higher temperatures ($T > T_{gel}$) show separate gelatin rich droplets in a dextran-rich matrix. Those quenched to low temperatures show a percolating, interconnected structure, frozen by gelation.

3.1. High temperature quenches

Samples quenched to 25°C show gelatin rich droplets in dextran rich phases, the morphology always appearing very similar to Fig. 2b. That this happens for all samples is unexpected — in those samples which have a higher overall concentration of gelatin it would be expected that the majority phase (the gelatin-rich phase) would be the continuous phase. This also contradicts the findings of Tanaka [24]. Tanaka has simulated (two-dimensional) phase separating systems in which there is an asymmetry in the two separating phases, by attributing different viscosities to each phase. He finds, in contrast to the results here, that the more viscous phase forms the continuous phase, while the less viscous phase forms droplets. In our case, the gelation means that the gelatin-rich phase is always the more viscous phase.

Comparison of samples quenched to the same temperature reveals that the evolution of the samples differs. In those quenched to 25°C the structure changes slowly (compared to the timescale of the early stages of phase separation) and the gelatin-rich droplets coalesce in the gelatin-poor sample (A) whereas in the gelatin-rich sample

(C) the droplets aggregate but no coalescence is observed. Sample B shows no movement of the droplets, but they do increase in size, indicating that the growth mechanism is that of evaporation–condensation, where material diffuses through the sample from smaller to larger droplets.

That the droplets move but do not coalesce in the gelatin-rich sample indicates that the gelatin in the droplets is gelled, as such any coalescence will take place slowly. It should be pointed out that coalescence is not prohibited by the gelation of the droplets since is it known that gelation continues for a long period of time, and may include a refinement of the structure, thus it may be possible for the droplets to coalesce given enough time. Coalescence, however, was not seen within the timescale of observation, ~2000 s. This also indicates that the coarsening of the structure, (from the interconnected morphology to separate droplets), must have been quite rapid, in order to reach such a stage before gelation occurred. The relative fluorescence of the two phases can also be seen to change indicating that, although the droplets must have a modulus high enough that they cannot coalesce, the compositions can still change to some extent.

In the case of the gelatin-poor sample, A, quenched to 25°C the droplets do coalesce, implying that the droplets are either not gelled, or very weakly gelled, such that the rearrangement of the gelatin network required for coalescence can easily occur. This takes place over ~200 s, relatively quickly compared to the timescale over which the droplets in the gelatin-rich sample, C, were observed only to aggregate. It should be noted, however, that only a few of those droplets observed actually coalesced, many passed close by or touched each other without coalescing or aggregating. This indicates that the dextran rich matrix in which they move must be relatively fluid, allowing the easy movement of the droplets since the droplets will only coalesce if the time of contact is long enough to allow the necessary rearrangement of the material.

There phenomena are counterintuitive, since for a given temperature the phases into which the sample separates are expected to be independent of the starting compositions and only the relative volumes of the phases should change. Thus we would expect, naively, that the gelatin in each sample would reach the same stage of gelation at a given time. That the gelatin droplets in the gelatin-rich sample C cannot coalesce whereas those in the gelatin-poor sample A can imply that there is more gelatin in the droplets in one case than in the other since although the gelation rate is concentration dependent, it is rapid; thus, even if the rate of phase separation and corresponding increase in gelatin concentration were slower in the gelatin-poor sample (which, in fact, is not expected since it is a deeper quench and implies a larger driving force to phase separate), over the timescale of observation the droplets do not reach the non-coalescing state of the gelatin rich sample. Thus we conclude that the traditional assumption that the equilibrium phases into which the samples separate are (for a given temperature) the same, is not applicable in this case.

3.2. Low temperature quenches

On the other hand, quenches to 15°C show a spinodal-like interconnected structure for all samples indicating that gelation freezes the sample morphology very quickly.

The transition from interconnected to droplet morphology occurs at different temperatures for different samples, depending on the rate of gelation of the relevant concentrations of gelatin at that temperature and the rate of separation. For sample B a droplet morphology is observed at 18°C, whereas for sample A an interconnected structure is still observed at 18°C. The gelatin poor samples would be expected to gel either at the same rate, or more slowly. Thus the transition from interconnected to droplet morphology in a gelatin poor sample would be expected to occur at a lower temperature rather than a higher one. This indicates that, at this temperature, the rate of gelation in sample A, must be faster than its phase separation, whereas for sample B the opposite must be true. Again this is counterintuitive since from the cloud point curve we see that for the compositions indicated and for a given quench temperature, the samples with less gelatin have the largest temperature difference, $|T - T_c|$. Thus, for any given temperature one should expect the driving force for the phase separation to be largest for these samples. This indicates how changing the rate of one mechanism can severely affect the rate of another.

3.3. Small angle light scattering results

The observations detailed above indicate that in the case of high temperature quenches the equilibrium compositions of the phases are different to those expected, even though the structures are similar, indicating that differences must have occurred on a similar timescale to the early separation process. This suggests that the mechanism of spinodal decomposition has been affected more than the images of the early phase separation imply. Small angle light scattering experiments were carried out to determine whether the Cahn–Hilliard theory of spinodal decomposition could describe the early phase separation.

The typical small angle light scattering (SALS) data for a sample can be seen in Fig. 3a. A peak in the intensity is always observed and, although its position is never stationary, its intensity increases exponentially, as would be expected for a spinodal decomposition, in agreement with the microscopy results. We examine first how well the phase separation process matches the predictions of the classical Cahn–Hilliard theory of spinodal decomposition. Although basic features such as the stationary peak predicted by the linear Cahn–Hilliard theory are not seen, the predictions for initial parameters, such as the value of $\mathbf{q}_{max}$, could be valid at the start of phase separation. The gelation process will also start as soon as the quench occurs, but the rate depends on the quench depth — at higher temperatures the pure gelatin

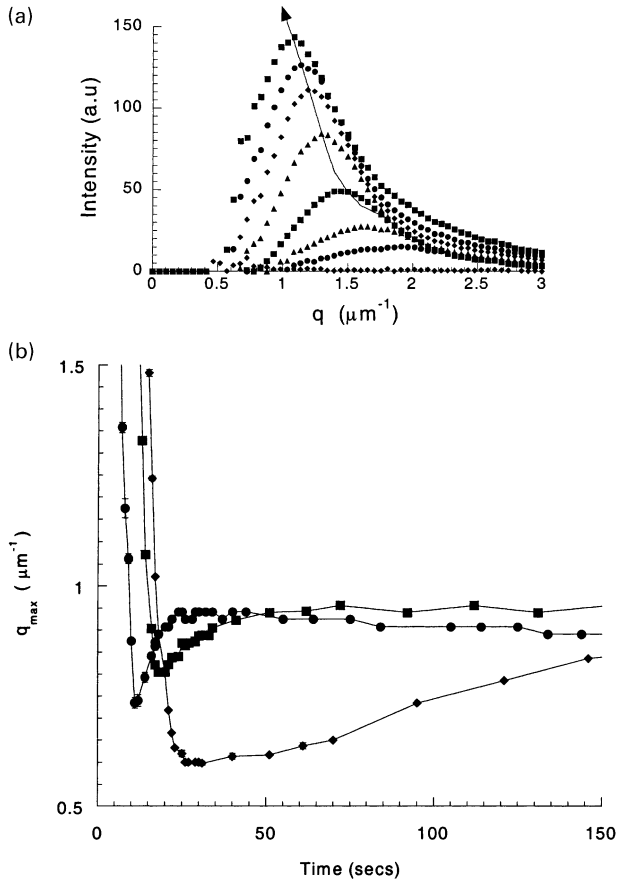

Fig. 3. (a) Typical small angle light scattering data showing a peak in the scattering pattern corresponding to a characteristic wavelength in the sample for sample B quenched to 18°C. The line indicates the peak movement, which can be seen to increase in intensity and move to lower wavevectors with time. Peak growth is shown here over 500 s; (b) variation of peak position with time showing the anomalous peak movement for sample C quenched to 18°C (circles), 15°C (squares), and sample D quenched to 15°C (diamonds).

solutions gel more slowly. The SALS data also shows that during the initial stages there is a critical wavevector, wavevectors larger than this do not grow. The presence of a critical wavevector, and a peak, which increases in intensity, are indicative of spinodal decomposition.

The ratio of the critical and peak wavevectors is predicted to be:

$$\frac{\mathbf{q}_{\max}}{\mathbf{q}_{\text{crit}}} = \frac{1}{\sqrt{2}} \tag{4}$$

The measured ratios are shown in Fig. 4. In spite of the non-linearity in the system, evident from the absence of a stationary peak, the predictions work remarkably well for most samples, though it can be seen that for the more off-critical mixtures the ratio is somewhat smaller. It is possible that for these samples the value of $\mathbf{q}_{\max}$ is smaller than the linear theory predicted, which is reasonable if the early stage has already passed and would also explain the lack of a stationary peak, but would indicate that the early stage

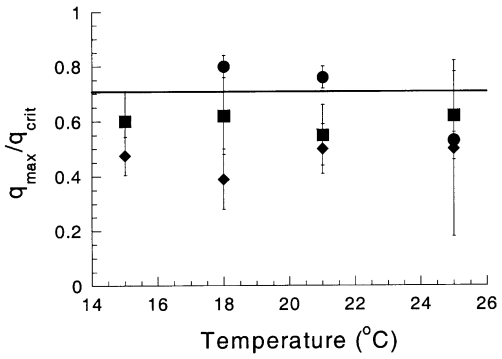

Fig. 4. Ratio of the measured critical and peak wavevectors for several samples: circles, squares and diamonds correspond to mixtures B, C and D respectively. The straight line indicates the value, $1\sqrt{2}$ predicted by the Cahn–Hilliard theory.

is very rapid and that the fluctuations do not show in the scattering pattern above the noise. It is also possible that the value of $\mathbf{q}_{\text{crit}}$ is larger than the measured — the large errors reflect the difficulty in determining this.

That the ratios, $\mathbf{q}_{\max}/\mathbf{q}_{\text{crit}}$, are so close to the expected value is in contrast to the predicted values of $\mathbf{q}_{\max}$. The dominant wavevector, $\mathbf{q}_{\max}$, in the initial stages of phase separation is predicted to be related to R_g, the polymer radius of gyration, via the equation [3,25]:

$$q_{\max} = \frac{1}{R_g}\sqrt{3(1 - T/T_{\text{crit}})} \tag{5}$$

The radius of gyration of the dextran is approximately 10 nm [26] and that of gelatin in solution is 35 nm [27]. The measured values of initial $\mathbf{q}_{\max}$ give $R_g \sim 300$ nm. Though the parameter R_g is not well defined (for gelatin chains in solution, the radius of gyration will vary depending on the quality of the solvent, the temperature etc. [28]; Pezron et al. [27] found inhomogeneities which were of the order of 50 nm in their solutions, the coil–helix transition and the presence of dextran might also affect range of interaction) this is quite a discrepancy. It is also possible that the screening of the gelatin due to the added salt may lead to a larger radius of gyration than expected. Although Eq. (5) does not take into account the fact that the polymers are in solution (rather than in the melt) a difference of the order of magnitude is very large, and it may be that the association of gelatin is already affecting the phase separation process by increasing the effective size of the gelatin chains.

The data from the samples showed an exponential increase in intensity with time in the very early stages, thus Cahn–Hilliard plots, of $R(q)$ vs. q, could be drawn. According to the linear theory, these should show a straight line, the intercept of which gives the effective diffusion coefficient ($= M|\mathrm{d}^2 f/\mathrm{d}\phi^2|$). None of the samples studied showed a straight line. This is to be expected since, as often found for phase separating polymer systems, the stationary peak predicted by the Cahn–Hilliard theory is

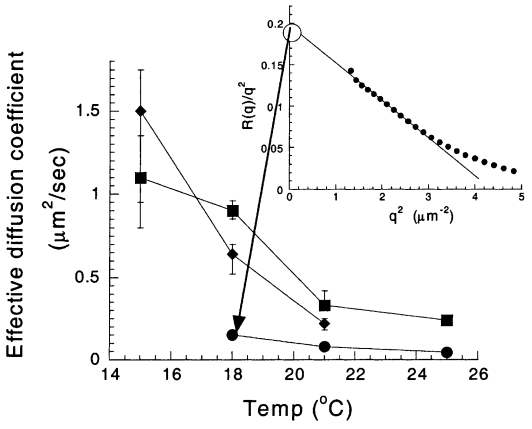

Fig. 5. Temperature variation of the magnitude of the effective diffusion coefficient, measured (or those samples with sufficient data) by a linear extrapolation of the low-**q** regime of Cahn–Hilliard plots to $q = 0$ (as shown by the inset). Circles, squares and diamonds correspond to mixtures B, C and D respectively. The lines are drawn as a guide to the eye.

not seen, indicating that the linear theory does not predict the sample evolution precisely. However, the theory is thought to hold well for small wavevectors [3] and thus a linear extrapolation for the low wavevector range can still be made (shown in the inset in Fig. 5). For the samples for which this extrapolation could be done, the variations of the effective diffusion coefficients thus determined are shown in Fig. 5.

It must be pointed out that it is the variation in the magnitude of the effective diffusion coefficient that is being considered. The effective diffusion coefficient itself is negative, thus giving rise to the term 'uphill diffusion' to describe the flow of material to those areas where it is already more concentrated. These results indicate that the effective diffusion coefficient decreases with increasing temperature. This is consistent with the predicted variation of $|\mathrm{d}^2f/\mathrm{d}\phi^2|$. As for the relative magnitudes of the coefficient for each sample, it would be expected that those samples that have more gelatin would have a lower coefficient, since for a given quench temperature, the samples with more gelatin would have a lower value of $|\mathrm{d}^2f/\mathrm{d}\phi^2|$ (since $|T - T_c|$ is smaller). Sample B has a smaller effective diffusion coefficient than the gelatin-rich samples, however the differences between the gelatin-rich samples are less well defined.

We examine whether the values of the effective diffusion coefficient, $M|\mathrm{d}^2f/\mathrm{d}\phi^2|$, are similar to the values we might expect for the diffusion coefficient, D, for polymers such as gelatin moving through a liquid. This is done by determining the value of D for a polymer chain in a liquid. This is given by the Stokes–Einstein diffusion coefficient [29]:

$$D = \frac{kT}{6\pi\eta l} \tag{6}$$

where k is the Boltzmann constant, η is the viscosity of the fluid in which a polymer of radius of gyration l is moving. In this case we have $T \cong 293$ K, $\eta \cong 10^{-3}$ Pa s, and $R_g \cong$ 300 nm, (here we use the value of l obtained from the measured values of $\mathbf{q}_{max}$ and Eq. (1)). Although, strictly, the Stokes–Einstein equation is only valid for dilute solutions, it suffices here to indicate the order of magnitude. Thus we have $D \cong 7 \times 10^{-13}$ m^2/s $\cong 0.7$ μm^2/s. However, we would expect the mobility to be modified by the thermodynamic factor of $\mathrm{d}^2f/\mathrm{d}\phi^2$, which is, of the order of magnitude, $|T - T_c|/T_c \sim 0.1$ lower, giving $D \cong 0.07$ μm^2/s. The values of the effective diffusion coefficient obtained above are in the range 0.05–1.5 μm^2/s, so the qualitative agreement is very good.

Thus, both microscopy and light scattering show that, while the initial phase separation is very similar to spinodal decomposition, there are differences: the main one being the change in the equilibrium compositions to which the phases separate (as indicated by the different states of gelation of the droplets in samples A and C quenched to 25°C). This increase in gelatin concentration in the droplets in sample C is as we would expect for a sample that had been quenched to a lower temperature. The higher mobility of the gelatin rich samples would also be expected for a deeper quench. We believe that, as the phase separation and gelation proceed, the quench is effectively deepening.

Confirmation of this comes from the light scattering data: the results for those samples B, C and D quenched to low temperatures show the peak in the scattered light moving first to low wavevectors, as expected, but then out again to larger values of **q**. This movement of the peak is counter-intuitive and means that characteristic size of the phase separated domains first grows, then shrinks again. We believe this too to be an effect of the gelation, which is found to begin very quickly in samples rich in gelatin. As mentioned earlier the parameter describing the interaction between gelatin and dextran has here, three cases: first (predominant at high temperatures) the gelatin-coil/dextran-coil interaction; second (coming into play after the coil–helix transition) the gelatin-helix/dextran-coil interaction, and third (as the gel forms) the gelatin-gel(associated helices)/dextran-coil interaction. Here we consider the latter in the Flory–Huggins manner, that is, also taking into account the entropic contributions, which are likely to change greatly with the gelatin conformation. There are more configurational constraints on a polymer made up of two 'gelled' chains than there are on the separated chains, just as there are more configurational constraints on a polymer of higher molecular weight. Thus the association of the gelatin chains is analogous to an increase in molecular weight, the differences in phase diagrams for polymers of different molecular weights are familiar and useful to consider here. A mixture of dextran and a higher molecular weight gelatin would have higher values of T_c, (because the configurational constraints on the gelatin are higher, making mixing less energetically favourable). Thus quenching the higher molecular weight mixture to the same temperature as the lower molecular weight one is, in fact, a deeper quench

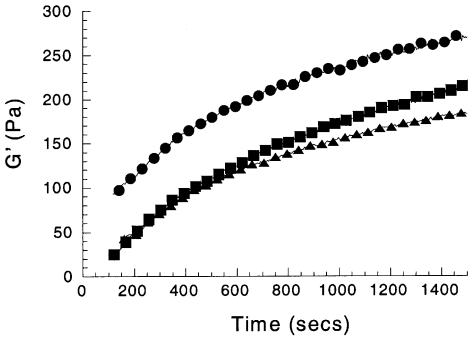

Fig. 6. Variation in the storage modulus, G' of samples quenched to 18°C. Triangles, circles and squares correspond to mixtures A, B and C respectively.

and would imply a higher value of $\mathbf{q}_{max}$. Analogously, in our case, as gelation occurs and the configurational constraints increase with time, the drive to phase separate becomes larger with time and the value of $\mathbf{q}_{max}$ will increase. At low temperatures the conformation change and the gelation occurs more quickly and is more comparable with the rate of phase separation than at higher temperatures, thus the effect on $\mathbf{q}_{max}$ is more pronounced and is seen in the anomalous movement of the peak at low temperatures. This increase in effective quench depth would also result in the equilibrium compositions being different, the gelatin rich phase would be expected to contain more gelatin for example, as the microscopy studies found for sample C quenched to high temperatures.

3.4. Rheology

This concept of the changing of the equilibrium compositions on gelation is supported by the rheology data. Although this cannot be directly compared with the optical studies due to the slower cooling rate, the trends are found to be consistent with the above hypothesis. It would be expected that the gelatin in the continuous phase contributes most to the modulus. As the quench deepens the amount of gelatin in the dextran-rich, continuous phase is expected to decrease, thus, ignoring for the moment the effect of temperature on the modulus, a deeper quench would be expected to produce a lower modulus. This is exactly what is found for samples quenched to the same temperature but with differing initial compositions, see Fig. 6. The gelatin-rich sample shows a lower modulus than expected, indicating less gelatin in the continuous phases (note that this is consistent with there being more gelatin in the droplets, as noted above). This implies that the gelatin-rich sample (C in this case) has equilibrium compositions that are characteristic of a sample quenched to a lower temperature. We believe this is due to the more rapid gelation of the gelatin chains (due to the higher concentration) and manifests itself as a polymer of higher molecular weight, which would have a higher value of T_c and thus is analogous to a deeper quench.

4. Conclusions

From the data we can distinguish several regimes:

1. First an early stage in which microscopy of simultaneously phase separating and gelling samples indicate that the initial mechanism of phase separation is that of spinodal decomposition. Small angle light scattering data also shows many universal characteristics of classical spinodal decomposition [19]. For all samples at early times the light scattering results are similar to those found for samples quenched well above the gelation temperature. Although the stationary peak predicted by the linear Cahn–Hilliard theory was not seen, we clearly identify a critical wavevector and a peak wavevector, the ratio of the two being close to the predicted value of $1\sqrt{2}$. The temperature dependence of the critical and peak wavevectors also corresponded well with that expected and the predicted values of the peak positions, determined from estimates of the radius of gyration of the polymers, were in good agreement with those measured. The initial increase in the intensity at each wavevector was found to be exponential in time, again, as predicted by the Cahn–Hilliard theory. Thus at early times the phase separation appears predominantly classical, even though the gelation process develops on a similar timescale.
2. At intermediate times the effect of the gelation is seen (note, this does not mean that gelation is not present earlier, merely that its effects are not seen until the intermediate stage). The gelation was found to affect phase separation by causing a time dependence of the peak position, such that, under certain conditions, the peak moved back to larger wavevectors. This is believed to be due to the conformational change and association of the gelatin helices on network formation. This effectively deepens the temperature quench such that the fastest growth occurs at larger wavevectors.
3. During the later stages the effect of the gelation is much more pronounced, due to the differing extents of gelation in the various samples affecting the coarsening mechanisms. Very different structures are seen — interconnected, spinodal-like structures at low temperatures and droplet structures with differing evolution (aggregation, coalescence) depending on the degree of gelation at high temperatures.

Thus the gelation does not affect the phase separation mechanism as much as was initially thought. Spinodal decomposition still occurs, but the effect of gelation is observed more strikingly in the final structures.

These findings present a consistent set of experimental

data waiting for an adequate theoretical description, an extension of phase-equilibria analysis to include the kinetic effects of elastic network formation.

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