Phase separation in the poly(γ-benzyl-α, L-glutamate)/benzyl alcohol system and its role in gelation

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Gelation in systems containing poly(γ-benzyl-α,L-glutamate) (PBLG) is well known, but two different mechanisms have been proposed to explain it. One attributes the gelation to crystallization, the other to phase separation via spinodal decomposition. In this paper we report a study of PBLG/benzyl alcohol (BA) gels. We have examined the possible types of phase separation that may occur when samples are held above the gel melting point. Three different types of phase separation may occur, corresponding to phase separation within the cap, the chimney and the broad biphasic parts of the Flory phase diagram. When these various phases cool, crystals (revealed by a melting endotherm in the differential scanning calorimetry) are formed in each of them, and the crystal melting point can be identified with the melting point of the unseparated gel. We are led to a picture of gelation in which phase separation occurs followed by crystallization, this picture bringing together the two existing models.

(Keywords: poly(γ-benzyl-α,L-glutamate); phase separation; gelation; crystallization; liquid crystal; Flory phase diagram)

INTRODUCTION

The polypeptide poly(γ-benzyl-α,L-glutamate) (PBLG) was first synthesized in the 1930s[1] by Courtaulds as a potential replacement for some of the natural fibres used in clothing. Although this scheme never came to fruition, PBLG was found to be a suitable model for rod-like polymers and has been used extensively in this capacity ever since.

PBLG is a polymer consisting of peptide monomers, each one of which has the same side-group $-(CH_2)_2-(C{=}O)-O-CH_2-C_6H_5$. It is thus a particularly simple analogue protein. In solution, it is possible for a hydrogen bond to form between the oxygen of a carbonyl group and the hydrogen of the amide group four groups further down the chain. The effect on the molecule's conformation is that the chain is wound into a coil which is characterized by the designation '3.6_{13}'. (This means that there are 3.6 residues (i.e. monomers) to one turn of the coil and that there are 13 atoms from one end of a hydrogen bond to the other as measured along the chain.) This type of coil is called the 'α-helix'. PBLG can only adopt the α-helix conformation in solvents which do not interfere with the hydrogen bonding and these solvents are accordingly called 'helicoidal solvents'. In these solvents the molecule behaves as a rather rigid rod because the hydrogen bond prevents chain flexibility.

The first theoretical work on solutions of rod-like polymers was done by Onsager[2] in 1949. He was motivated by the results from experiments on such anisotropic molecules as tobacco mosaic virus. He concluded that it was possible for rod-shaped particles to exist in anisotropic solutions at relatively low concentrations. Simply, the argument is that above a certain concentration it is better for the solution to arrange the rods in an ordered fashion rather than let them adopt any (random) configuration. The inevitable loss in entropy is paid for by the reduction in the inter-rod electrostatic interactions. This view was later confirmed by Isihara[3]. However, it was Flory[4], in 1956, who first suggested a phase diagram for solutions of rods. This is represented in *Figure 1*. At low concentrations, the solution is isotropic (I). At high concentrations, the solution is liquid crystalline (LC) as suggested by Onsager, i.e. it is anisotropic. Between these two one-phase regions, there is a biphasic region where the isotropic and liquid crystalline phases can co-exist. (The rules governing phase diagrams demand that such a region must exist, and since the isotropic and anisotropic phases must always be distinct, this biphasic region must be present at all temperatures below the melting point of the solid.) Flory's great contribution was to determine the temperature dependence of the phase boundaries. As can be seen from *Figure 1*, this can conveniently be split into two parts. At low temperatures, the onset of liquid crystallinity occurs at relatively low concentrations but does not become total until the volume fraction of polymer (v_2) is almost unity. However, at high temperatures, the onset occurs at a higher concentration and the transition to complete liquid crystallinity then occurs over a very small concentration range. These two biphasic regions are now often called the 'broad biphasic' and the 'chimney' regions respectively.

It is instructive to compare Flory's phase diagram with the more commonly encountered 'immiscibility dome' of simpler systems. The two phases which can co-exist in this case are both isotropic and differ only in concentration. As the tie-lines in the dome get shorter (with

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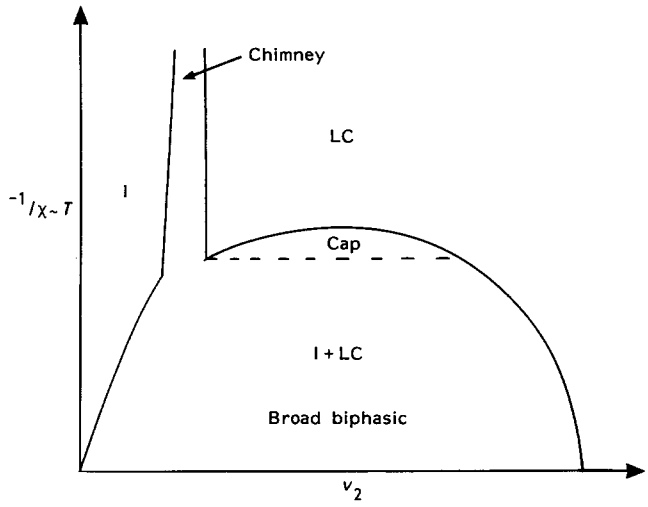

Figure 1 Phase diagram for a rigid-rod polymer, after Flory[4], showing the possibility of two coexisting liquid crystalline phases in the cap

changing temperature), the concentrations become less dissimilar until at the apex of the dome (the critical point), where the tie-line has zero length, the concentrations are identical. As noted above, this cannot occur for equilibrium between the isotropic and liquid crystalline phases in the liquid crystalline system. (Papkov[5] notes that the chimney's width can shrink to zero at $v_2 = 1$. This is equivalent to saying that when no solvent is present there is a phase transition from the (ordered) solid to the (isotropic) melt. This appears on the phase diagram as the chimney bending over and touching the line $v_2 = 1$ at one point.) A feature resembling the classical immiscibility dome can, however, be identified on *Figure 1* and this is the 'cap' on the high concentration side of the chimney. Flory himself tentatively observed this in his original paper and pointed out that if the cap does exist then it should manifest itself as two co-existing liquid crystalline phases. In this paper the region of biphasic equilibrium in which two anisotropic phases coexist will simply be referred to as the cap, and the biphasic region in which an isotropic phase coexists with an anisotropic phase will be referred to as the biphasic region, which will therefore encompass both the chimney and the broad biphasic.

The experimental evidence to support the existence of the cap has been slow in forthcoming. Russo and Miller[6] (working with the PBLG/dimethyl formamide (DMF) system) inferred evidence for the existence of the cap from observing the spacing of the 'fingerprints' arising from PBLG's cholesteric structure[7]. They noted that as a sample was cooled the spacing decreased slowly but then began to increase very quickly. Their interpretation was that initially their sample was in the one-phase region above the cap. On cooling, it moved towards the phase boundary and eventually crossed into the cap. Here a phase separation occurred and the spacing now seen was that of one of the phases (the fingerprints of the other not being visible at the magnification used). Hill and Donald[8,9], however, observed a phase separation more directly. Above ≈ 80°C they were actually able to identify two distinct anisotropic phases co-existing in the hot stage of the optical microscope. Their reservations were that since the entire experiment was done in the hot stage (i.e. both separation and observation), either the small size of the sample or the short duration of each experiment might have meant that equilibrium was not reached. In addition the constraints of the sample's geometry (namely its being in a dimpled slide) might have meant that surface effects were important. In this work, coexisting isotropic and anisotropic phases were naturally also seen.

However, for certain rod and solvent systems there is a facet of their behaviour which cannot be directly explained in terms of the Flory phase diagram. At low temperatures (i.e. below some gelation temperature, T_m) the system may 'gel', i.e. the system sets into a rigid, self-supporting structure which incorporates a certain amount of liquid. Miller[10] has reviewed some systems which do and do not gel. Precisely how a material like PBLG gels has not so far been unequivocally identified. The ideas presented in the literature, however, can be divided into two basic camps. The first is that the junction zones in the gel's three-dimensional structure are crystalline in origin. This may mean that the zones comprise polymer crystallites, but also encompasses the idea of 'crystallosolvates', i.e. crystalline structures that themselves' include some solvent. Proponents of this idea include Ginzburg *et al.*[11] and Sasaki and co-workers[12]. The second possibility is that the gel arises from spinodal decomposition in the original solute–solvent system. This idea is favoured by Miller and co-workers[13,14], who have found evidence for it from light-scattering, although it is not clear how they envisage the gel being stabilized after the decomposition.

Hill and Donald have looked at gels of PBLG in benzyl alcohol (BA) with differential scanning calorimetry (d.s.c.) and the Saunders and Ward technique for measuring rigidity modulus[15] and with polarized light microscopy[9]. Their main conclusion was that the gel properties of the PBLG/BA system are not related to its liquid crystalline nature (as featured in Flory's phase diagram) in any simple manner. For a range of concentrations, d.s.c. and modulus measurements showed a transition occurring at 50–60°C, which was identified as the gel melting point. Phase changes observed in the polarizing microscope took place at temperatures significantly different from this, the only change in structure observed at the gel melting point being a rounding of the sharp corners in cut slices of the gel, corresponding to the onset of flow. The phase changes seen optically, were consistent with the Flory phase diagram and, as mentioned above, they also saw microstructures strongly suggestive of two anisotropic phases coexisting.

Gelation is of course a phenomenon that occurs in a wealth of polymer systems; initially mechanisms for gelation tended to invoke the existence of crystals or specific interactions between polymer chains (e.g. helical junction zones in some biopolymers). Recently, gelation has been related to phase separation by Berghmans and others[16–18], who have proposed an interesting mechanism to explain the gelation observed in atactic polystyrene (*a*-PS), a polymer which cannot crystallize. Consider the phase diagram for a simple two-component system shown in *Figure 2*. Superimposed on it is the glass transition temperature as a function of concentration. As the temperature of the solution falls (to T_1, say), phase separation begins to occur and the solution separates into two phases with compositions c_1 and C_1. However, the more concentrated of these two phases eventually falls foul of the glass transition temperature. At T_2 (for

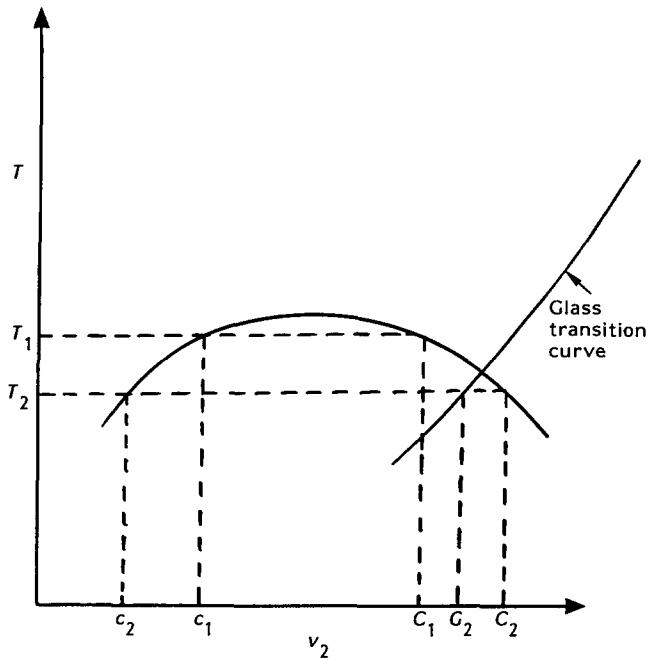

Figure 2 Schematic representation of the phase behaviour of a two-component system with an immiscibility dome intersected by the glass transition curve

instance), the solution would like to reach concentrations c_2 and C_2 to minimize its free energy. However, the position (T_2, C_2) is within the glassy zone and the high concentration phase is frozen at concentration G_2 ($<C_2$) before it can reach C_2. The result is that there are small glassy regions in the solution conferring rigidity on the system, mechanical connectivity is set up and it is deemed to have gelled. The theoretical argument for this is developed in more detail by Frank and Keller[19], who also show that in practice free energy considerations prevent c_2 quite being reached.

Before describing our experiments, we first emphasize that this paper does not purport to map out the PBLG/BA phase diagram. It is rather aimed at understanding the nature of gelation in a rigid rod liquid crystalline system. Mapping out the phase diagram is time-consuming at best (evidence the time Miller and co-workers[10,20,21] spent on exploring the PBLG/DMF phase diagram) and in addition can be severely complicated by the existence of trace amounts of non-solvent. To map out the diagram with total reliability requires first preparing the gels with very pure solvent and then preventing the gels becoming contaminated. As will be seen, our results (in common with those of Miller and co-workers[22]) suggest that trace amounts of water can be very important and, therefore, PBLG/BA gels ought to be handled in a dry atmosphere (such as N_2).

EXPERIMENTAL

BA was chosen as the solvent for this set of experiments. It is one of the two common solvents used for making PBLG gels and its principal advantage over its main competitor, dimethyl formamide (DMF) is that it forms gels at room temperature rather than below. BA, described as 99% pure, was purchased from the Aldrich Chemical Company. In general, it was used as supplied. However, a number of gels were made up with 'dry solvent'. Initially this meant that batches of the 99% pure BA were distilled under reduced pressure. (The boiling point of BA at room temperature is 205°C. The distillation apparatus was connected to a rotary pump and the BA found to boil at ≈95°C. This corresponds to a pressure of ≈10 mm Hg(1.3 kPa).) Later, commercially dried BA (also from Aldrich), quoted as containing <0.005% water and supplied under nitrogen, was used. Some BA was also deliberately contaminated with water to study the effect of changing the quality of the solvent.

The PBLG was supplied by Sigma Chemical Laboratory. The samples used in this paper had viscosity average molecular weights (derived by the suppliers) of 248 000, 260 000 and 345 000, most experiments being carried out on the lowest molecular weight. The concentrations of the gels were 15% volume/volume (v/v) for the two lower molecular weights and 2, 5, 8 and 10% v/v for the highest.

Details of the basic sample preparation have been given by Hill and Donald[15]. Most of the samples used in the experiments described here were so viscous that a magnetic stirrer was ineffective in mixing solvent and solute. Instead, PBLG and BA were put in a bottle together and the bottle then held in a hot oil bath for several hours and periodically turned. Immersion in an oil bath rather than merely standing the bottle on a hot-plate has the advantage that the sides of the bottle are heated as well as the bottom. Small amounts were then removed from the bottles as required. No sample was used until it had been aged for at least two weeks. The bottles used had ground glass necks, and as a further protection against contamination or solvent loss, the neck was wrapped with plastic film.

The initial experiments described in this paper were aimed at reproducing Hill and Donald's phase separation[9] in a bulk sample rather than just within the constraints of a dimpled slide. This necessitated using an oil bath. Samples were held in the bath by putting them into small-bore test tubes (capped with ground-glass stoppers) and then suspending the tubes in the bath from a specially designed support. Phase separations were searched for with the naked eye.

The methods used to examine the resulting phases were differential scanning calorimetry (d.s.c.), polarized optical microscopy and X-ray scattering. The differential scanning calorimeter was a Mettler DSC 30 controlled by a Mettler TA 3000 controller. The optical microscope was a Carl Zeiss Jenapol fitted with an Olympus OM4 camera. The samples were heated in a Linkam Scientific Instruments hot stage (model TH600) controlled by a Stanton Redcroft controller. The X-ray scattering was done in a powder camera using the K_α radiation of copper which has a wavelength, $\lambda = 1.54$ Å.

RESULTS

The work described here was started as an attempt to reproduce Hill and Donald's[9] results in the bulk, but a greater wealth of behaviour was observed, with three distinct phase separations being found in all. These will be referred to as A, B and C. Each of these three types manifested itself as one phase on top of another, separated by a visible boundary. Each pair of phases was examined with suitable techniques and the results are described below.

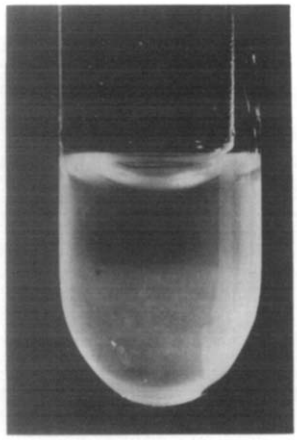

Figure 3 Bulk phase separation A at room temperature. N.B. the appearance is very similar at 80°C, but the quality of the photograph is reduced due to the need to photograph through hot oil

Figure 3 shows the first type of phase separation, designated A (this occurred in the 15% samples of 248 000 molecular weight). Both phases are viscous and at ≈80°C, where the separation occurs, both transmit light in the sense that neither are opaque (*Figure 3*). However, while the upper phase is translucent, the lower one might best be termed 'frosted'. When such a sample is cooled to room temperature both phases darken. The boundary between the phases is not sharp (cf. separations B and C below) and takes, typically, several days to appear. This phase separation has already been described elsewhere[23], but will be described briefly here since understanding of this phase separation is necessary to interpret the other two separations. Both phases in A are anisotropic, as can be seen by looking at them in the polarizing microscope. Typical textures are shown in *Figure 4*. It is notable that the lower phase shows 'fingerprints'. The X-ray spectrum yields little of interest other than a diffuse halo from the solvent. The d.s.c. for both top and bottom phases shows a melting endotherm at ≈60°C, plus a less distinct transition at 45–50°C (*Figure 5*).

The remaining two separations each consisted of an isotropic phase resting on an anisotropic phase. The boundary between the two phases in each case looked like a standard liquid meniscus in that it was well defined and rose where it met the walls of the tube. This should be compared with the phase boundary in A (*Figure 3*). Phase separation B was achieved for 5, 8 and 10% samples of 345 000 molecular weight at 110°C. When hot, the upper phase is completely transparent and very fluid. The lower phase is translucent and very viscous. However, at elevated temperature the lower phase will not support a ball bearing and so can be classed as a viscous liquid rather than as a solid. The lower phase does not alter in appearance on air cooling other than becoming slightly opaque, but it does now gel. The upper phase turns white on cooling and has the consistency of a very weak gel. On a more prosaic level, it might be said that this phase has the consistency of warm butter. If the sample is cooled very slowly (typically a few degrees a day), the upper phase remains a transparent liquid but now containing small white particles.

When examined at room temperature beneath the crossed polars of the optical microscope, the upper phase of B shows needle-shaped particles in an isotropic liquid.

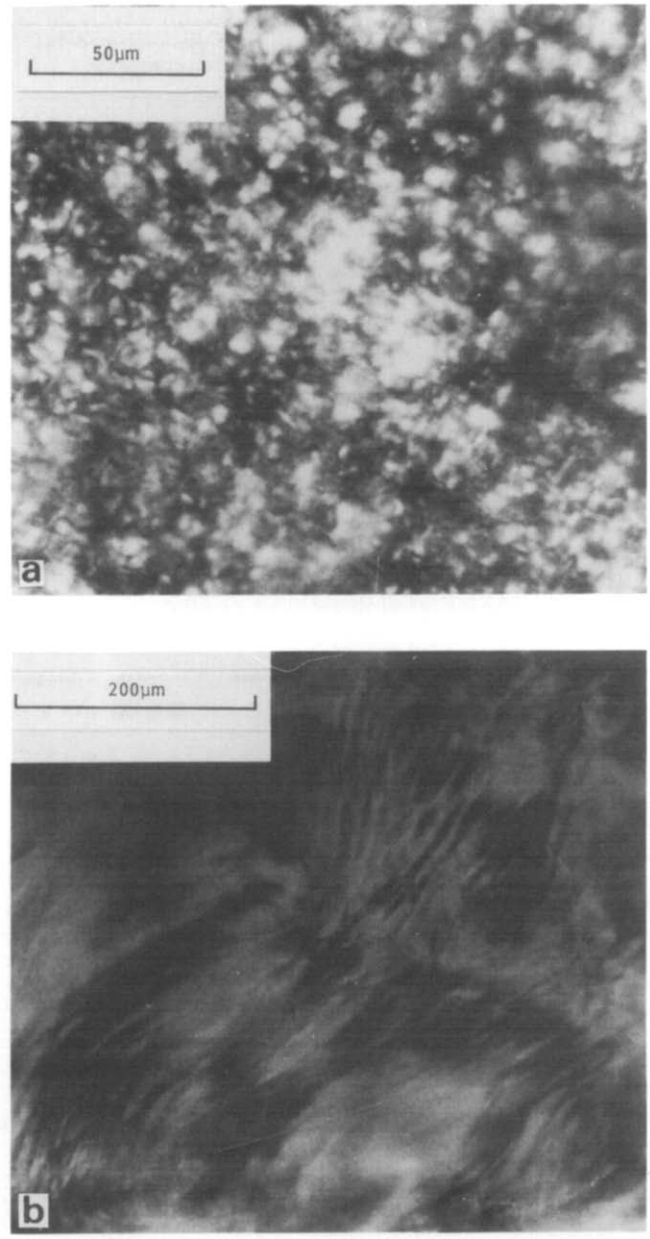

Figure 4 Optical micrographs under crossed polars of phase separation A: (a) top phase; (b) bottom phase

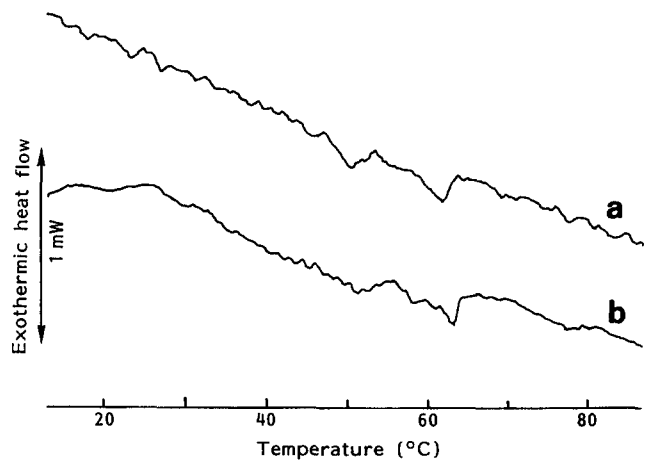

Figure 5 D.s.c. traces of phase separation A: (a) top phase; (b) bottom phase

(See *Figure 6a*, which is a photograph taken under crossed polars showing a top phase that has been taken out of the oil bath and left to cool in the atmosphere.) These particles disappear when the sample is heated to 60°C, which is the temperature at which a large endotherm is seen in the d.s.c. trace (*Figure 7a*). (Note the appearance of a weak second transition at ≈50°C as with phase separation A.) The X-ray pattern of the top phase at room temperature shows several sharp rings (*Figure 8a*). The strongest of these are at 4.0 and 3.6 Å* with weaker rings at 3.0, 2.4 and 2.2 Å. The bottom phase is anisotropic (*Figure 6b*) and similar in appearance to the top phase of separation A. It also has a dip in its d.s.c. trace at ≈60°C (*Figure 7b*). It shows weak rings in its X-ray pattern (*Figure 8b*). The strongest rings are at 4.1 and 3.6 Å, as for the top phase.

Phase separation C (*Figure 9*) occurred at 80°C for a 15% sample of 248 000 molecular weight which had been deliberately contaminated with 8% water. This phase separation also shows a well defined meniscus with a top

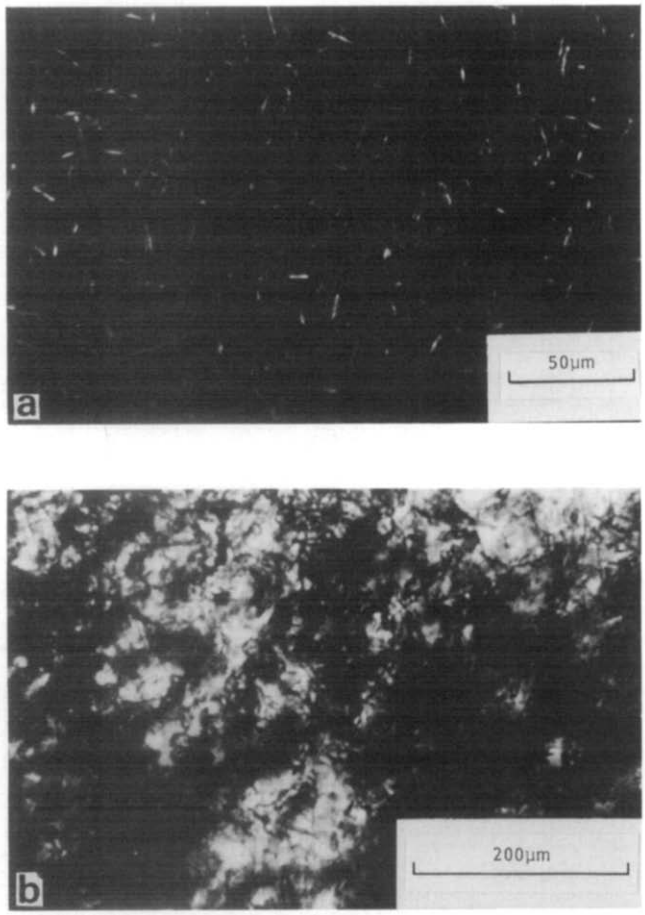

Figure 6 Optical micrographs under crossed polars of phase separation B: (a) top phase; (b) bottom phase

* 1 Å = 10^{-1} nm

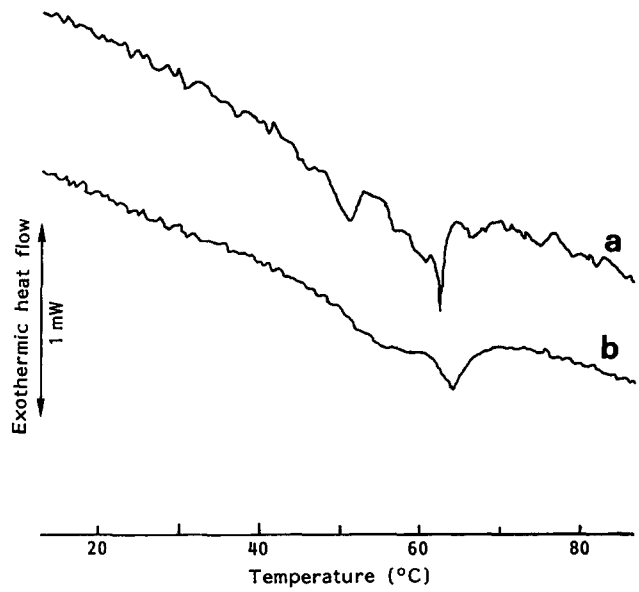

Figure 7 D.s.c. traces of phase separation B: (a) top phase; (b) bottom phase

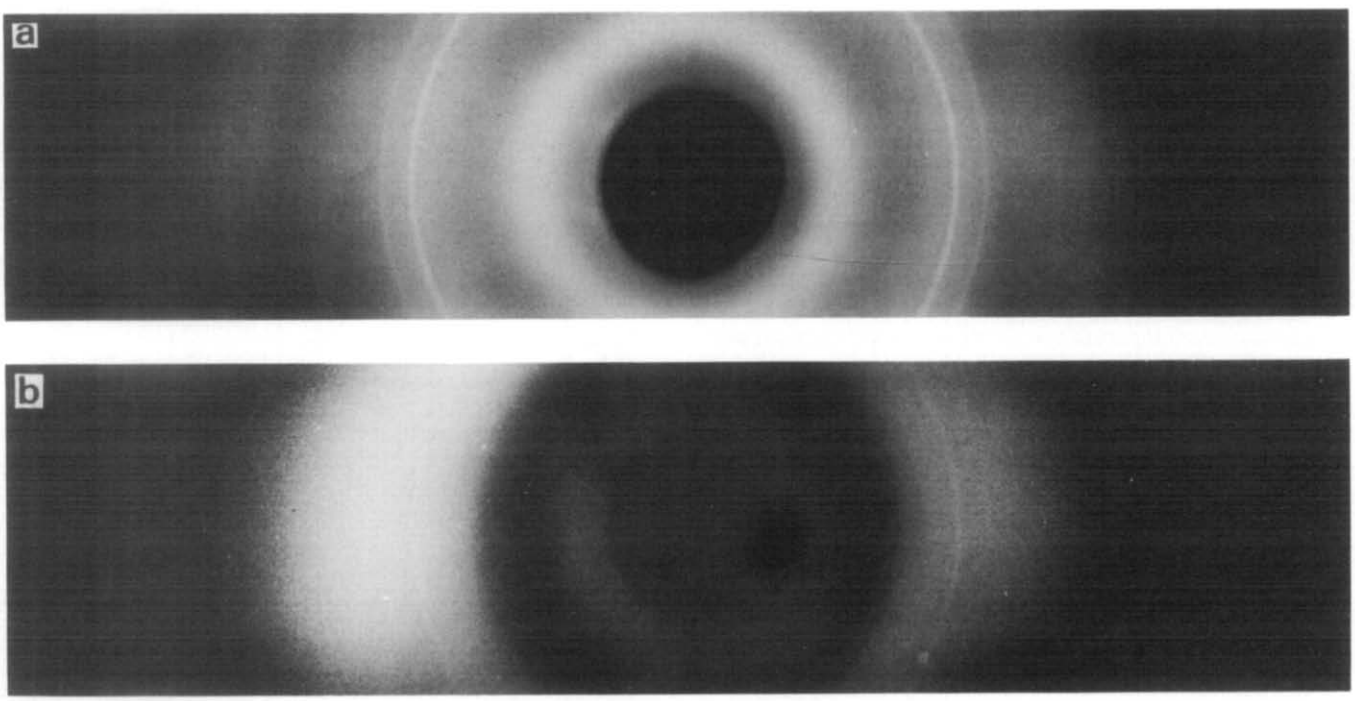

Figure 8 X-ray diffraction patterns of phase separation B: (a) top phase; (b) bottom phase

isotropic phase which appears virtually identical to that seen in B. The lower (anisotropic) phase however is 'frosted' (seen clearly in *Figure 9b*), like the lower phase in A. When cooled (*Figure 9b*) the top phase turns white (again suggesting the formation of some entity having the same size as the wavelength of light). However, it does not appear to 'set' in the same way as the top phase of B. It is notable, for instance, that the viscosity of the top phase of B was such that it had to be put into d.s.c. crucibles using a spatula, whereas the top phase from C could simply be poured in.

Figure 10a shows the appearance of crystals in the polarizing microscope when the top phase of C was air-cooled. After melting these crystals and then cooling the sample at $\leqslant 2°C\ min^{-1}$, a much larger number of crystals form (*Figure 10b*). X-ray diffraction of the top phase of C shows again the two sharp rings at the same spacing as in *Figure 8* (*Figure 11*). The only change in the lower phase on cooling is that it transmits a little less light. Distinct fingerprints with a spacing of typically $\approx 10\ \mu m$ (*Figure 10c*) are seen in the lower phase of C. This is in contrast to the upper phase of A and the lower phase of B which are clear, not 'frosted' and do not show fingerprints. The d.s.c. traces for both phases of C (*Figure 12*) once again show the dip at $\approx 60°C$ with a less prominent transition at $\approx 50°C$.

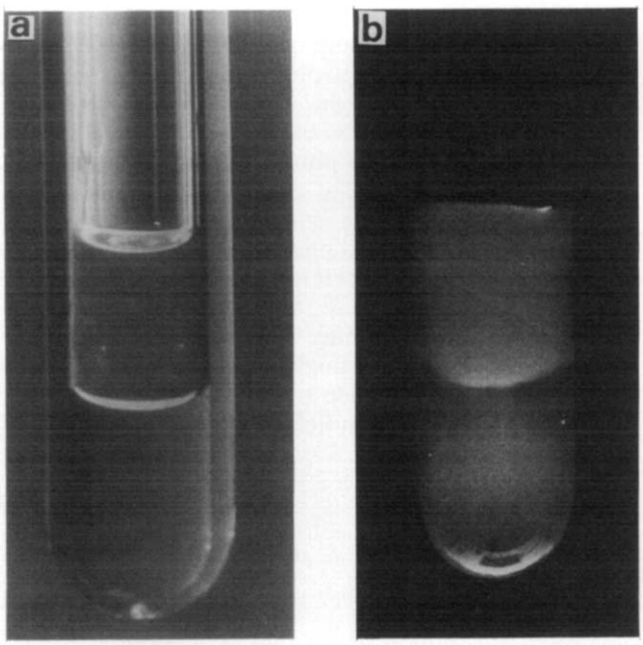

Figure 9 Bulk phase separation C at (a) 80°C and (b) room temperature. The top phase is transparent when hot, (a), but opaque when cold, (b)

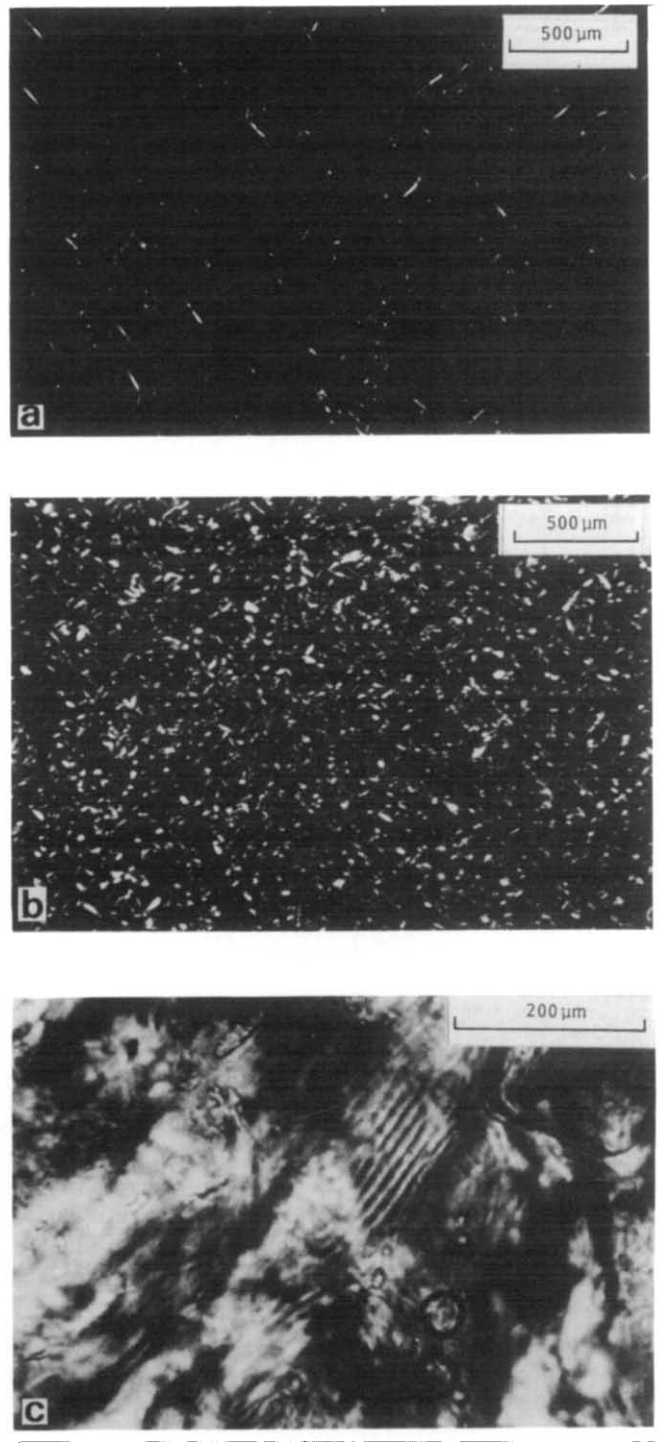

Figure 10 Optical micrographs under crossed polars of phase separation C: (a) top phase after air cooling; (b) top phase after cooling at $\leqslant 2°C\ min^{-1}$; (c) bottom phase (air cooled)

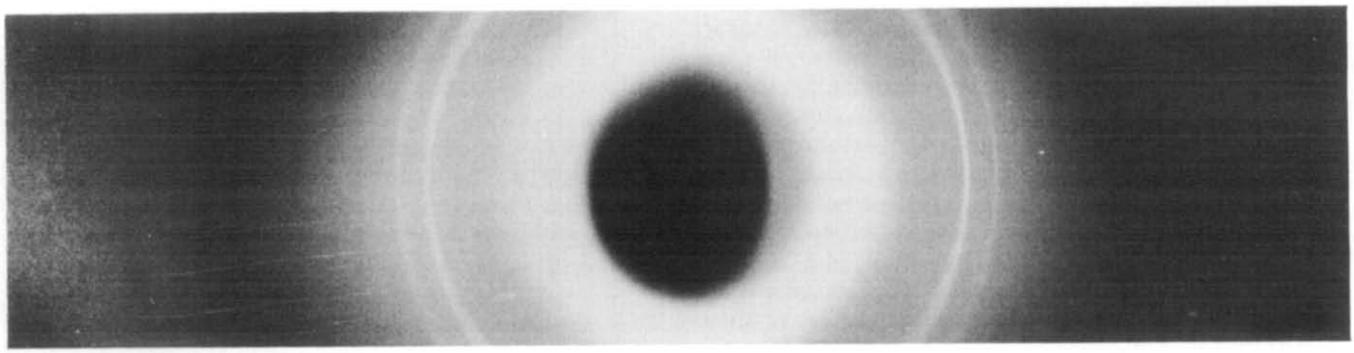

Figure 11 X-ray diffraction patterns of phase separation C, top phase

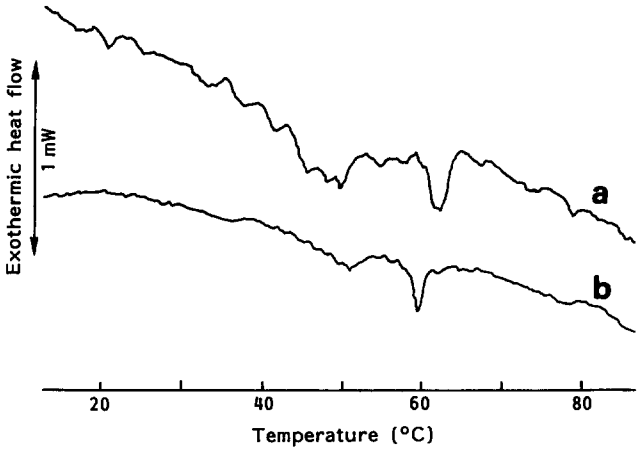

Figure 12 D.s.c. traces of phase separation C: (a) top phase; (b) bottom phase

The X-ray spectrum of the anisotropic phases (apart from the lower phase of B to a certain extent) showed no features other than the diffuse halo attributed to the solvent. This was also true of the original gels before phase separation. In all these phase separations the anisotropic phases suffer discoloration (to a shade of brown) if they are left at an elevated temperature for more than a few weeks. This has been presumed to be due to oxidation. It also occurs to gels which do not phase separate.

DISCUSSION

The phase separations described above all take at least a day to occur. This probably has two causes. First, the anisotropic phases are very viscous. Secondly, density differences between two compositions of PBLG and BA are unlikely to be particularly great. The phase separation A seems to be the separation into two liquid crystalline phases[23] first speculated upon by Flory when he was discussing the cap in his original paper[4]. In this case, the upper (clear) phase is the less dense one and it has the composition of the low concentration end of a tie-line passing through the composition of the original gel. The denser, lower phase (which has the frosted appearance) has the composition of the other end of the tie-line. The density differences between these two phases cannot be greater than that corresponding to the difference between the two ends of the cap, and this is consistent with the observation that A is very slow in appearing.

The two other phase separations involve an isotropic phase and, therefore, must be occurring in some part of the biphasic which has a tie-line reaching to the isotropic phase of *Figure 1*. As noted above, the lower phase of B does not show fingerprints and, when hot, is clear, whereas the lower phase of C does show fingerprints and is frosted. As discussed by Hill and Donald[9], the periodicity of fingerprinting in the highly concentrated phase is expected to be small, whereas in the less concentrated phase the observations of Sasaki *et al.*[12] suggest that fingerprinting may not be observed at all. These facts suggest that C occurs in the broad biphasic, because tie-lines in this region reach out to very concentrated LC phases, and that B occurs in the chimney, since the LC edge of the chimney is only at a relatively low concentration. The observation that the top phase of C is more dilute (less viscous) than that of B is consistent with this picture. A diagram of this interpretation of separations A, B and C is shown in *Figure 13*. Another fact which supports this picture is the observation of phase separation B at relatively low concentrations (albeit of the highest molecular weight) but not for the lowest concentration studied of 2%. It may be anticipated that if B does correspond to the chimney region, it should be achievable over a range of temperatures. This has not been attempted here.

Before further discussion of these phase separations, their suppression by using dried solvent and their relation to the gelation phenomena, it is perhaps helpful first to examine the literature for other reports of such phenomena. Ginzburg *et al.*[11], working on the PBLG/DMF system, find a phase separation for PBLG with molecular weight of 50 000 but not for PBLG with a molecular weight of 200 000. The phase separation was achieved at elevated temperature, and was into an isotropic and an anisotropic phase. That the high molecular weight sample did not phase separate was attributed to differences in the phase diagram for different molecular weights. As the molecular weight becomes smaller, the features on the phase diagram move down and to the right and, in particular, the chimney broadens. This broadening means that the two phases resulting from a separation occurring within the chimney differ more in concentration, and hence in density, than is the case for higher molecular weights and thus a phase separation can happen more easily.

Sasaki *et al.*[12] found a phase separation occurring when they made up a blend of two PBLG samples (of viscosity molecular weights 99 000 and 347 000) in BA. There are two important points to notice in this case. First, Sasaki *et al.* say that the separation took place over about a week. They blame this on the small density difference between the two phases. Secondly, their upper phase was isotropic and their lower one anisotropic. This suggests they have a phase separation which is taking place in the chimney. Since they have a deliberately polydisperse system, it might be expected that the chimney should be very wide and, therefore, the argument of Ginzburg *et al.* (as outlined above) can be seen to

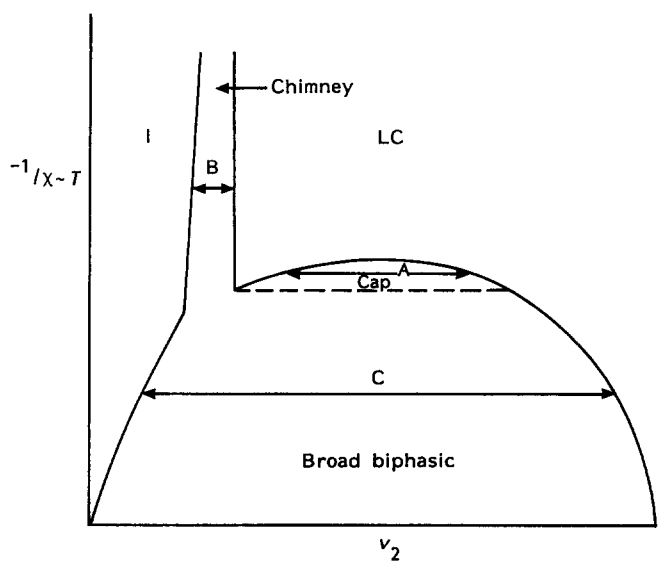

Figure 13 Schematic representation of the Flory phase diagram (see *Figure 1*) showing the different types of phase separation achieved

apply. Miller *et al.*[13,21] note in passing that they observed a meniscus form in the PCBL*/DMF system. Although they give no further details, in the light of the results discussed above, the meniscus suggests that this phase separation was also one occurring in the biphasic rather than in the cap.

Most recently, phase separation in the PBLG/dioxane/water and PBLG/benzene systems has been discussed[24]. This work specifically concentrated on compositions to the left of the biphasic chimney, but still has some relevance to the work presented here. In particular, this work considered the relationship between phase separation and gelation. The suggestion is that gelation occurs in two stages due to a combination of effects caused by crystallization (or, as they put it, the formation of solid PBLG or a PBLG-rich solid solution) and phase separation. This model, like the earlier suggestions of Miller *et al.*[14], seems to beg the question of what stabilizes the gel if phase separation has occurred but there are no crystals present. Jackson and Shaw[24] suggest that phase separation is occurring by nucleation and growth rather than spinodal decomposition, over the concentration range they consider.

The effects of water must now be considered before moving on to the way the results of the work presented here may be interpreted. For this it is possible to draw on earlier results to clarify the situation. Russo and Miller[22] have shown that the *effective* result of having a non-solvent such as water in the system is that the features on the phase diagram move to higher temperatures. (Three-component systems should, of course, be represented on a ternary phase diagram. However, it is very convenient if a modified two-component phase diagram can be used instead, a 'pseudobinary'). Thus temperatures and concentrations which formerly fell in the one-phase region (where there is no phase separation) now lie within the two-phase region where phase separation can occur. Similarly, Jackson and Shaw observed the pronounced effect of water as a non-solvent shifting the phase boundaries upwards, and thus increasing the temperature range over which two phases (in their case an isotropic and an LC phase) coexist. This gives a clue to the results reported here.

All the gels melt at $\approx 60°C$. When the solvent is dry, the two-phase/one-phase boundary must occur at a relatively low temperature. In particular, it may occur very close to (or even below) the gel melting temperature T_m. This means there is very little, if any, (v_2, T) space in which the gel can undergo bulk phase separation. If the temperature at which phase separation is attempted is too low (i.e. below T_m) then the gel is solid; if too high a temperature is used then the sample is in the one-phase region. If the position of the coexistence curve actually lies below T_m, then the system cannot undergo bulk phase separation: the crystallization would pin all, or nearly all the chains, in position inhibiting phase separation at a lower temperature. However, this is not to say that there could not be a gel between the binodal line and T_m, a single phase gel. This seems to be the case for those gels made up with carefully dried BA. Although these were immersed in the oil bath at a range of temperatures, none of them showed any sign of a phase separation. Adding water has the effect of lifting the phase boundary and producing more (v_2, T) space where phase separation can occur. In the case of the sample which was deliberately contaminated with water (separation C), the shift in phase boundary is so great that now 80°C lies within the broad biphasic, as opposed to the cap for the uncontaminated sample (separation A).

X-ray analysis of the isotropic top phase shows that crystals can form in the PBLG/BA system. Their appearance may depend on the cooling rate. Fast cooling gives few sites for nucleation and a low crystal density. This appears to lead to the possibility of chain continuity between crystals to give a gel. Slow cooling, on the other hand, leads to a high crystal density, but chains presumably are confined to essentially one crystal as the sample consists of a crystal suspension rather than a gel. Both the unseparated gel and the anisotropic phases give hardly discernible X-ray rings, suggesting their crystallinity is very poor. On the other hand, the isotropic and anisotropic phases as well as the unseparated gel all show the same melting behaviour, not only in the simple everyday sense but also as seen in the d.s.c. The seemingly ever present dip in the d.s.c. trace at $\approx 60°C$ suggests that there is some crystallinity present in the original gel and in the anisotropic phases but that it does not occur as large well formed crystals which can be detected by X-rays. This may be because the latter phases are more concentrated than the isotropic phase and the molecules cannot readily move independently of one another to form highly regular crystals.

The picture that emerges from this study is that the Flory phase diagram can be used to describe the different phases that form at different temperatures, and that crystals (albeit highly imperfect in some cases) may be present in both isotropic and anisotropic phases. The key question is, therefore, what role do these various phase separations play in forming the gel. As noted in the introduction, the literature contains two basic viewpoints for PBLG-based gels: either that crystals impart connectivity to the gel, or that phase separation via spinodal decomposition occurs. We believe it is possible to reconcile these two viewpoints using essentially the scheme of Berghmans (first described by Arnauts and Berghmans[16]), as subsequently refined by Frank and Keller[19] and Hikmet *et al.*[18]. A similar idea appears to underlie the recent work of Jackson and Shaw[24] (but in their case phase separation seems to be occurring by a nucleation and growth mechanism), although they have not formulated the picture in the same terms.

The mechanism of Berghmans and others for explaining the gelation of a-PS has been described in the Introduction. Stated baldly, the current picture of a-PS gelation is that phase separation occurs which is then arrested by vitrification. Given connectivity between the phases, this leads to a rigid gel structure. For i-PS the picture is that the phase separation is rather arrested by crystallization in the polymer rich phase and it is the crystals that rigidify the structure[25]. As suggested by Hikmet *et al.*[18] this kind of mechanism based on phase separation may have a far broader application than simply to PS, although this should not be taken to mean that phase separation is invariably involved in gelation. For instance, a similar mechanism[26] has been invoked in the gelation of amylose. In this case, although arrived at quite independently without reference to the Berghmans scheme, the suggestion is again that there is a phase separation followed by a slow crystallization in the

* PCBL is poly(ε-carbobenzoxy-α,L-lysine), which is similar to PBLG except that the side-group is $-(CH_2)_4-NH-(C{=}O)-O-CH_2-C_6H_5$

polymer rich phase. Similar ideas relating phase separation and crystallization have been put forward by Kawanishi *et al.*[27] for other polymers.

For the PBLG/BA system (at least at the concentrations we have studied) it seems that the correct picture is one in which phase separation occurs, followed by crystallization in both phases, and it is these two processes together which confer rigidity on the gel. This rationalizes the observation that the gel melting point coincides with the crystal melting point (as shown directly by observation of the melting of the needle shaped crystals in the top phase of separation B[28]), but also allows for the fibrillar microstructure attributed to spinodal decomposition observed by Miller *et al.*[13,14,29] (assuming that the PBLG/DMF system behaves in essentially the same manner). However, there is no necessity in this picture for the phase separation to proceed via spinodal decomposition, as long as the phase separation leads to a structure with sufficient connectivity between the phases to lead to a network. The only essential difference between this rigid rod polymer and more flexible polymer systems therefore resides in the greater complexity of the appropriate phase diagram, with a variety of different phase separations being possible.

This implies that, even for a fixed composition, different gels may be prepared by altering either the temperature at which the sample is prepared, moving, for instance, from the cap to the broad biphasic regions, or the cooling rate, which will change the size and connectivity of the crystals formed. How subtle the differences between the gels prepared in these various ways may be is not clear, but further work on their detailed rheological response should cast light on this point. However, what is clear is that it is not valid to use rheological data straightforwardly to identify the phase boundaries of the Flory diagram, as was done by Murthy and Muthukumar[30]. Furthermore, since the details of the gel can depend on cooling rate, it is clear that these gels cannot strictly be regarded as equilibrium structures, a point that has already been raised by Aubert[25] and Frank and Keller[19].

Although this study has been able to identify both the importance of phase separation (including conclusive evidence for the existence of the cap) and the presence of crystals, it leaves many questions still unresolved, particularly relating to the details of the crystals that form. The earlier work of Donald and co-workers[15,31] suggested that there might be two types of crystals present, one of which was capable of forming much faster than the other. It is clear that the crystals that do form are in general imperfect, rendering their detail largely obscure in X-ray diffraction patterns. Only those which grow in the dilute isotropic solution from phase separation B show several well defined rings. The lack of information in the diffraction patterns from other phases, as well as the unseparated gel, we attribute to the imperfection of crystals that form in viscous, concentrated phases, even after long times (although the time course of the development of the rings has not been explicitly followed). That crystals are nevertheless present in all cases we infer from the d.s.c. data.

The appearance of a double transition in the melting of the gel is puzzling. Jackson and Shaw[24] discuss the double transition observed by them for the dioxane/water system at some length. They attribute it to a melting first of crystals and then of the LC phase. Berghmans[17] and Morris and co-workers[32] suggest that two stages of molecular aggregation may occur: first binary associations that could in some systems correspond to helix formation, and then aggregation of these associates. This would likewise give rise to a two-stage melting process. For the PBLG/BA system, since we have shown that crystals can form in any of the possible phases but that they are likely to be small and imperfect in all but a dilute isotropic phase, it is possible that different crystal populations in the different phases melt at different temperatures. This picture could explain why low concentrations do not show two melting peaks[15].

The results show that if the (dilute) isotropic phase in separation B is cooled quickly, then a weak gel is formed. This suggests that phase separation may not always be necessary, and 'traditional' gelation via crystallization may occur when in a one phase region of the phase diagram, as long as the concentration of the polymer and the size of the crystals permit connectivity to occur. This possibility is akin to the 'region I' gels described by Aubert for i-PS[25]. For samples in which the water content is sufficiently low that the cap is lowered beneath the crystal melting point, this effect could also apply when in the one phase liquid crystalline region above the cap. Conversely, the existence of crystals pinning the growth of the different phases may explain why as-prepared gels do not show well defined isotropic and anisotropic regions, but typically appear uniformly birefringent (sometimes sitting in an isotropic pool[9]), unlike ungelled systems which may exhibit quite coarse biphasic structures.

The possibility that phase separation is a common mechanism for polymer gels has already been raised[18,33]. The observations of Tohyama and Miller[29] of a fibrillar microstructure attributed to phase separation have already led Guenet *et al.*[33] to suggest that rod-like systems may be included in this general picture, and Atkins[34] has also suggested that there may be a connection between liquid crystal formation and gelation. The results presented here support the view that in a stiff rod system there is an interplay between gelation and phase separation, and liquid crystal phases can be involved.

CONCLUSIONS

It has been shown that bulk phase separation of PBLG/BA gels may be achieved by holding at elevated temperature. Three different types of phase separation have been achieved: separation into two anisotropic phases in the cap of the Flory phase diagram, and separation into an isotropic and an anisotropic phase in both the chimney and broad biphasic regions. The system is, therefore, seen to accord with the general features of the Flory phase diagram. If the solvent is made too dry (solvent quality too good), then it may become impossible to achieve any phase separation if the phase boundary between the cap and the single anisotropic phase lies too close or (or below) the gel melting point. It appears that crystals may be present in any of the three phases although, except in the most dilute isotropic one, these are too imperfect to give useful diffraction information. However, all phases give an endotherm at $\approx 60°C$ in the d.s.c. This crystal melting temperature coincides with the melting point of the gel itself.

Given the evidence for phase separation and crystallization, we believe it is possible to reconcile the two different pictures for gelation in PBLG-based systems that exist in the literature, in terms of the mechanism put forward by Berghmans. In this picture we envisage phase separation (by any of the three routes depending on concentration and temperature) to occur, possibly by spinodal decomposition, followed by crystallization in both the phases that form. The crystallization (which may be limited) prevents the phase separation from proceeding to completion and means that an interconnected structure is set up with mechanical connectivity, i.e. a gel. The gels will not be true equilibrium structures and further work is planned to investigate the relationship between preparation route and final mechanical properties.

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