

THERMODYNAMICS AND DYNAMICS OF POLYPEPTIDE LIQUID CRYSTALS

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ABSTRACT

The prediction that molecular asymmetry alone is sufficient to force a phase transition from a disordered to an ordered phase in a system of rodlike particles has been investigated using α -helical synthetic polypeptides as the source of rodlike particles. To this end the temperature composition phase diagrams for the two component systems polybenzylglutamate (PBLG)-dimethylformamide (DMF) and polycarbobenzoxylysine (PCBL)-dimethylformamide are compared with the Flory lattice model for rigid impenetrable rods. The experimental systems exhibit and the theory predicts three distinct regions in the phase diagram: a narrow biphasic region in which isotropic and liquid crystal phases differing only slightly in composition coexist, a transition region over which solvent is increasingly excluded from the coexisting ordered phase and rods excluded from the coexisting isotropic phase, and a region where almost pure solvent coexists with a highly concentrated liquid crystal phase. A detailed comparison of the theoretical phase diagram for rigid, impenetrable rods with the experimental ones reveals discrepancies which can be attributed to the facts that the experimental rods are neither completely rigid nor impenetrable. Analysis of thermal data on PBLG-DMF indicates that the latent heat for the isotropic to liquid crystal phase transition is small and endothermic. We conclude that molecular asymmetry alone is sufficient to produce a phase transition to an ordered phase.

Dynamical data show that the bulk viscosity may be considerably lower in the ordered than in the disordered phase. Although rod motion in the liquid crystal phase is correlated, electron spin resonance data suggest that individual rod motion about its mean lattice position is greater than in the isotropic solution of equivalent concentration. Additional electron spin resonance studies show that the motion of small rods and of the polymer side-chains is little affected by the presence of a high concentration of long rods, whereas the tumbling of long rods is dramatically influenced by the presence of other long rods.

INTRODUCTION

The thermodynamics and dynamics of flexible linear polymer chains have been thoroughly investigated and are well understood. The flexibility of polymeric chains is highly variable, and depends on chemical constitution and secondary bonding. Stiff chain polymers form a small but interesting

set. They may have properties which are dramatically different from and not so well understood as their flexible relatives. Solutions of stiff chain polymers are known¹⁻⁶ to organize into an ordered liquid (liquid crystalline) phase at sufficiently high polymer concentration. Theoretical considerations⁷⁻¹⁰ indicate that molecular asymmetry alone may be sufficient to explain the stability of the ordered phase relative to the disordered (isotropic) phase

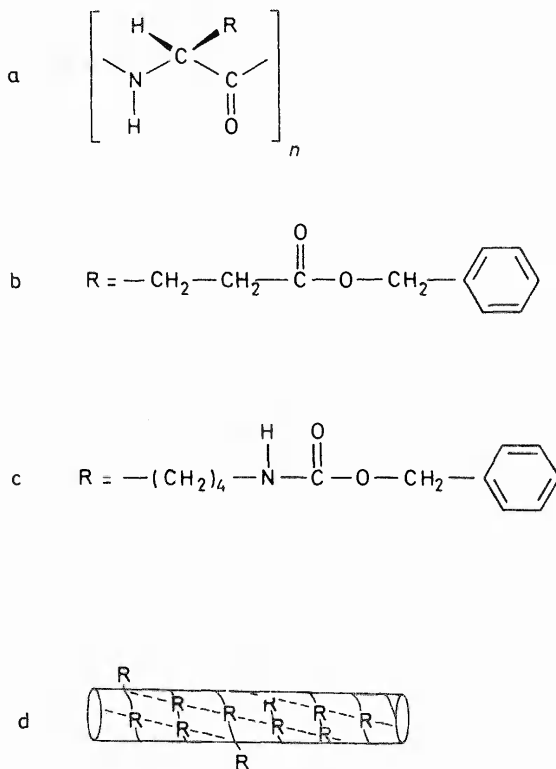


Figure 1. (a) monomeric unit of an α -L-polyamino acid; (b) γ -benzylglutamate side chain; (c) ϵ -carbobenzoxylysine side chain; (d) portion of an α -helix with attached side chains

The lattice theory of Flory¹⁰ also predicts that as the polymer-solvent interaction becomes increasingly unfavourable there is a sudden and precipitous increase in order and in rejection of solvent from the liquid crystal phase.

In aqueous systems the polymers which have been studied are polyelectrolytes. Addition of small electrolyte destroys the ordered phase indicating that electrostatic interaction is important in stabilizing the ordered phase. With these systems it is thus difficult to establish whether or not molecular asymmetry alone is sufficient to stabilize an ordered phase. The early observations with nonionic, helical polypeptides^{5,6} were carried out with

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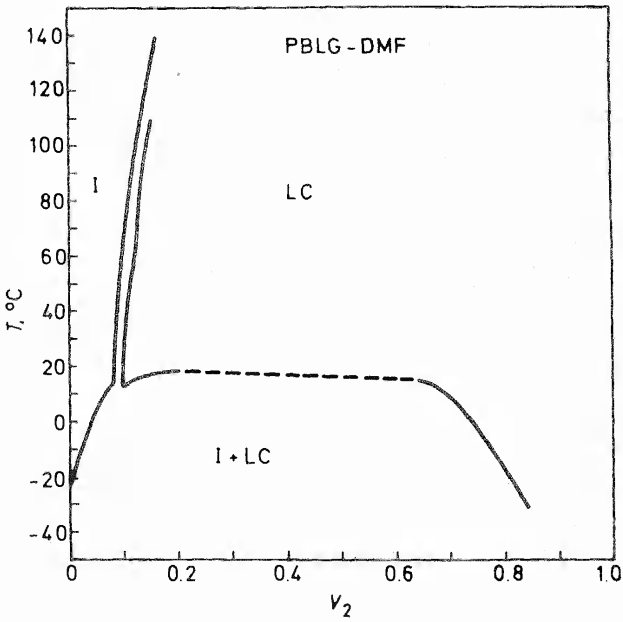


Figure 2. Temperature-composition phase diagram for PBLG (310000) in DMF^{16,17}. Dashed line indicates area of insufficient data

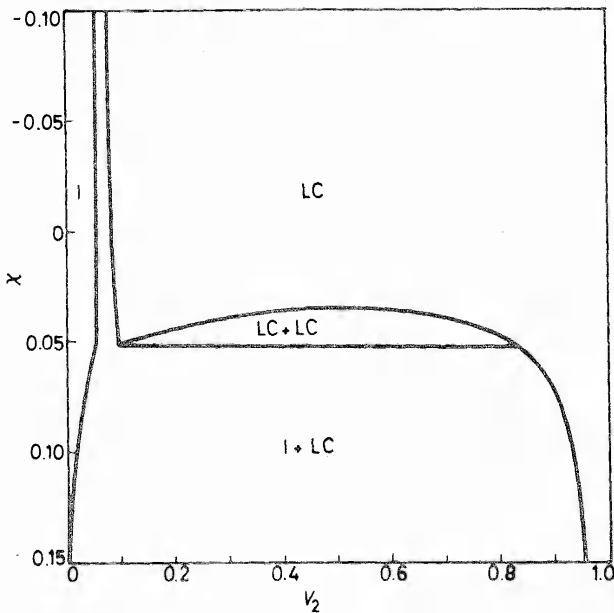


Figure 3. Lattice model phase diagram for rigid, impenetrable rods¹⁰ of axial ratio 150. Phases are isotropic (I) or liquid crystalline (LC)

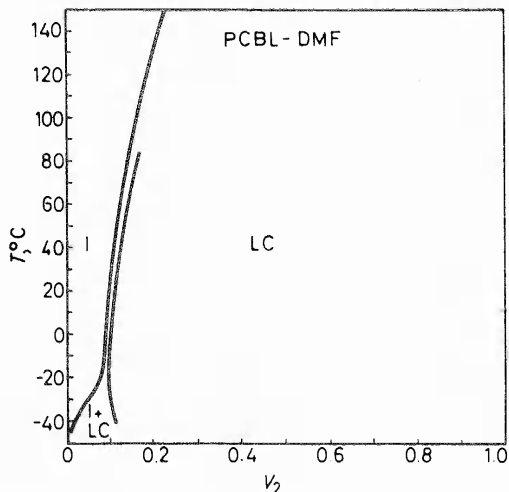


Figure 4. Temperature-composition phase diagram for PCBL (690000) in DMF¹⁸

polymer-solvent systems wherein the polymers are now known to have a tendency to associate even at low concentration¹¹ suggesting that in these systems too, factors other than molecular asymmetry are important. The peptide bond polymers (Figure 1a) seem attractive, however, as the side chain may be varied widely while the hydrogen bonded helical, rodlike conformation remains intact. Inasmuch as several helical polypeptides show little or no tendency to associate in dimethylformamide (DMF)¹¹⁻¹³ and that DMF remains liquid over a 250 Centigrade degree temperature range, we felt that the helical polypeptide-DMF system might be attractive for polymer liquid crystal investigation. Studies on the three component system polybenzylglutamate (PBLG)-DMF-nonsolvent indicate that the general features of the Flory phase diagram are observable¹⁴. The finding¹⁵ that the dilute solution osmotic second virial coefficient for PBLG in DMF had a substantial temperature dependence and became zero near room temperature made the two component PBLG-DMF system particularly attractive. It suggested a change from a thermodynamically good to poor solvent in an accessible temperature range, perhaps making a determination of the temperature-composition phase diagram and of the importance of molecular asymmetry versus polymer-solvent interaction experimentally possible.

A partial temperature-composition phase diagram for PBLG-DMF was published in 1971¹⁶, and has since been extended¹⁷ to cover an approximately 200 Centigrade degree range of temperature and the entire range of polymer composition. A variety of measurements—nuclear magnetic resonance, polarizing microscope, hydrodynamic and isopiestic—were necessary in order to determine the phase boundaries. The various techniques yield phase boundaries in substantial agreement. Figure 2 shows our current best effort for the PBLG-DMF phase diagram for molecular weight 310000 (weight average) having a rigid rod axial ratio of about 135. Figure 3 shows

the theoretical rigid, impenetrable rod phase diagram¹⁰ for an axial ratio of 150. *Figure 4* shows a partial phase diagram¹⁸ for the system polycarboxylysine (PCBL)-DMF for 690 000 molecular weight PCBL with a rigid rod axial ratio of about 190. The similarities are striking. The gross features of the Flory model are certainly evident.

RIGID, IMPENETRABLE ROD THERMODYNAMICS

It is of interest to pursue in more detail the various features of the Flory lattice model. The isotropic-liquid crystal phase equilibria for rigid, impenetrable rods was determined from equations 1-3.

$$\ln(1 - v_2) + (1 - 1/x)v_2 + \chi^I v_2^2 = \ln(1 - v_2^*) + (y - 1)v_2^*/x + 2/y + \chi^{I,C} v_2^{*2} \quad (1)$$

$$\ln v_2 + (x - 1)v_2 - \ln x^2 + \chi^I x(1 - v_2)^2 = \ln v_2^* + (y - 1)v_2^* + 2 - \ln y^2 + \chi^{LC} x(1 - v_2^*)^2 \quad (2)$$

$$v_2^* = [x/(x - y)][1 - \exp(-2/y)] \quad (3)$$

where x is the rod axial ratio, y the equilibrium degree of disorientation in the liquid crystal phase, χ the polymer-solvent interaction parameter, and v_2 and v_2^* the equilibrium volume fraction of polymer in the isotropic and liquid crystalline phases, respectively. Equations 1 and 2 were obtained by equating chemical potentials, and equation 3 by minimization of the free energy of the liquid crystal phase with respect to rod alignment¹⁰. The phase equilibrium may be described as consisting of three regions: a narrow isotropic-liquid crystal biphasic region when χ is near zero or negative, a transition region when χ becomes positive where the coexisting ordered phase becomes increasingly rich and the isotropic phase increasingly poor in polymer, and a region where essentially pure solvent is in equilibrium with a highly concentrated polymer solution when χ becomes sufficiently positive. In addition to isotropic-anisotropic equilibria there is a small region where equilibria between two liquid crystal phases minimizes the free energy of the system.

If the ordered phase is perfectly aligned the isotropic-liquid crystal phase equilibria are given¹⁰ by equations 4 and 5. The phase diagram which was

$$\ln(1 - v_2) + (1 - 1/x)v_2 + \chi^I v_2^2 = \ln(1 - v_2^*) - \ln(1 - v_2^* + v_2^*/x) + \chi^{I,C} v_2^{*2} \quad (4)$$

$$\ln v_2 + (x - 1)v_2 - \ln x^2 + \chi^I x(1 - v_2)^2 = \ln v_2^* - \ln(1 - v_2^* + v_2^*/x) + \chi^{LC} x(1 - v_2^*)^2 \quad (5)$$

calculated from these equations is shown in *Figure 5*, where the ordered phase has been forced to remain totally aligned at all concentrations. There is a qualitative difference between the shape of the phase diagrams in *Figures 3* and *5*. Although it is difficult to quantitatively connect the theoretical χ parameter with the corresponding experimental variable, temperature, it should be a monotonically varying function of temperature. The general correspondence of *Figure 3* and lack of correspondence of *Figure 5* with

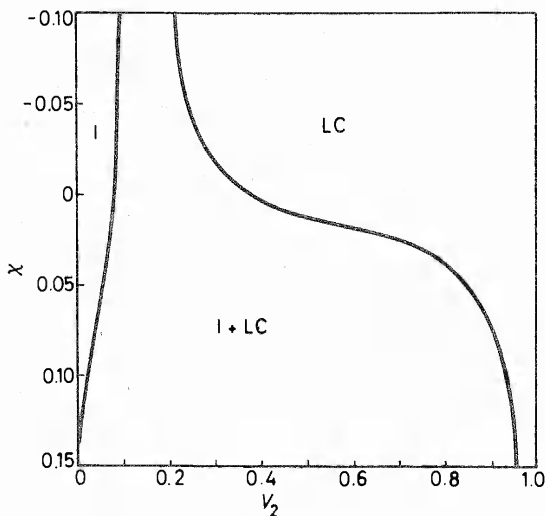


Figure 5. Lattice model phase diagram for rigid, impenetrable rods¹⁰ of axial ratio 150 in which the ordered phase is completely aligned

the experimental phase diagrams indicates that the disorientation entropy makes a significant contribution to the thermodynamics of the dilute, ordered phase and is largely responsible for the prediction of a narrow biphasic region that is effectively χ (or T) invariant over a wide range of χ values. The disorientation index y as a function of composition is shown in Figure 6, calculated from equation 3. In the narrow biphasic region the composition of the liquid crystal phase is 0.07–0.10, a region where y is extremely concentration dependent (axial ratio 150). Yet the calculated and experimental curves are in substantial though not complete (see below) agreement.

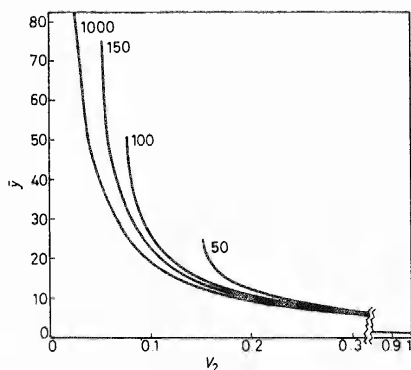


Figure 6. Index of disorientation as a function of composition for the rigid, impenetrable rod model¹⁰. Rod axial ratios are as indicated

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Therefore we conclude that the lattice model treatment of the disorientation is a rather good description in spite of its approximate¹⁰ and concentration sensitive nature. However, this choice of representing the phase equilibria is not unique in giving a phase diagram similar to *Figure 3*¹⁷.

The theoretical phase diagrams were calculated assuming χ^I was equal to χ^{LC} . If we consider χ to be entirely enthalpic and not an excess free energy the equality is equivalent to stating that the conversion of the isotropic phase to the liquid crystal phase at constant composition has no latent heat. It is of considerable importance to determine the existence and magnitude of the latent heat in the experimental systems, and its effect on the theoretical phase diagrams.

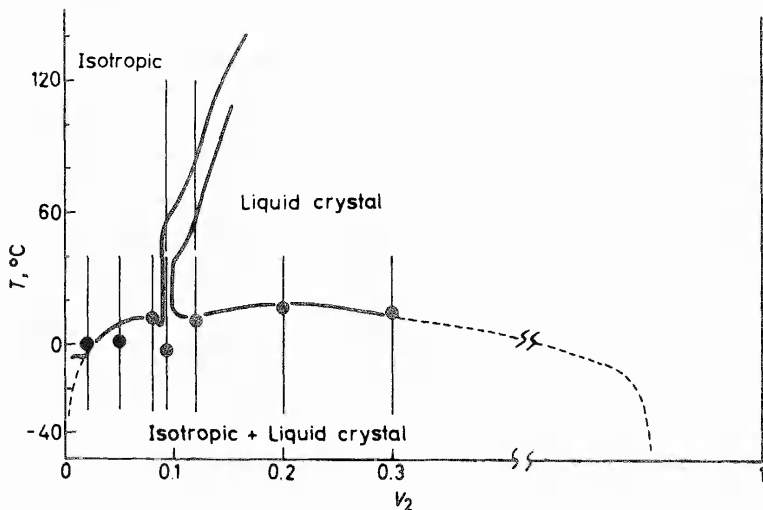


Figure 7. Schematic representation of the differential scanning calorimetry experiments¹⁹. Vertical lines indicate range of temperature scan. Circles indicate onset of endothermicity when scanning from low to high temperature

Differential scanning calorimetry (DSC) experiments on a 12 volume % solution whereby a PBLG liquid crystal solution was converted to an isotropic solution during the temperature scan gave no evidence of a latent heat¹⁹. However, DSC experiments were able to detect a thermal transition when a biphasic system at -30°C , composed of nearly pure solvent and highly concentrated polymer phases in equilibrium, was heated to give either an isotropic or liquid crystalline phase at $+40^{\circ}\text{C}$ ¹⁹. The experiments, shown schematically in *Figure 7*, yield heats which can be identified as the heat of mixing two widely different compositions. Similar experiments with PCBL-DMF are difficult due to the necessity of operating at temperatures near the solvent freezing point, and were inconclusive. Also direct heat of mixing measurements have not been successful due to the high viscosity of the concentrated polymeric solutions.

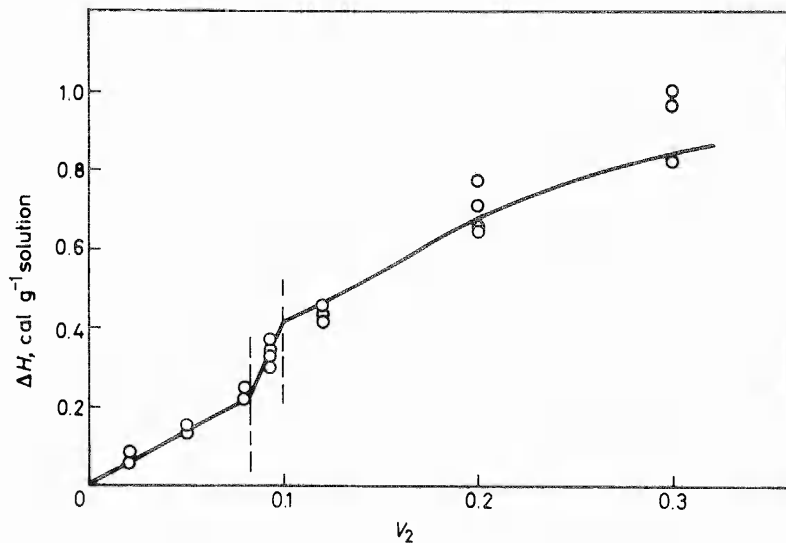


Figure 8. Heat of mixing as a function of PBLG (310 000) concentration. Open circles—experimental results¹⁹; solid line—calculated from equation 6 with $\chi^I = 0.50$, $\chi^{LC} = 0.70$. $T = 300$ K. $v_2^I = 0.001$, $v_2^{LC} = 0.84$; dashed vertical lines—phase boundaries at 15–20°C in the narrow biphasic region

The observed DSC heats, shown in Figure 8, are small and endothermic which suggests that a van Laar treatment of the heat of mixing is appropriate. If we assume that the χ parameter may be phase dependent but concentration independent within a phase, the heat of mixing in van Laar approximation will be given by equation 6

$$\Delta H_{\text{mix}} = RT(\chi^{I \text{ or } LC} n_1 v_2 - \chi^I n_1^I v_2^I - \chi^{LC} n_1^{LC} v_2^{LC}) \quad (6)$$

where there are n_1 moles of solvent in a solution of composition v_2 in the final state of the system, n_1^I and v_2^I refer to the initial isotropic phase, and n_1^{LC} and v_2^{LC} to the initial liquid crystal phase. The values of v_2^I and v_2^{LC} are fixed and obtainable from the phase diagram, and n_1^I and n_1^{LC} are fixed by material balance. Either χ^I or χ^{LC} is used in the first term depending on whether the final solution is isotropic or liquid crystalline, respectively. Although there are two unknown quantities in equation 6, χ^I and χ^{LC} , the experimental data can be fit with only a narrow range of χ values, typified by $\chi^I = 0.50$ and $\chi^{LC} = 0.70$. A theoretical line generated from equation 6 using these values is shown in Figure 8. Further refinement, such as a concentration dependent χ , is unwarranted.

Equation 6 adequately describes the data and furthermore gives unequal χ values. A latent heat exists and may be calculated in van Laar approximation at any composition from equation 7.

$$\Delta H_{I,C \rightarrow I} = RT(\chi^{LC} - \chi^I) n_1 v_2 \quad (7)$$

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The latent heat is slightly larger than that previously¹⁹ estimated when the low temperature phase boundaries were of unknown composition. For a 12 volume% solution equation 7 yields a value of 0.16 cal g^{-1} of solution or about 2 millicalories for our sample size, which should be detectable. However, we have a two component system and the phase transition must pass through a biphasic region which for the 12% solution is spread over 30 Centigrade degrees. We feel that the broad transition is probably responsible

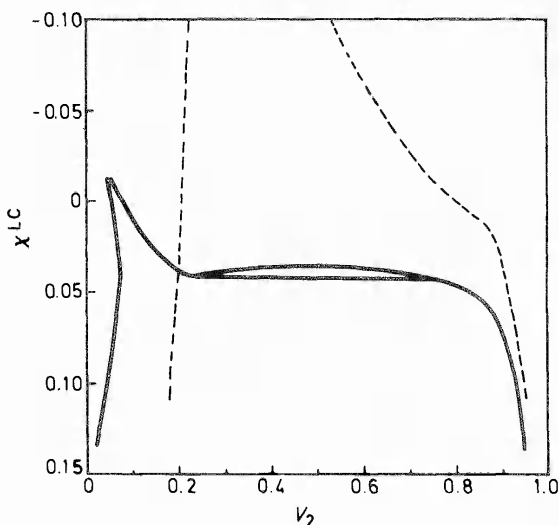


Figure 9. Rigid, impenetrable rod ($x = 150$) phase equilibria with unequal χ . Solid lines, $\chi^{LC} = 1.4\chi^I$; dashed lines, $\chi^{LC} = (210/T) - 0.68$, $\chi^I = (150/T) - 0.68$

for our inability to observe a latent heat directly, or else equation 7 overestimates it for unknown reasons.

We conclude that in the PBLG-DMF system a small latent heat exists, and is endothermic for conversion of the isotropic to the liquid crystal phase. The enthalpy thus serves to destabilize the liquid crystal phase. Consequently it must be stabilized by entropic considerations, which are dominated presumably by molecular asymmetry. The generality of this observation is unknown. The situation may be similar to the well-known entropy dominated process of rubber elasticity where the associated small energy changes may be positive or negative depending on the rubber.

We turn now to the effect of unequal χ on the rigid, impenetrable rod phase equilibria. The ratio $0.70/0.50$ is equal to 1.4 so we consider first the phase equilibria calculated from equations 1-3 with $\chi^{LC} = 1.4\chi^I$. The phase diagram is shown in Figure 9. In the negative χ region no solutions exist other than the ones shown. In the narrow biphasic region the calculated and experimental values are not even in qualitative agreement. We next

consider that χ should be considered as an excess free energy, and not as purely enthalpic. We further recognize that vapour sorption measurements indicate that there is a contribution to the solution thermodynamics from the entropy of solvent flexible side chain mixing²⁰⁻²². Thus there is experimental justification for considering χ to have an entropic component. Shown in *Figure 9* is a phase diagram calculated with χ^{LC} given by $210/T - 0.68$ and χ^{I} by $150/T - 0.68$. By this assignment the enthalpic components of χ retain a ratio of 1.4 and are equal to 0.70 and 0.50 at 300 K, and the entropic components are equal and chosen so that χ^{LC} becomes zero slightly above room temperature. This choice has completely wiped out any resemblance to the experimental phase diagram. With unequal χ the phase diagram is extremely sensitive to the manner in which χ^{I} and χ^{LC} vary. Excess entropy contributions must be carefully chosen in order to produce a narrow biphasic region resembling that in *Figure 3*. At this point they would have to be chosen arbitrarily.

SEMIFLEXIBLE, IMPENETRABLE ROD THERMODYNAMICS

We have emphasized up to now the similarities between the gross features of the experimental and theoretical phase equilibria. A closer inspection reveals that they have in fact considerable differences. In the theoretical diagram (*Figure 3*) the equilibrium concentrations in the narrow biphasic region are almost independent of χ , becoming slightly closer together as χ becomes more negative. Of the two lines the one bounding the isotropic phase is especially independent of χ . With both experimental systems the equilibrium concentrations move to larger values as the temperature increases though their difference remains almost constant. We believe the origin of this discrepancy lies primarily in the fact that the experimental rods are not rigid. Considerable experimental evidence has been accumulated which indicates that at room temperature neither PBLG nor PCBL behaves as a perfectly rigid rod²³⁻²⁵. The initial conclusion that PBLG was a rigid helix at room temperature seems likely to be due to an error in molecular weight calibration²⁴. And with each polymer the intrinsic viscosity drops considerably with increasing temperature^{17, 18}.

We can either consider a lattice model of semiflexible rods⁹, or a rigid rod model with a temperature dependent axial ratio. We prefer¹⁷ the latter and consider the axial ratio dependence of the rigid, impenetrable rod model. Inasmuch as the equilibrium concentrations are insensitive to χ in the narrow biphasic region it is convenient to consider their dependence on the axial ratio for the athermal case ($\chi = 0$). This is illustrated in *Figure 10*. The difference between v_2 and v_2^* increases as x decreases. In addition the difference between the theoretical v_2 and v_2^* is greater than the experimental values for all x equal or less than the rigid rod value of each polymer. It would take extremely large, negative χ values to bring the theoretical and experimental values into exact agreement. We can though take the experimental v_2 and v_2^* and from *Figure 10* determine to what apparent axial ratio they correspond. Results of such an approach are shown in *Figure 11*. Each of the values calculated from v_2 and v_2^* lies below the rigid rod value for the respective

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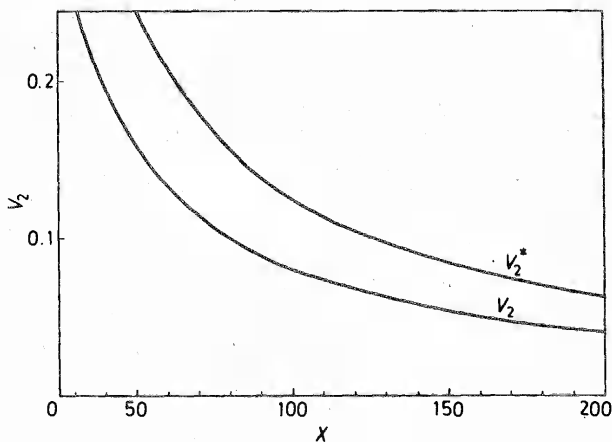


Figure 10. Equilibrium concentrations¹⁰ for rigid, impenetrable rods with $\chi = 0$. Lower curve—line bounding the isotropic phase (v_2); upper curve—line bounding ordered phase (v_2^*).

polymer, and the two sets of apparent axial ratios have a common temperature dependence. The temperature dependence is also the same for the two polymers which would not seem unreasonable as their helical backbones as well as their side chains through the γ -carbon are the same. The temperature dependence of the intrinsic viscosity is nearly the same for the two polymers^{17, 18}. The apparent axial ratios for PCBL fall in the range deduced

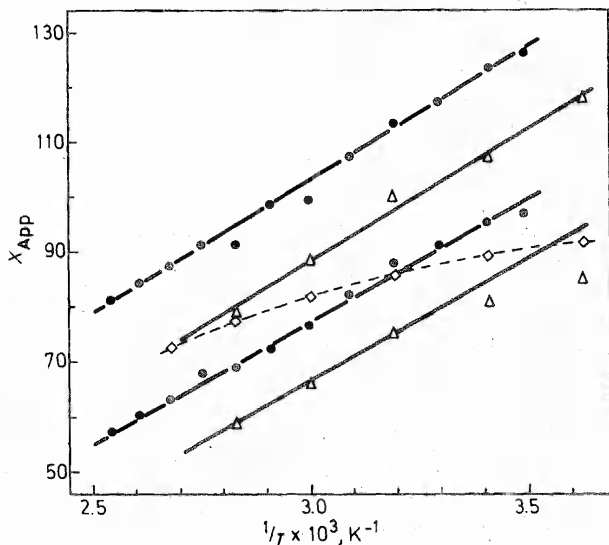


Figure 11. The apparent axial ratio as a function of temperature for PBLG (circles) and PCBL (triangles). Lower two sets—determined from v_2 (Figure 10); upper two sets—determined from v_2^* (Figure 10); diamonds—axial ratio of PCBL determined from intrinsic viscosity¹⁸

from intrinsic viscosity measurements although the temperature dependence is not the same. Though the agreement is not exact it is the similarity which leads us to conclude that the shift of the narrow biphasic region to higher concentration at increasing temperature is predominantly a function of increasing rod flexibility.

RIGID, PENETRABLE ROD THERMODYNAMICS

We have seen that the phase equilibria for the experimental systems depart from that predicted for rigid, impenetrable rods because the experimental rods are not rigid. We now consider the fact that the experimental rods are also not impenetrable. The side chains of PBLG (*Figure 1b*) and of PCBL (*Figure 1c*) constitute a sizeable fraction (75 and 79 wt. %, respectively) of the total cross section of the rod. Much evidence has accumulated^{20-22, 26} which indicates that unless the side chain is flexible and hence solvent penetrable the rod-like polymers will not dissolve, and the liquid crystal phase cannot be realized. The side chain flexibility introduces three factors: first, there is an entropy associated with flexible side chain-solvent mixing which contributes significantly to the solution thermodynamics; second, the polymer-solvent interaction is predominantly solvent interacting with flexible side chains, the main chain or hard core rod being shielded from contact with solvent; third, the rigid rod cross section in the previous discussions taken as the cross section with side chains of mean extension may not be appropriate for comparison with rigid rod theory. An accounting of the effects of flexible side chains has been attempted²⁷ patterned after the rigid, impenetrable rod approach. The phase equilibria for this model can be calculated from equations 8-10.

$$\ln(1 - v_2) + [1 - 1/x(1 + mc)]v_2 + [mc/(1 + mc)]\chi^1 v_2^2 = \ln(1 - v_2^*) + (y - 1)v_2^*/x(1 + mc) + 2/y + [mc/(1 + mc)]v_2^* + [mc/(1 + mc)]\chi^{1,c} v_2^{*2} \quad (8)$$

$$\ln v_2 + [x(1 + mc) - 1]v_2 - \ln x^2 + \chi^1 mcx(1 - v_2)^2 = \ln v_2^* + (y - 1 + mcx)v_2^* + 2 - \ln y^2 + 2mcx/y + \chi^{1,c} mcx(1 - v_2^*)^2 \quad (9)$$

$$v_2^* = [x(1 + mc)/(x - y)][1 - \exp(-2/y)] \quad (10)$$

where m is related to the number of side chains of length c per molecule and the other symbols are as identified previously. The phase diagram with mc equal to 0.15 is shown in *Figure 12*. This value of mc was chosen not only to be illustrative but also because it gives the best fit to vapour sorption isotherms²¹. The phase diagram is qualitatively similar to *Figure 3* except that the small liquid crystal-liquid crystal biphasic region has been eliminated. The narrow biphasic region extends to higher χ values because polymer-solvent interaction occurs only with the side chain component of the polymer. The distance over which the narrow biphasic region runs into the positive χ region depends of course on the magnitude of mc .

In common with the rigid, impenetrable rod model when the polymer-solvent interaction becomes increasingly unfavourable there is a point where the system separates into a solvent rich isotropic phase and a solvent poor ordered phase. The ordered phase has a polymer composition of 85-95% by

the impenetrable rod model, but a much lower concentration by the penetrable rod model. In the PBLG-DMF system the ordered phase goes from 70-80% polymer during the transition region. Allowing the rod to be semi-flexible but impenetrable, as in the previous section, will not bring the theoretical values into agreement with the experimental ones. The introduction of flexible side chains which alone interact with the solvent softens the exclusion of the solvent. We believe the flexible side chains contribute significantly to the transition region phase equilibria.

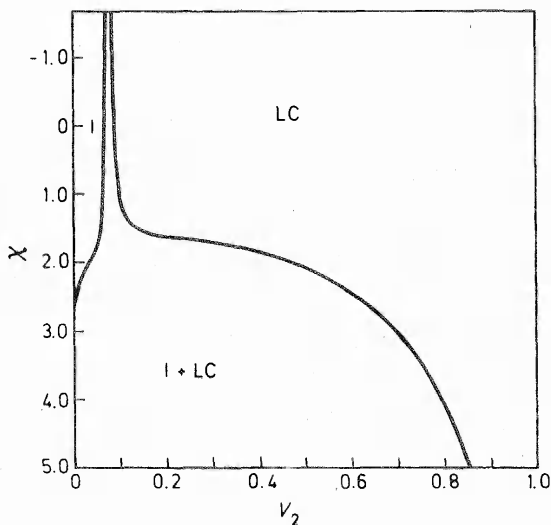


Figure 12. Lattice model phase diagram for rigid rods with flexible, permeable side chains²⁷. Axial ratio is 150 and mc is 0.15

The flexible side chains also permit a more unfavourable polymer-solvent interaction before the transition region is reached. Our dilute solution osmotic pressure¹⁵, vapour sorption^{20, 21} and DSC results¹⁹ in PBLG-DMF each indicates a substantial positive heat of mixing at room temperature where a single phase isotropic solution is stable to several volume % polymer, and where a single ordered phase is stable to several volume % solvent. Whereas it is not easy to rationalize these observations by the impenetrable rod model, it is easy to do so with the flexible side chain model. With PCBL only vapour sorption measurements have been made. In contrast to PBLG the conclusion with PCBL is that the polymer-solvent mixing is essentially athermal at room temperature²⁰. Since the transition region for PBLG lies just below room temperature we might infer that the transition region for PCBL should lie at lower temperatures. This turns out to be realized, as the transition region is 30-40°C lower for PCBL. We would also predict on the basis of vapour sorption measurements²⁰ that polycarbobenzoxyornithine-DMF should have a transition region below that of PBLG.

Finally we turn to the narrow biphasic region. As with the impenetrable rod model the line bounding the isotropic phase is effectively independent of χ , and that bounding the ordered phase nearly so. As with the rigid, impenetrable rod model we set $\chi = 0$ and investigate the apparent axial ratio dependence of the equilibrium concentration. We find that they lie close together and are in better agreement than those in *Figure 11*. However, the temperature dependence is similar to the impenetrable rod forced fit. The flexible side chain model affects the disorientation index, and hence would be expected to have a small effect on the narrow biphasic region.

LIQUID CRYSTAL-LIQUID CRYSTAL EQUILIBRIA

The rigid, impenetrable rod model predicts a small region where the minimum free energy of the system is represented by two liquid crystal phases

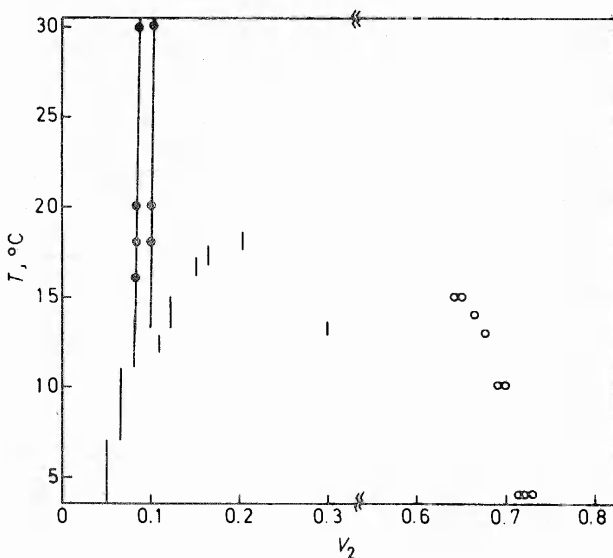


Figure 13. Expanded view of PBLG (310 000)-DMF phase boundary measurements relevant to the existence of an upper critical solution temperature

in equilibrium. Flory thought this biphasic region might disappear in a refined treatment¹⁰. This biphasic region has an upper critical solution temperature which occurs at about 50 volume %. The critical composition is almost molecular weight independent and thus behaves very differently from its counterpart in random coil solutions.

In *Figure 13* is an expanded scale view of pertinent phase boundary measurements for PBLG. Although there is need for a good method to determine phase boundaries in the 25-60 volume % region, the shape of the

regions we have measured requires that the liquid crystal-liquid crystal region must exist. Consequently an upper critical solution temperature must exist for the PBLG-DMF system, which gives rise to the possibility of critical opalescence. On occasion solutions of 15-30 volume% which have been standing at temperatures slightly below room temperature are visually white and opaque, yet when viewed under a microscope show no precipitation. The significance of these observations awaits a quantitative study.

NATURE OF THE PHASE TRANSITION

In our discussions we have assumed that the phase transition was first order, which is also assumed by the Flory lattice model. However, many order-disorder transitions are thermodynamically second, and not first order. It is of interest to consider the evidence relative to the order of the transition.

In the PBLG-DMF system we have provided indirect evidence that there is a small latent heat. In neither system has a latent heat been observed directly. We can be more definite, however, concerning a density difference at least in the narrow biphasic region of the PCBL-DMF system. Particularly in the 10-15 volume% region a sharp meniscus develops with the ordered phase being more dense. As such a biphasic solution is warmed the meniscus is displaced towards the bottom and disappears coincident with crossing the phase boundary shown in *Figure 4*. Even when the visually sharp meniscus is present it is not unusual to find, upon microscopic examination, spherical droplets of liquid crystal in the isotropic phase. With PBLG we have never observed a meniscus by eye in the narrow biphasic region which must indicate that the density difference is very small. In a polarizing microscope one can see that the ordered phase does have a tendency to settle. The ordered phase which is dispersed in the isotropic phase takes on a spherical shape indicating a difference in surface tension. In neither system have we observed a meniscus when a solution enters or is in the wide biphasic region. This may be a kinetic problem (see below).

The similarity of the theoretical and experimental phase diagrams, the existence of a distinct meniscus in one system and the indirect observation of a latent heat in the other give good evidence that we are working with first order transitions in the sense of discontinuous first derivatives of the free energy.

DYNAMICS—BULK FLOW THROUGH THE NARROW BIPHASIC REGION

The viscosity of solutions of PBLG is strongly concentration and shear rate dependent^{6, 14, 28-30}. Shown in *Figure 14* is the concentration dependence for several temperatures taken at a shear rate of 19.4 s^{-1} . The phase boundaries are clearly evident. The viscosity drops by a factor of 4 in crossing from the isotropic to the liquid crystal phase. Increasing the shear rate diminishes this effect. At high shear rates the distinction between the viscosity of isotropic and liquid crystal solutions disappears³⁰ probably because the high shear rate simply orders the isotropic phase. Conversely extrapolation to

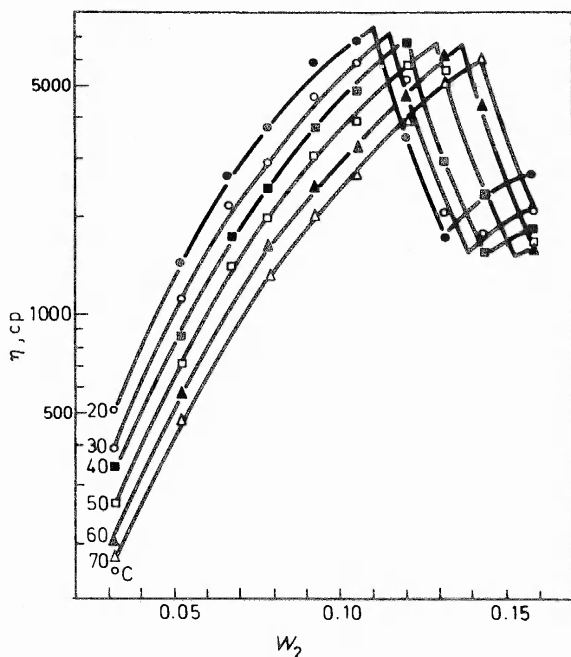


Figure 14. Concentration dependence of the viscosity of PBLG (310 000)-DMF solutions. The shear rate was 19.4 s^{-1} .

zero shear rate heightens the difference. In the isotropic phase the different temperatures produce a family of parallel lines which may be shifted to give a common curve²⁷. At a fixed temperature and composition the shear stress and shear rate are linearly related on a log-log plot in both phases indicating that a 'power law' is obeyed. As one might expect, the liquid crystal phase shows a much greater deviation from Newtonian flow. The only point we wish to make here is that ordering the rods into a mean parallel arrangement leads to considerably easier bulk flow. This effect is so large that it can be visually observed.

DYNAMICS—BULK FLOW NEAR THE TRANSITION REGION

The temperature dependence of the viscosity of 9.2 and 14.4 wt % PBLG-DMF solutions at fixed shear rate is shown in Figure 15. With the 9.2% solution, isotropic at room temperature, the viscosity shows a normal temperature dependence until about 17°C where it begins to show a very high temperature coefficient. At low temperature the behaviour of the 14.4% solution, which is liquid crystalline at room temperature, is similar. It is the low temperature behaviour which concerns us at the moment. The sudden increase in viscosity as the temperature is lowered occurs with all concentrations and is independent of whether one is cooling an isotropic or a liquid

POLYPEPTIDE LIQUID CRYSTALS

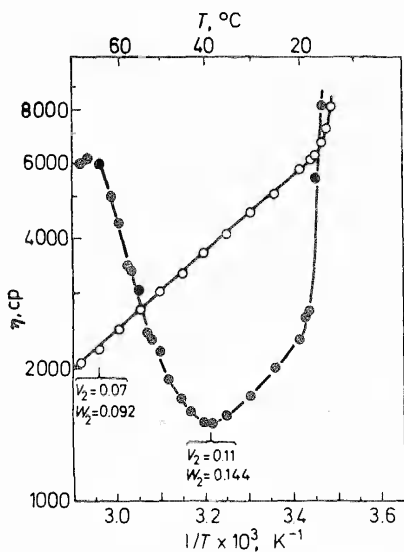


Figure 15. Temperature dependence of the viscosity of PBLG (310 000)-DMF solutions. The shear rate was 19.4 s^{-1} .

crystalline phase. The viscosity quickly rises above 10 000 centipoise, the maximum we could measure, and becomes time dependent. The solution eventually behaves as if it were a gel even in solutions containing less than 1 % polymer.

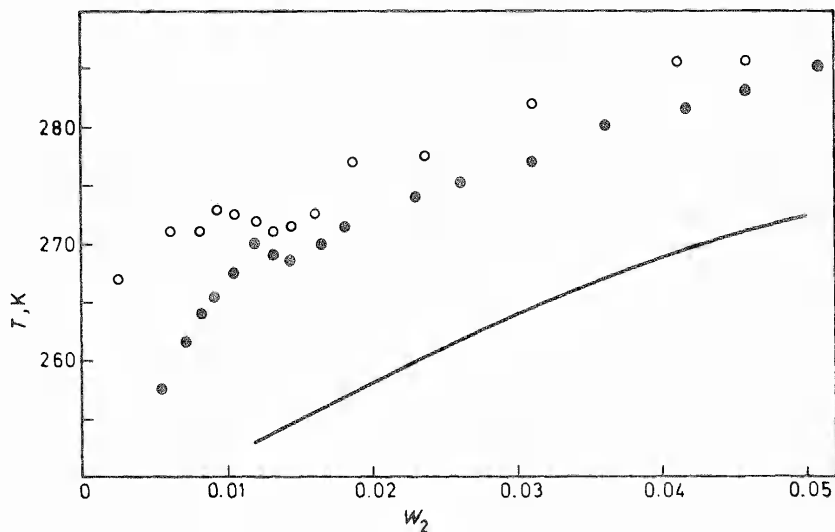


Figure 16. Concentration dependence of the gelation of PBLG (268 000) by visual observation¹⁵ (open circles—onset; filled circles—completion). Solid line is phase boundary determined by polarizing microscope¹⁷ for PBLG (310 000)

In our earliest study of PBLG-DMF we noted the gelation by a simple qualitative, visual observation¹⁵, *Figure 16*. We were unable previously to observe with assurance the appearance of the ordered phase at low concentration, which we know now was due to inadequate temperature control of the microscope stage at low temperature¹⁵ as well as cell design¹⁶. We can see from *Figure 16* that the visual appearance of a rigid, gel-like solution correlates well with the phase boundary, but anticipates it by several degrees. The viscosity measurements do likewise. With the PBLG-DMF system we now have ample evidence to show that as the transition region in the phase diagram is approached a dramatic change takes place in bulk flow, which precedes the phase boundary as determined by polarizing microscope or nuclear magnetic resonance measurements. In the PCBL-DMF system, where the transition region is shifted 30-40°C, a few qualitative observations indicate a similar phenomenon. The appearance of a low concentration gel-like phase may be a convenient method for deducing at what temperature a given polymer-solvent system approaches the transition region. Be that as it may it is still necessary to explain the existence of a very low concentration gel-like solution in a system of rod molecules already containing maximum intramolecular order. The fact that no meniscus appears upon crossing the transition region phase boundary may be related to this phenomenon. At present we do not understand these observations.

DYNAMICS—INDIVIDUAL ROD MOTION

In the liquid crystal phase there is a correlation of rod motion otherwise the periodicity lines so easily observed⁶ would not be present. Each rod has a mean lattice position parallel to its neighbours. Actually the mean positions are not quite parallel as the liquid crystal phase is cholesteric and not nematic in the absence of external fields³¹⁻³⁴. The twist results from the fact that the polymer is a collection not of molecular rods but of either left or right handed helices. In a given solvent changing the handedness of the helix changes the handedness of the cholesteric screw axis. With screws of a given handedness the cholesteric screw axis may be continuously varied and even reversed by appropriate choice of solvent, a phenomenon which does not appear to be understood. The cholesteric twist is so slight, however, that the mean position of the rods may be considered parallel for our discussion here.

In addition to the cooperative end-over-end tumbling of the rods each rod has a certain amount of motion about its equilibrium position. We saw that local motion made a significant contribution to the thermodynamic stability of the liquid crystal phase. It is of interest to measure this local motion. The axial ratio of a typical experimental polymer is so large that if the centre of mass of the rod were constrained to its equilibrium position, a rotation of only a degree or less would result in physical overlap even in the most dilute liquid crystal phase. The experimental system, of course, does not have this constraint. The distribution of rod axes has been measured indirectly in a 17.5 wt% solution of PBLG in dichloromethane by nuclear magnetic resonance³⁵. These studies indicate a substantial spread in rod orientation with 13% lying more than 20° out of parallel alignment with the applied

POLYPEPTIDE LIQUID CRYSTALS

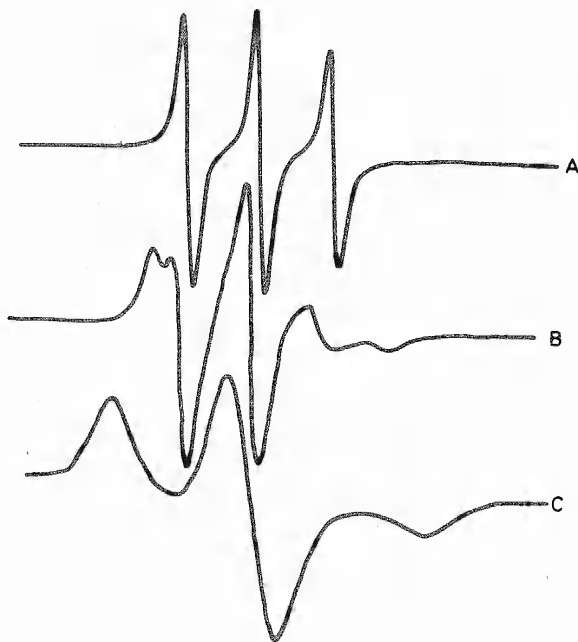


Figure 17. Nitroxide e.s.r. spectra classified as isotropic (A), axially symmetric (B), or rigid glass (C)

magnetic field. In the absence of the magnetic field the amount of disorder should be even larger. It was furthermore found that when a system of aligned rods was placed in a magnetic field their realignment occurred cooperatively and not individually.

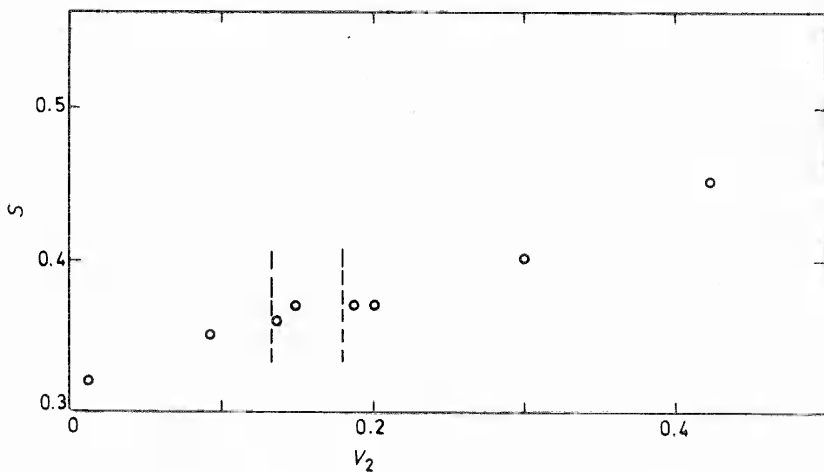


Figure 18. Order parameter (S) as a function of PBLG (122000) concentration for nitroxide labelled PBLG at 25°C³⁶. Vertical dashed lines indicate approximate positions of phase boundaries

Table 1. Rotational relaxation of nitroxide in end labelled PBLG (12 000) at 25°C.

Labelled PBLG Conc., vol. %	Nonlabelled PBLG Conc., vol. %	Mol. wt	DMF Conc., vol. %	τ_c^* s
1.0	0	—	99	3.0×10^{-10}
1.0	20	74 000	79	7.0×10^{-10}
1.0	20	310 000	79	7.5×10^{-10}
1.0	20	450 000	79	8.2×10^{-10}

*Calculated according to reference 39.

We have tried to measure the local motion by covalently attaching a nitroxide free radical to the end of each rod. The shape of the e.s.r. spectrum is sensitive to the motion of the nitroxide. The spectra may for our purpose be classified as an isotropic, an axially symmetric, or a rigid glass spectrum (Figure 17). For a 122 000 molecular weight PBLG molecule the axial ratio is sufficiently large to yield an axially symmetric spectrum at all compositions except pure or nearly pure polymer³⁶. From the spectrum the magnitude of the microorder parameter³⁷ S may be deduced. The results are shown in Figure 18. The e.s.r. magnetic field is too weak to orient the rods³⁶ so these results should more nearly represent the mean orientation in the absence of an applied field. However, the mean orientation of the rods is considerably larger than the S values deduced from the e.s.r. spectra, as a contribution from internal motion of the nitroxide has not been removed. This internal motion is not concentration dependent and the relative values of the order parameter are meaningful. If the results for the isotropic phase are extrapolated into the liquid crystal phase, the polymer molecules in the ordered phase are seen to have more local freedom than the same composition isotropic phase. This strengthens our belief that molecular asymmetry alone is responsible for stabilizing the ordered over the disordered phase at least in the PBLG-DMF system. Additionally the concentration dependence of the order parameter in the liquid crystal phase is in semiquantitative agreement with the disorientation index γ of the rigid, impenetrable rod model³⁶.

Table 2. Rotational relaxation of nitroxide in side chain labelled PBLG (310 000) in DMF at 25°C.

Polymer concentration		Molar solvent		η^* centipoise	Phase	τ_c^\dagger s
Volume %	Weight %	Molar (Monomer)	polymer			
6.4	8.4	32		3700	Isotropic	1.9×10^{-10}
12	15.5	16		2100	Liquid Crystal	2.6×10^{-10}
71	77	0.9		$> 10^4$	Liquid Crystal‡	6.8×10^{-10}
86	89	0.4		$> 10^4$	Liquid Crystal‡	1.8×10^{-9}
100	100	0		—	Solid	2.0×10^{-8}

*At a shear rate of 19.4 s^{-1} .

†Calculated according to reference 39 (first four), or reference 40.

‡Actually a 'wet' solid which is presumably ordered.

Studies on low molecular weight, end labelled PBLG are informative with regard to the hindrance of rod motion due to molecular asymmetry. In very dilute solutions 22 000 molecular weight PBLG rotates sufficiently rapidly to give an isotropic type spectrum (*Figure 17A*). As the concentration is increased to only a few per cent polymer the e.s.r. spectrum undergoes a qualitative change to an axially symmetric spectrum (*Figure 17B*), a result of reduced end-over-end tumbling³⁸. That this is not some sort of 'general viscosity' effect can be seen from a series of measurements on a 12 000 molecular weight end labelled sample which has a rigid rod axial ratio of about five. The rotational correlation times, not corrected for any internal nitroxide motion, are shown in *Table 1*. The short spin labelled rod does rotate more freely in dilute solution, but is not affected greatly by the presence of 20 per cent of high molecular weight PBLG. Each of the 20 per cent solutions is liquid crystalline, and also differs in bulk viscosity as a result of the molecular weight difference. As the rod axial ratio approaches unity the rod motion should reflect the local viscosity and not the bulk viscosity which is dominated by the long rods.

Free nitroxide in PBLG-DMF solutions has a rotational correlation time which is only slightly dependent on phase or bulk viscosity³⁸. This is consistent with the observations on short rods. These results lead us to believe that the motion of the solvent, DMF in our case, is not significantly phase dependent. This cannot be strictly true, otherwise direct polar coupling observed in the solvent proton magnetic resonance should be observed also in the isotropic phase. However, the rotational relaxation of a nitroxide attached to a glutamate side chain is little affected by bulk properties over a wide range of concentration, as can be seen in *Table 2*. These as well as nuclear magnetic resonance results²⁶ show that exceedingly little solvent is necessary in order for the helix side chain and the solvent to be in rapid motion.

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