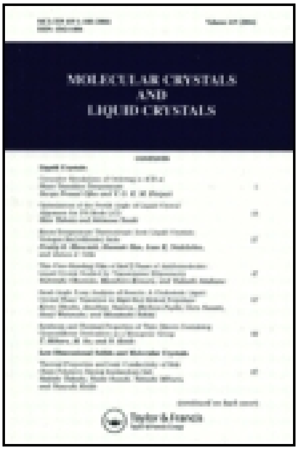

Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

Phase Transitions in Polypeptide Liquid Crystals: A Reentrant Isotropic Phase

R. Subramanian [a] , R. J. Wittebort [a] & D. B. Dupré [a]

[a] Department of Chemistry, University of Louisville, Louisville, Kentucky, 40292

To cite this article: R. Subramanian , R. J. Wittebort & D. B. Dupré (1983): Phase Transitions in Polypeptide Liquid Crystals: A Reentrant Isotropic Phase, Molecular Crystals and Liquid Crystals, 97:1, 325-336

To link to this article: http://dx.doi.org/10.1080/00268948308073161

Mol. Cryst. Liq. Cryst., 1983, vol. 97, pp. 325–336
0026-8941/83/9704–0325/$18.50/0

Phase Transitions in Polypeptide Liquid Crystals: A Reentrant Isotropic Phase*

R. SUBRAMANIAN, R. J. WITTEBORT and D. B. DuPRÉ

Department of Chemistry, University of Louisville, Louisville, Kentucky 40292

(Received January 20, 1983)

Optical and C-13 NMR examinations of concentrated solutions of poly-γ-benzyl-L-glutamate (PBLG) in a mixed solvent system containing a denaturant acid, show that this polymer liquid crystal has both a low and high temperature isotropic phase. The latter is due to thermal disruption of long range orientational order of the elongated macromolecules. The former reentrant isotropic phase is a result of an intramolecular helix to random coil transition, which leads to a macromolecular conformation inconsistent with liquid crystallinity.

INTRODUCTION

Reentrant phases are a curiosity in liquid crystal physics as they appear to violate the premise that molecular order should increase with decreasing temperature. In some cyano compounds, for example, a smectic phase formed from cooling from higher temperature nematic structure reverts to the nematic phase at a still lower temperature.[1] Reentrant nematic phases also can be formed under the influence of pressure.[2] A reentrant isotropic phase in a discotic mixture has also been reported.[3] The possibility of a reentrant isotropic phase of a polypeptide liquid crystal was discussed theoretically[4] and pursued experimentally.[5] If a polypeptide such as poly-γ-benzyl-L-glutamate (PBLG) is dissolved in a binary solvent mixture containing a nonhelicogenic component, the α-helix of the macromolecule can be disrupted. This disruption is due to the attack on the amide

---

*Presented at the IXth International Liquid Crystal Conference, Bangalore, India, December 6–10, 1982.

hydrogen-bonded secondary structure of the macromolecule by agents such as dichloroacetic acid (DCA) and trifluoracetic acid (TFA) and results in conversion of the macromolecule to a randomly coiled conformation. The loss of polymer chain elongation is inconsistent with liquid crystallinity and an isotropic phase should result. One of the peculiarities of PBLG in such solvents is that the random coil is stable at *lower* temperatures.[6–8] This is a result of balance of energetic and entropic effects of the system (polymer plus solvent) as a whole. At higher temperatures the denaturing solvent is thrown off the polymer chain and the free energy of the solution is minimized by formation of rod-shaped, α-helical polymer molecules. The decrease in the entropy of the intramolecularly ordered α-helices is more than compensated for by the disorder of previously bound denaturant molecules now introduced as free agents in the surrounding solvent.

The polypeptide α-helix itself is stable to very high temperatures (~200°C) and in fact the polymer decomposes before reaching a helix-coil transition by this mechanism.[9] Long range orientational order of the rod shaped particles in the liquid crystal state is destroyed however around 70°C.[10] Consequently, in the presence of a denaturing solvent, solutions of PBLG prepared so as to be liquid crystalline at room temperature may form an isotropic phase on both heating and cooling. Reentrance to the isotropic phase on cooling is expected to occur at a lower temperature than the helix-coil transition point observed in dilute solution of the polymer due to induced chain rigidity concepts discussed by deGennes and Pincus[11] and the configurational partitioning ideas explored by Flory.[12] The sequence of events is represented schematically in the phase diagram of Figure 1 where the descending arrow follows the high temperature isotropic → liquid crystal → low temperature isotropic phase transitions. The phase diagram is after that of theory[4] where the high temperature isotropic transition has been tied to our previous measurement[10] of the temperature dependence of the order parameter in this lyotropic mesophase. The predicted low temperature reentrance point (~20°C) is based on certain thermodynamic parameters of the helix-coil transition and an assumed form of the intermolecular potential function. As we will see the actual reentrant isotropic phase occurs at a much lower temperature than predicted by theory.

Reported below are our experimental observations of the reentrant isotropic phase of PBLG in dichloroethane/dichloroacetic acid solution. The transitions were monitored in the polarization microscope and confirmed by C-13 NMR studies of the conformational state of the macromolecule. As this polymer liquid crystal is of the cholesteric (spontaneously twisted) variety, pitch versus temperature measurements were also made that indicate that the liquid crystal enters an isotropic phase at both high (~70°C) and low (~−20°C) temperatures.

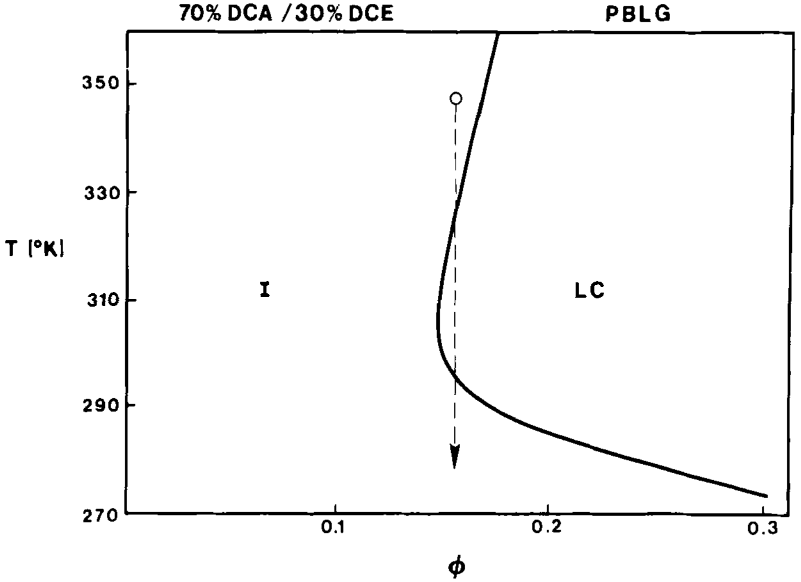

FIGURE 1 Schematic of the (T, ϕ) phase diagram of concentrated PBLG solutions in a mixed solvent system containing a denaturant acid. ϕ is the volume fraction of polymer. The descending arrow indicates a path from the high temperature isotropic phase of orientational disorder of the elongated macromolecules into a liquid crystal phase and finally to reentrance to a conformationally induced, low temperature isotropic phase. The diagram is after the theory of Rajan and Woo.[4] I: regions where the isotropic phase is stable; LC: liquid crystal region.

EXPERIMENTAL SECTION

All studies reported here were performed on solutions of PBLG of viscosity molecular weight 130,000 in mixtures of dichloroethane (DCE) and dichloroacetic acid (DCA). The latter solvent is known to induce a sharp helix to random coil transition in dilute solution of this polymer around room temperature when present in the range of 75% acid by volume.[6]

Optical observations were made between the crossed polars of a Nikon polarization microscope equipped with a photomicrographic attachment. Samples were prepared in 1 and 2 mm pathlength spectrophotometric cells and allowed to age for several weeks to assure complete solubilization of the polymer. The temperature of the cell was controlled and varied by circulating a fluid from a constant temperature bath through a special housing on the microscopic table.

Optical rotation measurements were performed on a Perkin-Elmer 241MC polarimeter in 2mm pathlength spectrophotometric cells. Measurements of the specific optical rotation, [α], in degrees per decimeter per g/100 ml were made at the Na-D line (λ = 589.1 nm). The sample was equilibrated at each temperature for one hour and data points presented below are the mean values obtained from heating and cooling cycles.

Natural abundance 90 MHz C-13 NMR spectra were obtained on a Nicolet NT-360 spectrometer at the Regional NSF NMR instrumentation Facility located on the University of Illinois Urbana campus. All spectra were obtained with the sample stationary (non-spinning mode). T_1 values were estimated from the delay time for the null magnetization in the $(90\text{-}\tau\text{-}180)_n$ inversion recovery sequence. The 90° and 180° excitation pulse widths were determined to be 18 μsecs and 38.5 μsecs, respectively. The spectrometer field was locked to an external methanol-d_4 signal and chemical shifts were measured using *p*-dioxane as the external reference. The samples were allowed to thermally equilibrate for 45 min in the spectrometer at each temperature prior to obtaining a spectrum.

RESULTS AND DISCUSSION

Optical observations

PBLG liquid crystals in the mixed solvent system containing DCA and DCE, 75:25 percent by weight, adopt a largely planar texture around room temperature when left to equilibrate overnight from a cooling cycle. The adoption of such a uniform texture in thick specimens is unusual in our experience with this polymer liquid crystal and must be attributed to this solvent system. No special preparation of the cell interwall surfaces was made. Figure 2 shows the edge of a broad uniform planar area where cholesteric striations appear. The latter features are due to views of the twisted organization at nonzero angles to the pitch axis. The planar domains could be crossed out by rotation of either analyzer or polarizer of the microscope.

Samples were heated and cooled in search of isotropic phase transitions. A 20.6% (wt/vol) liquid crystal in 75:25% (wt/wt) DCA/DCE mixed solvent showed an unequivocal cholesteric to isotropic transition in the temperature range $T_{CH\text{-}I}$ = 68–72°C. Complete extinction between crossed polars was observed at this higher temperature transition point which is due to loss of long range orientational order of the long axes of the rod shaped macromolecules. An increase in the acid composition lowers the transition point somewhat. The results for four preparations with differing acid composition are shown in Table 1.

FIGURE 2 Photomicrograph of edge of broad planar region of a PBLG (MW = 130,000) liquid crystal 20.6% wt/vol in a 75:25wt% mixture of DCA and DCE.

TABLE I

Pitch and Upper Transition Temperatures of PBLG Liquid Crystals Under Study. PBLG (MW = 130,000) in DCE and DCA Solution.

% Acid (by wt.)	0	50	75	80
Concentration of Polymer (%wt/vol)	18.3	19.7	20.6	21.3
High Temperature T_{Ch-I}(°C)	80-84°	—	79-82°	~76°C
Room Temperature Pitch (μm)	~70	~52	~40	~40

The polymer did not survive repeated heating and cooling cycles at the higher DCA concentrations. Degradation of the polymer after four cycles in the 75–80% acid range was evident by a shift in the color of the preparations from clear to dark brown and a general broadening of all IR spectral features of the liquid crystal. The samples however remained birefringent.

On cooling from room temperature, the 75% DCA sample exhibited prominent cholesteric striations around 15°C. At about −7°C these striations diverge beyond the field of view of the microscope and the sample, which was previously brightly colored in polarized light, became pale yellow. At −14°C spherulites began to appear and the sample took on a greyish hue. The spherulites grew both in size and number as the temperature was further reduced. Complete extinction was not observed however at the lowest temperature available to us (~−20°C in this apparatus). All the observations made just above this temperature suggest an imminent transition to an isotropic phase.

Measurements of the pitch (P) of the cholesteric phase as a function of temperature also support this assertion. It has been previously demonstrated[13] that the pitch of PBLG liquid crystals increases linearly with increasing temperature and tends to approach a zero slope (temperature independence) with small additions of another helix denaturant, trifluoroacetic acid (TFA). These measurements were performed in a limited temperature range around room temperature (10° to 70°C) and at TFA concentrations of less than 10%. Figure 3 illustrates the temperature dependence of the pitch for the 20.6% wt/vol sample between −17°C and 52°C. Cycling of the sample in and around room temperature causes a disruption of the planar texture and brings more periodicities of the helical axis into view. Samples were allowed to equilibrate at each temperature for about 30 minutes. Photomicrographs of the striations were taken at three different parts of the sample where at least 6–10 approximately parallel

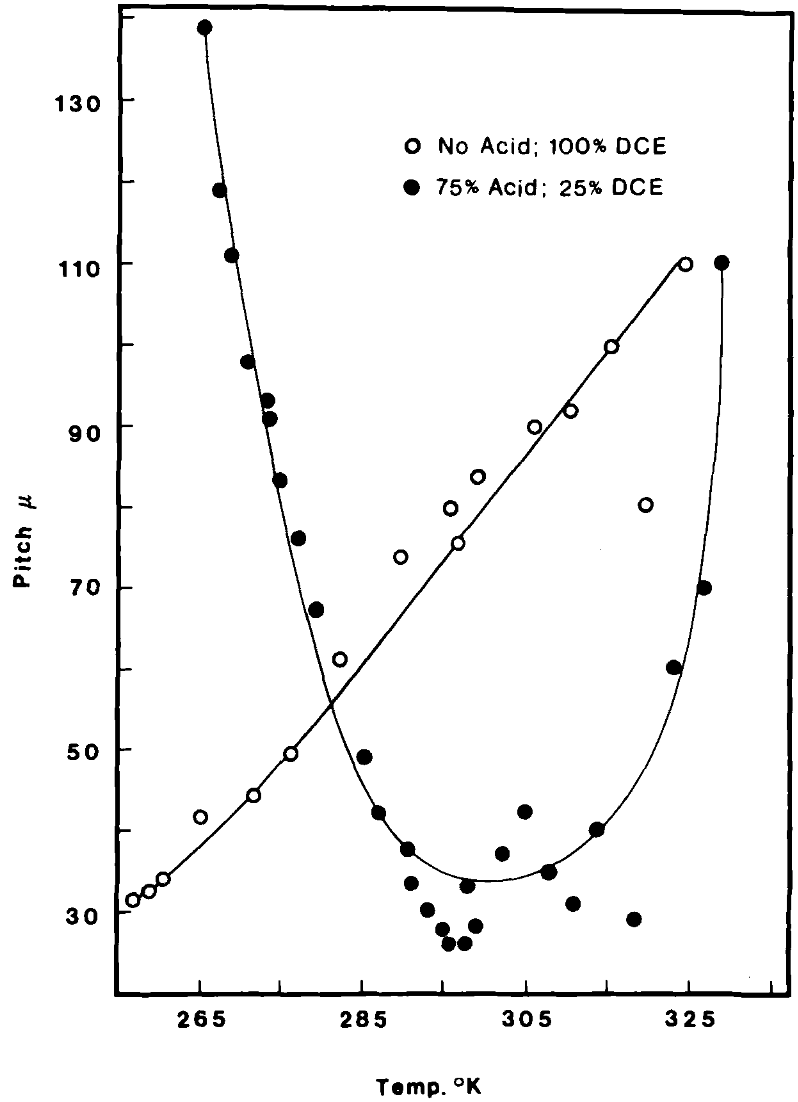

FIGURE 3 Temperature dependence of the pitch of two PBLG cholesteric liquid crystals of similar concentration with and without the presence of denaturant acid. Closed circles (●), PBLG (20.6% wt/vol) in 75:25wt% DCA/DCE solution. Open circles (○), PBLG (18.3% wt/vol) in DCE. The molecular weight of PBLG in both preparations is 130,000.

striation lines could be found. The striation separations were compared with a photograph of a stage micrometer at the same magnification and their average spacing taken as P/2. Figure 3 shows two sets of data on this polymer liquid crystal with and without the denaturant acid. In the presence of 75% DCA the pitch is seen to diverge to very large values at both high and low temperatures which is an indication of transition to either a nematic or an isotropic phase. The higher temperature transition is definitely to an isotropic phase, as verified in the polarization microscope. The similar behavior of the pitch at lower temperatures along with the other optical observations is suggestive of reentrance to an isotropic phase.

Scatter in the data around room temperature is attributable to the tendency of the liquid crystal to form a planar texture with helical axis parallel to the line of view. Reversion of tilted helical regions to the planar orientation causes distortion of the striation pattern and error in the resultant pitch measurements.

Figure 3 also contains data for a preparation of PBLG at a similar concentration (18.3% wt/vol) in DCE but without the denaturant acid. This data shows the normal linear dependence on temperature expected for a neutral solvent. The high concentration of DCA in the first sample is clearly inducing a liquid crystal transition of a different sort, presumably a result of the conversion of PBLG macromolecules into random coils. Definitive evidence of this conformational change and isotropic reentrance is provided by C-13 NMR studies discussed below. The spectra demonstrate that the macromolecular α-helix is converted into a random coil at lower temperatures (~ -20°C) even at high concentrations of polymer, and thus must force the formation of an isotropic phase.

C-13 Nuclear Magnetic Resonance

Figure 4 illustrates the temperature dependence of the C-13 NMR spectra of 10% and 22% PBLG solutions in the mixed solvent system DCA/DCE (75:25% wt/wt). The intense resonance centered at 168.3 ppm in Figure 4a and at 169.3 in Figures 4b and 4c is due to the carbonyl group of the solvent component DCA. The downfield resonances are due to PBLG carbonyl groups, either from the peptide backbone or the side chain ester. Spectral assignments and approximate T_1 values for these carbonyl resonances are listed in Table II. At 28°C where the concentrated (22%) PBLG solution is known to exist in a cholesteric phase, the NMR sample appears bright when viewed between crossed polarizers, as expected. The C-13 spectrum (Figure 4a) shows a narrow resonance with a broad overlapping or upfield shoulder. The T_1 value of the narrower component is estimated to be approximately 1 sec. (The resolution and sensitivity of the instrument is insufficient to determine whether there are two directly overlapping features or a sharp feature with a broad upfield shoul-

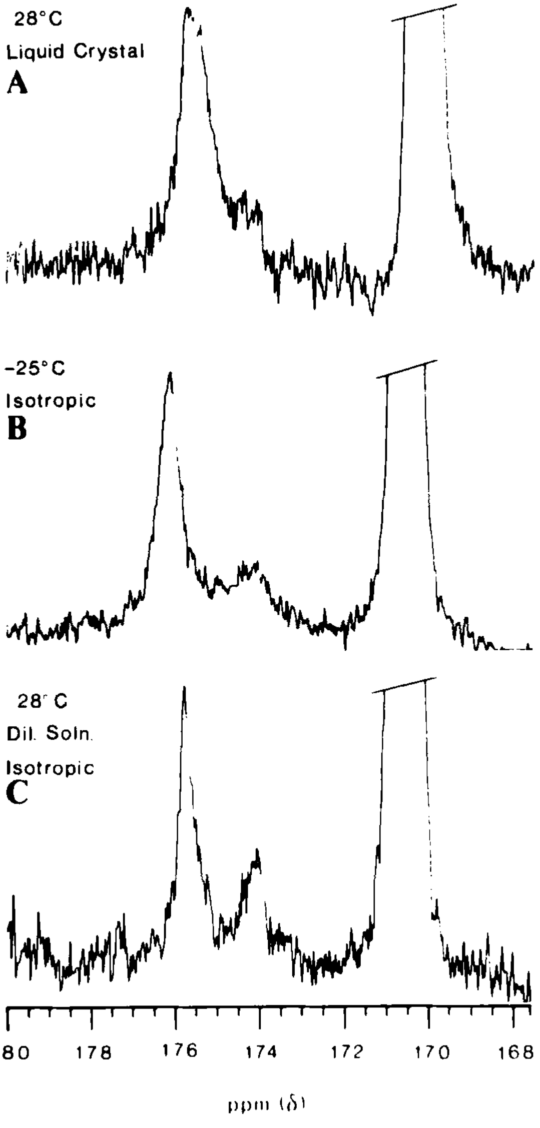

FIGURE 4 Downfield region of 90 MHz C-13 NMR spectra of PBLG (MW = 130,000) in mixed solvent of DCA and DCE (75:25 wt/wt). Spectra A and B are of the concentrated solution (22% wt/vol) of the polymer, while spectrum C is of the dilute solution (10%) in the same solvent.

TABLE II

C-13 NMR Assignments and T_1 Values for PBLG (MW = 130,000) Solutions in DCA/DCE (75/25 wt/wt).

Group C-13	LC 28°C	Reentrant Isotropic −25°C	Dilute Solution Isotropic (Random Coil) 28°C
Ester carbonyl	174.5 ppm	175.0	174.6
Amide carbonyl	174.5-173.8 ppm	173.0	172.8
DCA carbonyl	168.3 ppm	169.3	169.3
T_1 (ester)	~1 sec	≅1 sec	—
T_1 (amide)	—	≳3 sec	—

der.) On lowering the temperature to −25°C, Figure 4b, the broad shoulder shifts upfield by 0.8 to 1.5 ppm. Additional spectra show that the onset of this upfield shift occurs at ~ −10°C. At −25°C the T_1 value for the downfield narrower resonance is 2 sec whereas for the broad resonance T_1 is $\geqslant$3 sec. Because of the small recycle time of 12 sec used in these experiments some loss of intensity of the broad resonance ($T_1 \gtrsim 3$s) relative to the narrow one may have occurred due to saturation. On the basis of relative linewidths and the measured T_1 values, the sharp downfield peak at 174.5 ppm is identified with the polymer ester carbonyl functionality which would be expected to have a faster relaxation time as it is a pendent to the main polymer chain. The broader upfield feature is assigned to the motionally more restricted peptide carbonyl of the polymer backbone. In Figure 4c a spectrum obtained from a dilute (10%) PBLG solution at 28°C is shown. Under these conditions PBLG exists in an isotropic phase and consequently the NMR sample appears dark between crossed polarizers. The observed C-13 spectrum, Figure 4c, shows chemical shifts for the two resonances equivalent to those obtained for the concentrated sample at −25°C but with reduced linewidths.

Increasing the viscosity of the sample by either lowering the temperature or increasing the polymer concentration increases the T_1 values of both the polymer carbonyl resonances. Thus we can conclude that these experiments are in the slow correlation time limits where the T_1 value and the linewidths are proportional to the effective motional correlation time. This is immediately expected in these highly viscous, high molecular weight (130,000) samples. Consequently the broader upfield resonance is assigned to the motionally more restricted peptide backbone carbonyl group (longer correlation time) and the narrower line with the shorter T_1 value is assigned to the more mobile (shorter correlation time) side chain ester carbonyl group.

The effect of polypeptide helix-coil transitions on C-13 chemical shifts has been investigated by Bradbury *et al.*[14] and Chaiken.[15] For peptide carbonyls they observe upfield shifts of 1.5 to 2.0 ppm concurrent with intramolecular helix to coil transitions. Thus there are two lines of NMR evidence for the formation of a low temperature reentrant isotropic phase for liquid crystalline solutions of PBLG. First, on lowering the temperature from 28°C (cholesteric phase) to −25°C the peptide carbonyl spectrum changes to one equivalent to that obtained from an isotropic sample (Figure 4c), except for the linewidths. The broader lines in Figure 4b are due to the greater viscosity from the lower temperature and higher polymer concentration. Secondly, the ~1.5 ppm difference in the chemical shifts between the peptide carbonyls for the dilute and concentrated solutions of this polymer (Figures 4c and 4b, respectively) is just that observed for helix-coil transitions in other peptide systems. Since PBLG in the random coil state is necessarily isotropic, the presence of a reentrant isotropic phenomenon in this sytem is confirmed.

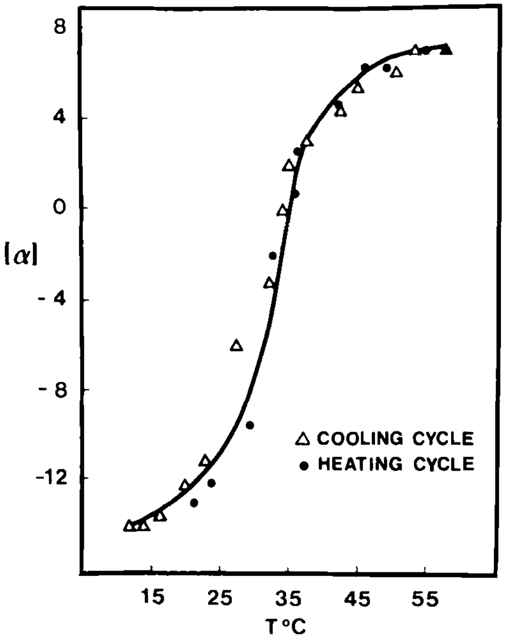

FIGURE 5 Temperature dependence of the specific optical rotation $[\alpha]_{589.1\,nm}$ of a dilute solution of PBLG (10% wt/vol) in 75:25wt% DCA/DCE. The polymer molecular weight is 130,000 as above. At the temperature of spectrum C in Figure 4 above, the polymer is in the random coil conformation [*cf.* negative sign of the optical rotation and Ref. 6].

A confirmation that the isotropic sample of spectrum C contains the polymer in the random coil configuration is presented in Figure 5 which is a plot of the temperature dependence of the optical rotation of this dilute polymer solution. At the temperature of the C-13 NMR measurement in Figure 4c, PBLG is definitely in the random coil conformation in this binary solvent. ($[\alpha]^{28°C}_{589\,nm} = -9.8°$ per decimeter per g/100 ml. The negative sign indicates a coil conformation.)[6]

Note that the reentrant phenomenon discussed in this article is induced by an intramolecular conformational change and is fundamentally different from reentrant phases previously reported in thermotropic liquid crystals.

Acknowledgments

This work was supported in part by the National Science Foundation under grant DMR-8112975 and the National Institutes of Health under grant HL 24364. Use of the University of Illinois NSF Regional Instrumentation Facility, grant NSFCHE 79-10100, is also acknowledged. A special NSF travel grant DMR-8244358 to D. B. D. to attend the IXth International Liquid Crystal Conference in Bangalore is also gratefully acknowledged. We wish to thank Dr. Richard Fernandes for many helpful suggestions at various stages of this work.

References

1. P. E. Cladis, *Phys. Rev. Lett.*, **35**, 48 (1975).
2. P. E. Cladis, R. K. Bogardus, W. B. Daniels and G. N. Taylor, *Phys. Rev. Lett.*, **39**, 720 (1977).
3. C. Destrade, P. Foucher, J. Malthete and N. H. Tinh, *Phys. Lett.*, **88A**, 187 (1982).
4. V. T. Rajan and C.-W. Woo, *Phys. Rev. A.*, **21**, 990 (1980).
5. R. Subramanian, R. J. Wittebort and D. B. DuPré, *J. Chem. Phys.*, **77**, 4694 (1982).
6. P. Doty and J. T. Yang, *J. Amer. Chem. Soc.*, **78**, 498 (1956).
7. M. Calvin, J. Hermans, Jr. and H. A. Scheraga, *J. Am. Chem. Soc.*, **81**, 5048 (1959).
8. V. S. Ananthanarayanan, E. Leroy and H. A. Scheraga, *Macromolecules*, **6**, 553 (1973).
9. A. K. Gupta, C. Dufour and E. Marchal, *Biopolymers*, **13**, 1293 (1974).
10. R. W. Duke, D. B. DuPré and E. T. Samulski, *J. Chem. Phys.*, **65**, 2748 (1977).
11. P. G. deGennes and P. Pincus, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.*, **18**, 161 (1977).
12. P. J. Flory, *Macromolecules*, **11**, 1138 (1978).
13. D. B. DuPré, R. W. Duke, W. A. Hines and E. T. Samulski, *Mol. Cryst. Liq. Cryst.*, **40**, 247 (1977).
14. L. Paolillo, P. A. Temussi, E. Trivellone, C. Crane-Robinson and E. M. Bradbury, *J. Chem. Soc. Chem. Comm.*, **335**, (1972).
15. I. M. Chaiken, *J. Biol. Chem.*, **249(4)**, 1247 (1974).