PII: S0032-3861(97)00009-8

Re-entrant isotropic transition of polypeptide liquid crystal

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(Revised 11 December 1996)

In the presence of denaturing solvent, solutions of polypeptide prepared so as to be liquid crystal at room temperature may undergo an anisotropic–isotropic re-entrant transition at low temperatures where the intramolecular helix–coil transformation occurs. Considering that the molecular conformation of isotropic phase follows Zimm and Bragg's notion for the helix–coil transition, a theoretical approach to the low-temperature re-entrant phenomenon based on the Flory lattice model was attempted in this work. The phase behaviours predicted have provided a reasonable picture for the low-temperature transitions. The calculation results also show that the re-entrant transitions receive an appreciable influence from the activity of denaturing solvent and molecular weight. Finally, a comparison was made with those data reported in the literature. © 1997 Elsevier Science Ltd

INTRODUCTION

The molecular conformation of a polypeptide such as poly(γ-benzyl L-glutamate) (PBLG) is maintained in the α helix in numerous organic solvents that support intramolecular hydrogen bonding. Above a critical volume fraction of polymer that depends on the aspect ratio of this helix, randomness of orientation about the long axes of the macromolecule is lost and a lyotropic liquid crystal is formed.

If the PBLG is dissolved in a binary solvent mixture containing a non-helicogenic component such as dichloroacetic acid (DCA) or trifluoroacetic acid (TFA), the helical stability is frequently mediated by the presence of the acid component in the solvent. The random coil form of the polymer is predominant at lower temperature where acid molecules are firmly attached to the amide linkages. As the temperature is increased, however, the parasitic acid is thrown off the polymer and the chain is able to wind up into a helix. The helical form can bring about the formation of a liquid crystalline phase, if its concentration is sufficient. On cooling down such an anisotropic solution, the denaturant solvent may attack the hydrogen bond network of the helical conformation, and convert the α helix into a random coil. Since the random coil polymer chain is geometrically inconsistent with the long-range orientational order of the liquid crystal, an anisotropic-to-isotropic transition could be observed at lower temperature. On the other hand, upon heating the above anisotropic solution, the α helix backbone becomes flexible due to the thermal energy; however, the polypeptide α helix itself is usually stable to very high temperatures. Thus, the liquid crystalline phase tends to be less stable. An anisotropic-to-isotropic transition takes place eventually at the point where the free energy of the ordered phase becomes equivalent to that of the conjugated isotropic phase. Consequently, in the presence

of a denaturing solvent, solutions of PBLG prepared so as to be liquid crystal at room temperature may form an isotropic phase on both heating and cooling. The anisotropic-to-isotropic phase transition upon cooling, the so-called re-entrant transition, was earlier reported by Subramanian et al.[1-3], and studied extensively in recent work[4-7]. The re-entrant isotropic phase occurs because the coiled chain is unable to support the liquid crystalline ordering, but the transition to the isotropic state occurs at a temperature lower than the helix–coil transition temperature measured in dilute solution.

Theoretical considerations for the possibility of a re-entrant isotropic phase of a polypeptide liquid crystal have been described in the literature. Rajan and Woo[8] prescribed a theory based on the de Genne–Pincus concept[9], which assumes an attractive orientation-dependent dispersion interaction between α-helical rod molecules. Their consideration is essentially a one-component development and does not take into account the possibility of two phases in equilibrium with one another at certain conditions of polymer concentration and temperature that the phase rule allows for in lyotropics. On the other hand, Flory and Matheson[10-12] constructed a lattice theory to predict liquid crystal formation coupled with the coil–helix transition of polymeric molecules in solution. The free energy change for the coil–helix transition has been incorporated in the lattice scheme by adopting an expression such as

$$-\sum mx(\theta \ln s - \rho \ln \sigma) \qquad (1)$$

where s and σ denote, respectively, the statistical weight for a unit in the helical state relative to the coil state and the weight factor for initiation of a sequence, m represents the number of repeat units involved in a helical sequence, θ is the fraction of polymer units in the

helical conformations and ρ is the fraction of units that mark the beginning of a helical sequence. The marked broadening of the biphasic gap and its shift to higher volume fractions were predicted as a result of the enhanced flexibility of polymer chains. However, the fact that intramolecular conformational changes take place in the low-temperature isotropic phase has not been considered, and no explanation can be made about the re-entrant isotropic transition by this model. Recently, a theory based on the above lattice model has been put forward by Lin *et al.*[7] on the assumption that the polymer–solvent interaction parameter, χ, takes a negative value in both isotropic and anisotropic phases. The calculated result which rests mainly on analysis of the χ parameter shows that different polymer–solvent interactions in the isotropic and anisotropic phases could play a significant role in the re-entrant transition. But the conformation changes in the isotropic phase have not yet been taken into account.

In the present study, a theoretical approach with respect to the re-entrant phenomenon was attempted on the basis of the Flory–Matheson lattice model, and the essential concept of the previous work is considered i.e. the χ parameter is negative[7]. A further improvement to theory was made under the consideration that the molecular conformation in the isotropic phase is temperature-dependent and follows the notion of Zimm and Bragg[13] for the statistical thermodynamics of the helix–coil transition. The phase behaviour calculated on this basis has provided a reasonable picture for the low-temperature anisotropic–isotropic transition. Influences of acid activity and molecular weight on the re-entrant isotropic transitions were also discussed. Finally, the theoretical predicted results have been compared with published experimental observations.

RESULTS AND DISCUSSION

Theoretical background

Free energy expressions applicable to a polymer chain which can assume either rodlike or random-coil conformations have been developed by Flory and Matheson within the framework of the lattice model[10–12]. The partition functions derived for a binary system are solved for the equilibrium condition, i.e.

$$(\mu_s - \mu_s^0)/RT = (\mu_s' - \mu_s^{0\prime})/RT \qquad (2)$$

and

$$(\mu_p - \mu_p^0)/RT = (\mu_p' - \mu_p^{0\prime})/RT \qquad (3)$$

where μ_s and μ_p designate, respectively, chemical potentials of the solvent and the polymeric solute composed of x segments, the prime denoting the anisotropic phase. Chemical potentials of the components in an isotropic phase are given by[12]

$$(\mu_s - \mu_s^0)/RT = \ln(1 - v_p) + (1 - 1/x)v_p + \chi v_p^2 \qquad (4)$$

and

$$(\mu_p - \mu_p^0)/RT = \ln v_p + (x - 1)v_p + \chi x(1 - v_p)^2$$
$$+ mx\ln[1 - \rho/(1 - \theta)] \qquad (5)$$

where v_p is the volume fraction of the polymer component and χ is the familiar solvent–polymer interaction

parameter. Since the polymer chain is assumed to occur in a highly helical form in the anisotropic phase (see the section below), θ may be replaced by unity and ρ is appropriately set equal to $1/mx$ as specified by Flory and Matheson[12]. The obtained chemical potentials for the fully helical nematic phase are

$$(\mu_s' - \mu_s^{0\prime})/RT = \ln(1 - v_p') + v_p'(y - 1)/x$$
$$- \ln[1 - v_p'(1 - y/x)] + \chi v_p'^2 \qquad (6)$$

and

$$(\mu_p' - \mu_p^{0\prime})/RT = \ln v_p' + v_p'(y - 1) - \ln f_1$$
$$+ \chi x(1 - v_p')^2 - mx\ln s - \ln \sigma \qquad (7)$$

where y is the disorientation index. These expressions are similar to the results given in the early version for the nematic solutions of rigid rods[14] apart from the presence of the terms $-mx\ln s$ and $-\ln \sigma$. These differences reflect the choice of a new reference state[12]. Auxiliary relations required for the equilibrium conditions in the individual phase are

$$f_p = \int_0^{\pi/2} \sin^p \psi \exp(-\alpha \sin \psi)d\psi \qquad (8)$$

$$\alpha = -(4/\pi)x\ln[1 - v_p'(1 - y/x)] \qquad (9)$$

$$y = (4/\pi)x(f_2/f_1) \qquad (10)$$

$$s = (1 - \rho/\theta)[1 - \rho/(1 - \theta)] \qquad (11)$$

$$\sigma = \rho^2/(\theta - \rho)(1 - \theta - \rho) \qquad (12)$$

Numerical calculations[12], under the assumption that θ is independent of the χ value, or equivalently temperature, show that relaxation of rigidity in the isotropic phase renders the transition more gradual from the broad biphasic range to the narrow gap characteristic of systems of rigid rods as the value of the χ parameter becomes more positive. The transition range is shifted simultaneously to higher concentrations.

Molecular conformations in anisotropic and isotropic phase

Both theoretical calculations and experimental observations suggest that polypeptide chains remain in a highly rigid α-helical conformation as long as they are involved in the liquid crystal phase. According to the calculations reported by Flory and Matheson[12], for systems in which the degree of helicity θ and the mean helical axis ratio $\theta/\rho m$ are sufficient to engender stable anisotropy, the free energy invariably reaches its minimum value at complete helicity. Partially helical anisotropic states may be dismissed for any systems at equilibrium.

Experimental observations[4–7], based on the deuterium quadrupolar splitting data obtained by the ^2H n.m.r. method, indicate that PBLG takes a nearly perfect α-helical conformation arranged in a highly ordered fashion when the molecule exists in the PBLG/DCA/ dichloroethane (EDC) liquid crystal state. An intermediate partially helical configuration is unstable. The nearly complete exclusion of heterogeneous species from the given phases inevitably enhances cooperativity in the helix–coil transition in an anisotropic state.

Based on the discussion above, the molecular conformations of the liquid crystal phase are assumed to take a rigid rod state in the present studies. On the other hand, as for the chain conformations in the isotropic phase, the molecules are able to assume either a rodlike or random-coil state under different conditions, notably other temperatures and in the presence of denaturant solvent.

The conformational state of a polypeptide chain in an isotropic solution can be treated theoretically by the methods of statistical thermodynamics. In the theoretical approach considered by Zimm and Bragg[13], the statistical weight of a helical residue, s, can be related to a change in the Gibbs free energy ΔG_0 for the helix formation

$$s = \exp(-\Delta G_0/RT) = \exp(-\Delta H_0/RT + \Delta S_0/R) \tag{13}$$

where ΔG_0 is broken up into intrinsic enthalpy and entropy changes, ΔH_0 and ΔS_0, and which are usually around $-1000\,\mathrm{cal\,mol^{-1}}$ and $-2\,\mathrm{cal}\,(\mathrm{mol\,K})^{-1}$, respectively for PBLG polypeptides[15]. The other parameter of importance, σ, has been shown to be around 10^{-4} for polypeptides[15]. For high molecular weight polypeptides, the helix fraction θ is given by

$$\theta = 1/2 + (s-1)/2[(1-s)^2 + 4\sigma s]^{1/2} \tag{14}$$

Since the defined ΔH_0 is negative, s will increase as the temperature goes down[16]. The helical form is thus stable at low temperatures. The helix-to-coil transformation described by this model is normal (helix → coil with increasing temperature). However, inverse (coil → helix as the temperature increases), even multiple (coil → helix followed by helix → coil as the temperature increases) transitions are usually seen in acid-mediated solutions of synthetic polypeptides[17,18]. To properly account for the inverse or multiple transitions, the role of the solvent must be written explicitly. It is frequently assumed that the organic acid molecule binds with the amino acid residue when the residue is exposed in the coiled conformation. To reflect the binding impediment, the factor of s could be replaced by[15]

$$s_c = s/(1 + Ka) \tag{15}$$

where a is the acid activity proportional to the concentration of acid, and K is another equilibrium constant due to the binding which occurs through hydrogen bonding to exposed amide linkages in the coiled state. K can be written in an expression of the form

$$K = \exp(-\Delta G_c/RT) = \exp(-\Delta H_c/RT + \Delta S_c/R) \tag{16}$$

where $\Delta H_c < 0$, $\Delta S_c < 0$. By this model, two transitions (as inverse followed by a normal transformation as temperature is increased) can be predicted for certain values of the thermodynamic parameters and acid activities, as sketched in *Figure 1*. The polymer concentration influence on the intramolecular conformational transformation in an isotropic solution is weak, as reported in the literature[19], and such an effect is ignored in the present study.

The polypeptide α helix itself is generally stable to very high temperatures (about 200°C); in fact, the polymer decomposes before reaching a helix–coil transition by this mechanism. However, as shown by the intrinsic
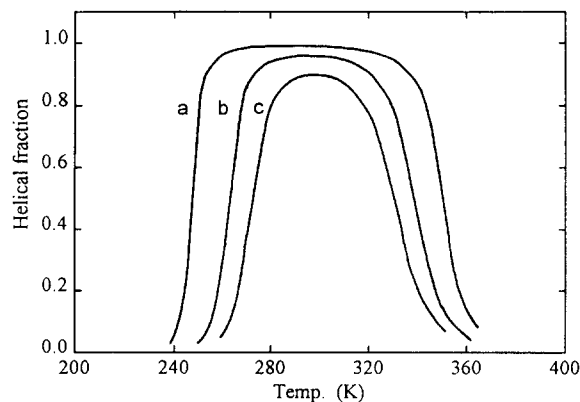

Figure 1 Temperature dependence of the helical fractions in the isotropic phase at three acid activities: (a) 0.075, (b) 0.085 and (c) 0.09. Here $\Delta H_0 = -1000\,\mathrm{cal\,mol^{-1}}$, $\Delta S_0 = -2.5\,\mathrm{cal}\,(\mathrm{mol\,K})^{-1}$, $\Delta H_c = 3000\,\mathrm{cal\,mol^{-1}}$, $\Delta S_c = -6.67\,\mathrm{cal}\,(\mathrm{mol\,K})^{-1}$, and $\sigma = 10^{-4}$

viscosity studies[20], the α-helical backbone tends to be much more flexible with an increase in temperature to about 100°C. Such a relaxation of chain flexibility plays an important role in the high-temperature anisotropic–isotropic phase transition, and may be artificially manifested by the high-temperature helix–coil transition as described above. With respect to the low-temperature helix–coil transitions, many investigations have been done with various techniques[15].

Numerical calculations

In the Flory theory, χ is the parameter defined as the interaction between polymers and solvents, and is responsible for the temperature dependence of phase equilibrium conditions. The transition associated with the low-temperature helix–coil conformational changes in the isotropic phase is known to be induced by the presence of the acid component, i.e. as the temperature is lowered the denaturant molecules start attacking the hydrogen bond involved in the α-helical architecture, and finally convert the α helix into a random coil. As suggested by Abe[5], the χ parameter in the present condition should be considered to be negative over the whole temperature range employed, and corresponds to a good solvent, in which the enthalpy decrease contributes to the free energy reduction on solution. In this work, the χ value which is usually regarded as an inverse measure of temperature is also taken to be of the form $A + B/T$, with A and B specified arbitrarily as -1.667 and 352.

The relationships that determine the conditions for the stable coexistence of a fully helical nematic phase in equilibrium with the conformational variable isotropic phase can be obtained by equating equation (4) to equation (6) and equation (5) to equation (7) with auxiliary relations of equations (8)–(12). The molecular conformations of the isotropic phase as manifested by the degree of helicity θ are followed by equations (13)–(16). A typical result of numerical solutions of these relationships is demonstrated by curve a in *Figure 2* where both χ and temperature are plotted against the volume fractions v_p and v'_p in the coexisting phase. On the left-hand side of the diagram (low v_p) all solutions are isotropic. Within the intermediate χ value or temperature range, a single anisotropic phase forms when the polymer concentration becomes higher. The transition from the
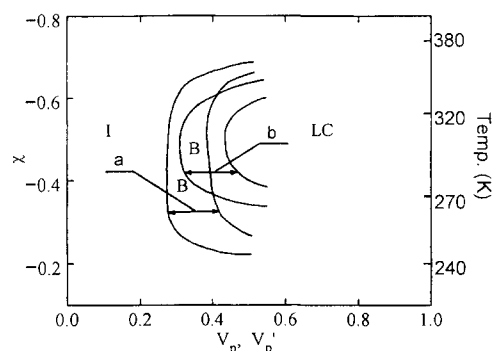
Figure 2 Phase diagram calculated for (a) $a = 0.075$ and (b) $a = 0.09$, in which the invariant parameters are $\Delta H_0 = -1000\,\mathrm{cal\,mol^{-1}}$, $\Delta S_0 = -2.5\,\mathrm{cal\ (mol\,K)^{-1}}$, $\Delta H_c = 3000\,\mathrm{cal\,mol^{-1}}$, $\Delta S_c = -6.67\,\mathrm{cal}$ $(\mathrm{mol\,K})^{-1}$, $\sigma = 10^{-4}$ and $x = 100$ (I, isotropic phase; B, biphasic region; LC, liquid crystalline phase)

isotropic of the anisotropic phase is bridged by a biphasic chimney. As the temperature goes down, or equivalently χ turns positive, v_p and v_p' tend to be higher, and eventually no anisotropic phase can be formed. In other words, with a decrease in temperature, an anisotropic-to-isotropic re-entrant transition caused by the intramolecular helix–coil transformation is predicted. In this re-entrant isotropic region, the rigid helix anisotropic phase is in equilibrium with an isotropic solution comprising flexible chain molecules. At higher temperatures, a gradual blend of the chimney towards a higher concentration region is demonstrated. Such a bending is related to the enhanced chain flexibility in the isotropic phase caused by the thermal energy at elevated temperatures as normally observed in a real polymer system[21]. In this work, the relaxation of rigidity in the isotropic state is artificially described by the helix–coil transition at higher temperatures.

The effect of acid activity a is also illustrated in *Figure 2*. The binodals shown have been calculated for (a) $a = 0.075$ and (b) $a = 0.090$. The other thermodynamic parameters used in the numerical calculations are indicated in the caption. As can be seen from *Figure 1*, the acid activity plays an important role in determining the molecular flexibility in the isotropic phase. Higher acid activity gives rise to a flexible chain rigidity and a narrow region of the helix conformational state. With increasing acid activity, the critical equilibrium is shifted to higher volume fraction, and the lower temperature re-entrant isotropic transition tends to take place at higher temperatures.

It is also instructive to look at a plot, shown in *Figure 3*, in which both high- and low-temperature anisotropic–isotropic transition temperatures are portrayed as functions of acid activity at a given polymer concentration. Since the intramolecular helix–coil transition can be induced by the increasing amount of denaturant agents, and the coiled chain conformation is unable to support the liquid crystalline ordering, an anisotropic-to-isotropic transition with increasing acid activity is predicted, as traced out by the arrow in *Figure 3*.

The dependence of molecular weight is illustrated in *Figure 4* by binodal pairs for (a) $x = 100$ and (b) $x = 200$. With decreasing x value, the liquid crystal–isotropic miscibility gap tends to broaden and shift towards higher volume fractions in both the high- and low-temperature transitions.

Comparison with experimental results

The phase behaviour of PBLG in a denaturant solvent mixture of DCA/EDC has been reported by Subramanian *et al.*[1–3]. An extensive study based on optical observation and n.m.r. measurement was described recently[4–7]. The phase diagrams shown as a function of PBLG volume fraction in the mixed solvent DCA/EDC obtained by Subramanian *et al.* and Lin *et al.* are reported in *Figure 5*. Two anisotropic–isotropic transitions occur at high and
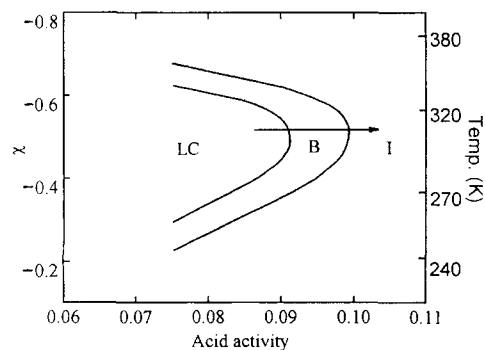

Figure 3 Plot of χ values and phase transition temperatures *versus* acid activity at a given polymer volume fraction of 0.45. Other thermodynamic parameters used in the calculation are similar to those in *Figure 2* (I, isotropic phase; B, biphasic region; LC, liquid crystalline phase)
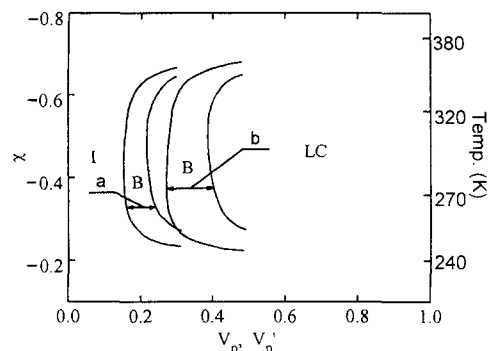

Figure 4 Phase diagrams calculated for (a) $x = 100$ and (b) $x = 200$, in which the invariant parameters are $\Delta H_0 = -1000\,\mathrm{cal\,mol^{-1}}$, $\Delta S_0 = -2.5\,\mathrm{cal\ (mol\,K)^{-1}}$, $\Delta H_c = 3000\,\mathrm{cal\,mol^{-1}}$, $\Delta S_c = -6.67\,\mathrm{cal}$ $(\mathrm{mol\,K})^{-1}$, $\sigma = 10^{-4}$ and $a = 0.075$ (I, isotropic phase; B, biphasic region; LC, liquid crystalline phase)
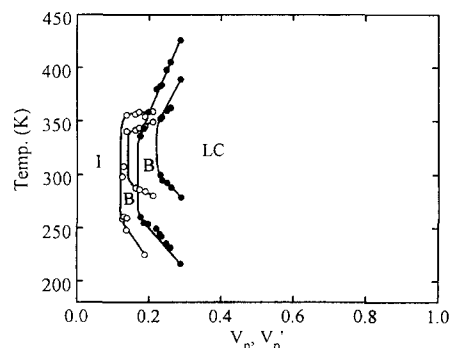

Figure 5 Experimental phase diagram for PBLG/DCA/EDC. Open circles indicate the values obtained for PBLG ($M_v = 13.0 \times 10^4$) dissolved in DCA/EDC (85.3/14.7 w/w) according to Subramanian *et al.*[3]. Also shown by solid circles are those for PBLG ($M_v = 6.2 \times 10^4$) dissolved in DCA/EDC (80/20 w/w) as reported by Lin *et al.*[4,5]. I, isotropic phase; B, biphasic region; LC, liquid crystalline phase
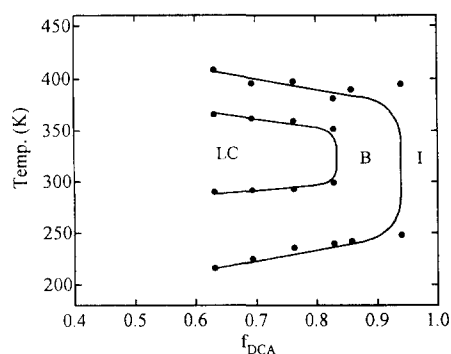
Figure 6 Experimental phase boundary curves of PBLG/DCA/EDC solutions as functions of acid composition at a given polymer volume fraction of 0.25, where f_{DCA} (mole fraction) = DCA/(DCA + EDC) according to Lin *et al.*[6,7]. I, isotropic phase; B, biphasic region; LC, liquid crystalline phase

low temperatures, with the latter a result of the intra-molecular conformation transformation. The helix–coil transition in the isotropic phase upon cooling was detected by ^{13}C n.m.r. by Lin *et al.* An acid-induced liquid crystal to isotropic transition was also examined[6,7]. The result is replotted in *Figure 6*. With increasing acid content, liquid crystal phase tends to be destabilized along both the high- and low-temperature boundaries. In the range of high acid concentration, no liquid crystal phase could be detected at all temperatures due to the coiled molecular conformations being unable to sustain the anisotropic ordering.

Comparison of *Figure 2* with *Figure 5* and *Figure 3* with *Figure 6* illustrates a good qualitative agreement of the lattice theory with observations on the PBLG/DCA/EDC systems. Verification is also provided by the effect of molecular weight. As shown by *Figure 5*, on increasing the M_w from 6.2×10^4 to 13.0×10^4, the isotropic to liquid crystal transition range is shifted to lower polymer concentrations. This is qualitatively in line with the theoretical calculation of *Figure 4*. However, the critical volume fractions v_p and v_p' over the whole temperature range predicted by the theory seem too high. This may be attributed to neglect of the orientation-dependent inter-actions of the anisotropic phase, which would play an important role in stabilizing the liquid crystalline phase. The quantitative comparison between theory and experiment is also hindered by relating the lattice-dependent parameters to the conventional measurements of quantities, such as $\chi - T$ relationships.

As stated by Flory in the 1950s[22], the lattice model, in spite of its artificiality, has proved most successful in the treatment of liquid crystallinity in polymeric systems[23]. The versatility of this theory has permitted its extension to polydisperse systems[24,25], to mixtures of rodlike polymers with random coils[26] and to some of the many kinds of semirigid chain[27]. The subject studied in this work is one of such example. Although the isotropic interaction plays an important role in the re-entrant transition as reported by Lin *et al.*[7] through analysis of the effect of different χ parameters in the isotropic and anisotropic phases, the better agreement between the theory and the experimental

results achieved in the present study indicates that the dominant factor responsible for the re-entrant isotropic transition appears to be asymmetry of molecular shape. A correct representation of molecular conformation in the isotropic phase was found to be of primary importance in predicting the phase behaviour of liquid crystal polymers.

Also of great interest in this study is the re-entrant phase, which is a curiosity in liquid crystal physics as it appears to violate the premise that molecular order should increase with decreasing temperature.

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