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# Liquid Crystallinity in *para*-Substituted Poly γ-benzyl-L-glutamates

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The liquid crystalline behavior of poly γ-*p*-fluorobenzyl-L-glutamate, poly γ-*p*-trifluoromethylbenzyl-L-glutamate, poly γ-*p*-nitrobenzyl-L-glutamate, and poly γ-benzyl-L-glutamate has been investigated. The first two polymers are new materials while the synthesis of the latter two has been reported in the literature. Only the liquid crystalline properties of poly γ-benzyl-L-glutamate have been previously reported and this study was initiated in order to determine if electron withdrawing substituents in the para position would influence the liquid crystal behavior of these polymers. Poly γ-benzyl-L-glutamate was used as the reference standard to which the other three were compared. Under the experimental conditions employed, the equispacings for poly γ-benzyl-L-glutamate were 4–6 μm, poly γ-*p*-fluorobenzyl-L-glutamate, 6–8 μm, poly γ-*p*-trifluoromethylbenzyl-L-glutamate, 7–9 μm, and poly γ-*p*-nitrobenzyl-L-glutamate, 11–15 μm. The equispacings for the para nitro polymer show the largest difference from the unsubstituted benzyl ester polymer, with approximately twice the value than that for poly γ-benzyl-L-glutamate.

The results of this study have shown that, in the poly glutamic acid benzyl ester system, the presence of a strong electron withdrawing group in the para position of the benzyl function does influence the liquid crystal properties of this polymer system.

*Keywords: para-substituted poly-γ-benzyl-L-glutomates, -$NO_2$, F, -$CF_3$ liquid crystallinity, interspacings*

## INTRODUCTION

Proteins and synthetic polyamino acids that possess extensive sequences of the α-helix conformation can, under appropriate conditions of molecular weight, concentration, and temperature, exhibit liquid crystal behavior. The rigidity of the α-helical backbone facilitates the alignment of the polymer chains in a manner that can result in the formation of a liquid crystal system.[1–4] When the chain alignment differs in each plane of the system so that the stacked planar layers form a helix, the system will exhibit the characteristics of a cholesteric liquid crystal. These systems can also exhibit nonlinear optical properties that can be used in a variety of practical applications. Extensive studies of the liquid crystalline behavior of proteinaceous materials have not been reported.

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A few studies on the structure and properties of polypeptide liquid crystals have been reported.[1-7] Elliot and Ambrose,[8] in the 1950's, reported that solutions of the polyamino acid, poly γ-benzyl-L-glutamate [PBLG], exhibited liquid crystalline properties.

In many polypeptide systems, mesomorphic forms can be produced by heating the materials already in a particular solid phase and, with some substances, liquid crystallinity can be achieved by the addition of a limited amount of an appropriate solvent.

The biphasic phenomenon or occurrence of self-ordering in solutions of rigid rodlike molecules is known to be concentration dependent.[9-13] This behavior can be seen in some polypeptides where there is a critical concentration range in which the liquid crystal and the isotropic phase can coexist. As the concentration of a solution of the polyamino acid, poly γ-benzyl-L-glutamate [PBLG] is increased, it separates into two phases. Only one of these phases is birefringent and the birefringent phase is the one that has the higher concentration of PBLG. Phase separation occurs at a specific PBLG concentration denoted by the letter A. The resulting system is stable over a concentration range commonly denoted as A to B. When the concentration of PBLG is raised above concentration B, only the birefringent phase is observed. The concentration A delineates the appearance of the birefringent phase and when the concentration B is exceeded, the uniform birefringent phase is completed.[1-4]

Except for the studies on PBLG, the only other studies of polyglutamate esters that have been carried out are on the methyl, ethyl and some longer chain aliphatic esters. The rationale for the present study is based on our interest in determining how a change in the side chain dipole of a polyglutamate ester system influences both the liquid crystal properties and the nonlinear optical properties of that system. In the present study we report the effect of attaching strong electron withdrawing groups in the para position of the benzyl function to determine its influence on the liquid crystal properties of the polymer. The substituent nitro, fluoro, and trifluoromethyl groups should be sufficiently electronegative to allow the polarity of the γ-benzyl side chain to make a significant difference relative to the unsubstituted poly amino acid ester. Poly γ-benzyl-L-glutamate served as a reference for the substituted analogs and permitted a comparison of the transition behavior from the isotropic phase to the continuous birefringent phase as defined by the polymer concentrations A and B.

## EXPERIMENTAL PROCEDURE

The polymers poly γ-*p*-fluorobenzyl-L-glutamate [PGLU(pFB)], poly γ-*p*-trifluoromethylbenzyl-L-glutamate [PGLU(pTFMB)], poly γ-benzyl-L-glutamate [PBLG], and poly γ-*p*-nitrobenzyl-L-glutamate [PGLU(pNB)] were synthesized using the procedure reported by Hanby[14] and modified by Ledger and Stewart.[15] Typical protocols are given below.

### Preparation of γ-benzyl-L-glutamate

L-Glutamic acid (5 g, 0.0339 mole) and benzyl alcohol (33.3 ml, 0.32 mole) were mixed under a blanket of nitrogen. After a few minutes, concentrated hydrochloric acid (5 ml, 0.16 mole) was added, with stirring, at room temperature. This mixture was refluxed gently for 3 hours at 100°C, until the L-glutamic acid completely dissolved. After the amino acid had dissolved, the solution was cooled and stored overnight at room temperature. If the solution was still clear, it was diluted with ethanol (66.7 ml) and treated with 10 ml of pyridine to neutralize it. After dilution and neutralization, the solution was stored in an ice bath overnight. The resulting precipitate was collected on a Buchner funnel and washed with ethanol and ether. This procedure yielded a white precipitate of γ-benzyl-L-glutamate. The product was dried overnight under vacuum at 35°C. The crude product was recrystallized by dissolving it in 100 ml of water at 90°C and then a sufficient amount of sodium bicarbonate was added to bring the solution to a pH of 7. The solution was allowed to stand at room temperature for 4 hours and then stored at 5°C. The product, γ-benzyl-L-glutamate, was filtered and dried under vacuum at room temperature. Percent yield: 25–30% m.p. 170–173°C, reported[15]: 169–170°C.

Proton NMR in $d^4$-acetic acid: α-CH (4.16 ppm, triplet), β-$CH_2$ (2.32 ppm, multiplet), γ-$CH_2$ (2.70 ppm, triplet), $CH_2$ in benzyl (5.14 ppm, singlet), phenyl protons (7.356, 7.343 ppm, multiplet).

### Preparation of γ-benzyl-L-glutamate *N*-carboxy anhydride

A suspension of γ-benzyl-L-glutamate (10 g, 0.042 mole) in freshly dried and distilled 1,4-dioxane (51.3 ml, 0.60 mole) was prepared, and nitrogen was passed into the suspension for 15 minutes. The temperature of the solution was slowly increased to 60°C, and phosgene gas was bubbled into the suspension. In 1–2 hours, the mixture became homogeneous, and its white color changed to yellowish white. Excess phosgene was removed by purging the solution with nitrogen. Undissolved material was removed by filtration and the filtrate purged with nitrogen a second time. The filtrate was concentrated to 10 ml in vacuo at a maximum temperature of 50°C. The oily phase was diluted with a small amount of chloroform (1 g/1 ml), treated with charcoal, and filtered. The addition of *n*-hexane was carried out dropwise, until the cloud point was reached. The solution was stored at 4°C overnight. The white precipitate was collected on a filter, washed with *n*-hexane, and dried in a vacuum oven. Repetition of the precipitation step yielded the purified product. Yield 7.6 g, 70% m.p.: 90–95°C reported[15]: 96–97°C.

Proton NMR in $CDCl_3$: NH (6.259 ppm), α-CH (4.368 ppm), β-$CH_2$ (2.27, 2.13 ppm, multiplet), γ-$CH_2$ (2.61 ppm, triplet), $CH_2$ in benzyl, (5.145 ppm, singlet), phenyl protons (7.36 ppm).

### Polymerization of γ-benzyl-L-glutamate *N*-carboxy anhydride

To a solution of γ-benzyl-L-glutamate *N*-carboxy anhydride (5 g, 0.019 mole) in 100 ml of dry dioxane at 40°C was added 0.76 ml of a solution of triethylamine in dioxane, 0.25 mmole/ml. A second addition of 0.76 ml of initiator solution was

added so that the monomer to initiator ratio was 50. The reaction mixture then was stored in a desiccator over $CaCl_2$ or $CaSO_4$. After 5 days, the reaction mixture was slowly added to 250 ml of ethanol with vigorous stirring. A white fibrous material formed and this was separated from the suspension by centrifugation. The polymer was collected on a Buchner funnel, washed with ethanol and ether and dried in a vacuum oven for several days. Percent yield 92%.

### Preparation of *p*-nitro-γ-benzyl-L-glutamate

Sodium copper L-glutamate was prepared as described in the literature.[14,15] The sodium copper L-glutamate (4 g, 0.01 mole) was dissolved in 200 ml of water and a solution of *p*-nitrobenzyl bromide (4.32 g, 0.02 mole) in dimethylformamide (200 ml) was added, with stirring, to the water solution at 45–50°C. This resulting blue suspension was diluted with 500 ml of acetone after 30 hours, and filtered through a Buchner funnel. The collected precipitate was washed with acetone and water to remove unreacted *p*-nitrobenzyl bromide and sodium copper L-glutamate. The dried copper complex was dissolved in boiling EDTA (pH 4.5) and boiled for 30 min. The hot solution was filtered to remove any undissolved residue, and then stored at 5°C overnight. The resulting yellowish γ-*p*-nitrobenzyl-L-glutamate (1.65 g) that precipitated was filtered and dried in a vacuum oven at 50°C for 24 hours. Percent yield: 29%, m.p. 170–175°C; reported[14,15] 171–172°C.

### Preparation of γ-*p*-nitrobenzyl-L-glutamate *N*-carboxy anhydride

A suspension of γ-*p*-nitrobenzyl-L-glutamate (0.5 g, 1.77 mmole) in 20 ml of dioxane was stirred for 10 minutes. Then nitrogen was passed into the suspension for 15 minutes. The suspension was slowly heated to 50°C and triphosgene (0.53 g, 1.785 mmole) was added in a single portion. Elevation of the temperature to 50°C should proceed slowly! After two hours, the mixture became almost homogeneous. The solution was kept at 40°C and nitrogen was passed through for two hours in order to remove any residual phosgene. The solution was filtered, and the solvent evaporated to give an oily residue. A small amount of ethyl acetate was added, with stirring, to dissolve the oil. When the solution became homogeneous, *n*-hexane was added dropwise to the cloud point, and the purged solution stored at 5°C overnight. Repetition of the precipitation step yielded γ-*p*-nitrobenzyl-L-glutamate *N*-carboxy anhydride, 0.46 g, 84% yield. m.p.: 101–104°C; reported[15] 100–101°C.

Proton NMR in DMSO-d[6]: NH (9.12 ppm, singlet), α-CH (4.48 ppm, quartet), β-$CH_2$ (2.10, 1.95 ppm, multiplet), γ-$CH_2$ (2.61 ppm, triplet), $CH_2$ in benzyl (5.26 ppm, singlet), two protons in phenyl (7.66, 7.64 ppm, doublet), another two protons in the phenyl ring (8.25, 8.23 ppm, doublet).

### Preparation of Poly γ-*p*-nitrobenzyl-L-glutamate

The γ-*p*-nitrobenzyl-L-glutamate *N*-carboxy anhydride (0.31 g, 1 mmole) was dissolved in 20 ml of freshly dried dioxane and a solution containing 0.25 mmole of triethylamine in 0.08 ml dioxane was added as the initiator. The solution was placed in a desiccator over calcium sulfate and kept for several days. The reaction was

then diluted with ethanol to precipitate the polymer. The polymer was collected on a fritted glass filter, washed with hot ethanol, cold ethanol, ether and then dried under vacuum at room temperature. Yield 0.3 g, 93%.

Analysis, Calc'd for $(C_{12}H_{12}N_2O_5)_n$: C, 54.55; H, 4.58; N, 10.60;
Found: C, 54.31; H, 4.58; N, 10.41

Proton NMR in DMF-d[7]: NH (8.60 ppm, broad), α-CH (4.25 ppm, singlet), β-$CH_2$, γ-$CH_2$ (2.942, 2.347 ppm, (2.575 ppm, broad), $CH_2$ in benzyl (5.294 ppm, singlet), two protons in the phenyl ring (7.66, 7.68 ppm, doublet), another two protons in the phenyl ring (8.26, 8.28 ppm, doublet), Dioxane (3.5 ppm, singlet), DMF (2.74, 2.91 ppm, strong).

### Preparation of γ-*p*-fluorobenzyl-L-glutamate

The monomer γ-*p*-fluorobenzyl-L-glutamate, was prepared using the same procedure that was employed for the synthesis of γ-*p*-nitrobenzyl-L-glutamate. Yield 25%, recrystallized from water, m.p. 165–169°C.

Proton NMR in DMSO-d[6]: OH (7.92 ppm, singlet broad), α-CH (4.08 ppm, quartet), β-$CH_2$ (1.98, 2.41 ppm multiplet, γ-$CH_2$ (2.12 ppm, multiplet), $CH_2$ in benzyl (4.47 ppm, singlet), two protons in the phenyl ring (7.16, 7.13 ppm, multiplet), another two protons in the phenyl ring (7.32, 7.34 ppm, multiplet).

### Preparation of γ-*p*-fluorobenzyl-L-glutamate *N*-carboxy anhydride

The anhydride was prepared by a modification of the procedure for the preparation of the *p*-nitro anhydride. The γ-*p*-fluorobenzyl-L-glutamate was suspended in dry tetrahydrofuran, heated to 50°C, and 20 ml of toluene containing 20% phosgene was added. Workup and isolation of the anhydride was the same as for the *p*-nitro anhydride. Yield was 90%, m.p. 60–65°C.

Proton NMR in $CDCl_3$: NH (6.92 ppm, singlet), α-CH (4.42 ppm, singlet), β-$CH_2$ (2.15, 2.30 ppm multiplet), γ-$CH_2$ (2.577 ppm, triplet), $CH_2$ in benzyl (5.09 ppm, singlet), two protons in the phenyl ring (7.07, 7.04 ppm, multiplet), another two protons in the phenyl ring (7.32, 7.28, 7.23 ppm, multiplet).

### Preparation of Poly γ-*p*-fluorobenzyl-L-glutamate

The polymerization was carried out essentially as described for the polymerization of γ-*p*-nitrobenzyl-L-glutamate *N*-carboxy anhydride. The yield of polymer was 85%.

Analysis, Calc'd for $(C_{12}H_{12}NO_3F)_n$: C, 60.76; H, 5.10; N, 5.90
Found: C, 60.66; H, 5.12; N, 5.69

Proton NMR in $CDCl_3$: NH (8.30 ppm, singlet), α-CH (3.955 ppm, singlet), β-$CH_2$, γ-$CH_2$ (2.51, 2.20 ppm, broad), $CH_2$ in benzyl (5.00 ppm, singlet), two protons in the phenyl ring (6.99 ppm, singlet), another two protons in the phenyl ring (7.26 ppm, singlet).

### Preparation of γ-*p*-trifluoromethylbenzyl-L-glutamate

The γ-*p*-trifluoromethylbenzyl ester was prepared using two methods. Direct esterification of the amino acid with trifluoromethyl benzyl alcohol and *p*-toluenesulfonic acid as the catalyst in benzene solvent gave the desired derivative in 30% yield after workup. The desired ester was also prepared from the sodium-L-copper glutamate and α′-bromo- α,α,α, -trifluoro-*p*-xylene in DMF solution. Typical yields were 30% regardless of the synthetic method. Recrystallization from water gave a m.p. of 161–165°C.

Proton NMR in DMSO-d$^6$: OH (7.9 ppm, singlet), $NH_2$ (5.42 ppm, broad singlet), α-CH (4.08 ppm, quartet), β-$CH_2$ (2.30, 1.95 ppm multiplet), γ-$CH_2$ (2.13 ppm, multiplet), $CH_2$ in benzyl (4.59 ppm, singlet), two protons in the phenyl ring (7.55, 7.53 ppm, doublet), another two protons in the phenyl ring (7.70, 7.68 ppm, doublet).

### Preparation of γ-*p*-trifluoromethylbenzyl-L-glutamate *N*-carboxy anhydride

The anhydride was prepared as described for the preparation of γ-*p*-fluorobenzyl-L-glutamate *N*-carboxy anhydride. A second synthetic route was also employed with the modification that triphosgene was used instead of the 20% solution of phosgene in toluene. The product was reprecipitated several times from ethyl acetate by the addition of *n*-hexane. Yield was 75%, m.p. 50–52°C.

Proton NMR in $CDCl_3$: NH (6.45 ppm, singlet), α-CH (4.40 ppm, singlet), β-$CH_2$ (2.26, 2.13 ppm multiplet), γ-$CH_2$ (2.61 ppm, triplet), $CH_2$ in benzyl (5.17 ppm, singlet), two protons in the phenyl ring 7.46, 7.43 ppm, doublet), another two protons in the phenyl ring (7.63, 7.60 ppm, doublet).

### Preparation of Poly γ-*p*-trifluoromethylbenzyl-L-glutamate

Polymerization of the anhydride was carried out as described for the previous systems. The resulting polymer was purified by dissolving it in THF and precipitating the polymer in a large excess of diethyl ether. Yield 90%.

Analysis, Calc'd for $(C_{13}H_{12}NO_3F_3)_n$: C, 54.36; H, 4.21; N, 4.88
Found: C, 54.26; H, 4.29; N, 4.71

Proton NMR in $CDCl_3$: NH (8.35 ppm, singlet broad), α-CH (3.99 ppm, singlet), β-$CH_2$, γ-$CH_2$ (2.693, 2.32, 2.15, ppm broad triplet), $CH_2$ in benzyl (5.08, 5.05, 5.02, 4.98 ppm, quartet), two protons in the phenyl ring 7.30, 7.28 ppm, doublet), another two protons in the phenyl ring (7.49, 7.47 ppm, doublet).

The molecular weight of the polymers was determined from the intrinsic viscosity using the Mark-Houwink equation with α, and *k* values as reported by Doty *et al.*[16] for PBLG.

The cell for observing the liquid crystal behavior under an optical microscope is illustrated in Figure 1. The cell thickness is adjusted to prevent the spherical liquid crystal droplets which form from twisting or distorting. Good resolution of the surface structure or inner structure, when viewed under the microscope, depended on the sample thickness. The cells was constructed with a thickness of 0.076 and

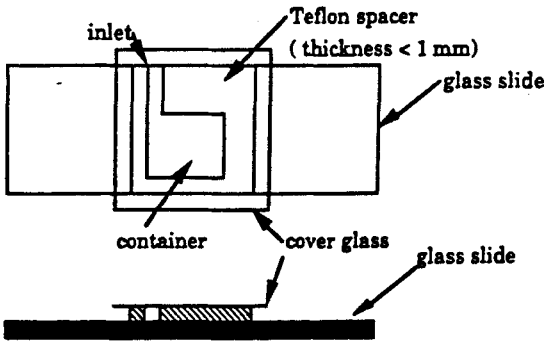


FIGURE 1 Cell for observing the liquid crystal behavior of para substituted poly-γ-benzyl-L-glutamates under the polarizing microscope. The width and length of the container are 1 inch × 1 inch.

TABLE I

| Polymer | Solvent | Intrinsic Viscosity | Molecular Weight |
|---|---|---|---|
| PBLG | DCA | 0.388 dl/gm | 49,900 |
| PGLU(pNB) | DCA | 0.354 | 52,000 |
| PGLU(pFB) | DCA | 0.299 | 42,000 |
| PGLU(pTFMB) | DCA | 0.157 | 20,000 |

The molecular weight and intrinsic viscosity of each polymer used in the present study.

0.102 mm by cementing an etched Teflon spacer with the noted thickness to a microscope slide with high performance epoxy adhesive. A cover glass was bonded to the Teflon spacer to prevent leakage of the solution. The polypeptide solution (concentation 15–25%) was injected at the top inlet. As soon as the cell was filled, all the edges and holes were sealed by application of silicone rubber cement. Any outgassing from the cement did not appear to affect the formation of the liquid crystal phase. The photographs were taken within two hours of sample preparation. The liquid crystalline phases were monitored under an optical microscope (Olympus BH-2).

## RESULTS AND DISCUSSION

The molecular weights of the polymers synthesized for this study were determined from intrinsic viscosity measurements and these results are present in Table I. The use of $\alpha$ and $k$ values for related but different polymers assumes that these values will be unchanged or minimally affected by the presence of the nitro, fluoro and triflormethyl groups at the para position of the benzyl ester.

The characteristics of the lyotropic liquid crystalline behavior of the three polypeptides [PGLU(pFB), PGLU(pNB), and PGLU(pTFMB)] were investigated with respect to their phase transition concentrations A and B.

### Phase Transition of PGLU(pNB) in 1-Methyl-2-pyrrolidinone

PGLU(pNB) in 1-methyl-2-pyrrolidinone (NMP), at a concentration of 15% (wt/v), showed no liquid crystalline domains. Increasing the concentration to 21% (wt/

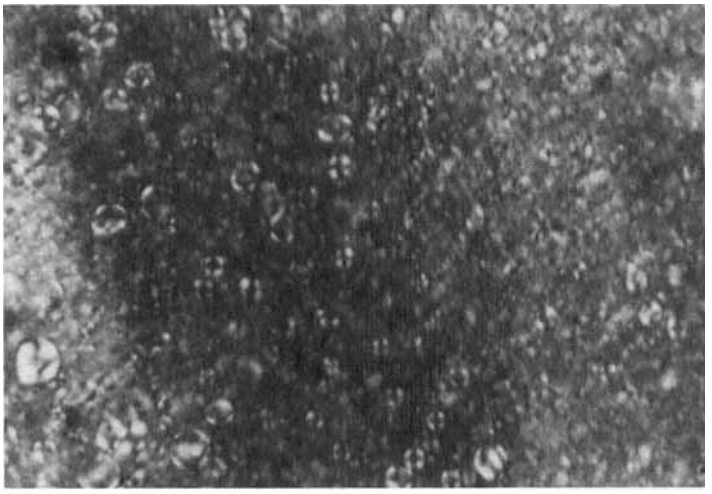

FIGURE 2 Liquid crystal behavior of PGLU(pNB), at 25°C, showing an early stage of the biphasic region under the polarizing microscope. Solvent is 1-methyl-2-pyrrolidinone. Mag X50. See Color Plate I.

v), resulted in the growth of a small anisotropic phase at the edge of the cell. In this solvent the droplets exhibit a well-developed Maltese Cross pattern indicative of the spherical symmetry of the droplet (Figure 2). The smallest spherical droplet observed had a diameter of 20 μm, and the largest spherical droplet had a diameter of 50 μm.

The disruption of the nematic droplets and their coalescence is attributed to Brownian motion induced collisions of the small spherical particles with each other as well as collisions with the cell wall. Large spherical droplets (dia. > 50 μm), were sometimes distorted and, by coalescing, tended to form a large nematic droplet. Nevertheless, small floating spheres with a Maltese Cross pattern dispersed in the isotropic phase, were observed in 21% (w/v) solutions. When the polymer concentration was increased to 30% (wt/v) the solution had already passed through the transition concentration, A. Samples prepared from a more concentrated solution (30–35% [wt/v]) than the sample concentrations in the pretransitional region, contained large, fully developed spheres. The largest spheres, that were not disrupted or coalesced, had a diameter of 85 μm. Although the solution consisted of the uniform birefringent phase of the solute in some regions of the cell, the large particles with defined annular lines were still present in the dispersed region. At this stage, the largest liquid crystalline droplets exhibiting equidistant lines had a diameter of 250 μm.

In the initial stage of the biphasic region, as seen in Figure 3, the liquid crystalline phase develops at the interface between the birefringent and the isotropic phases rather than near the center of the cell. In addition to the birefringent liquid droplets, there is also a region of schlieren like texture indicative of a multidomain structure. However, at a distance from the birefringent area, the spherical liquid crystalline domains are still present. Observations over an extended period of time revealed that the units with a Maltese Cross pattern and annular lines were distorted or disrupted and formed a group of large birefringent droplets. The largest liquid

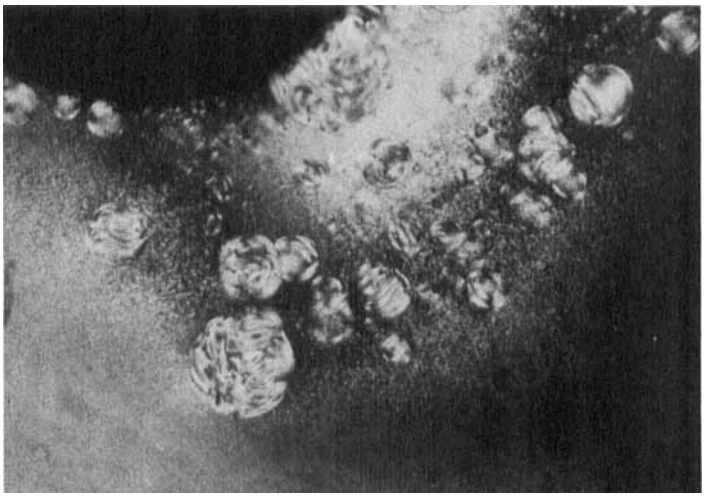

FIGURE 3 Liquid crystal behavior of PGLU(pNB), at 25°C, showing a late stage of the biphasic region under the polarizing microscope. Solvent is 1-methyl-2-pyrrolidinone. Mag X100. See Color Plate II.

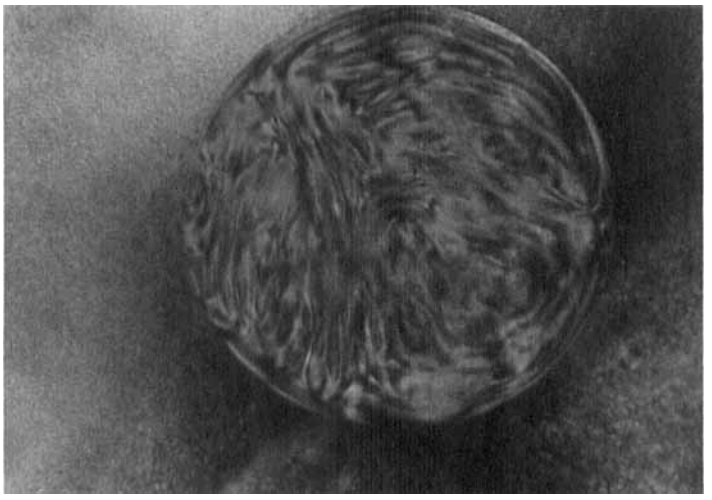

FIGURE 4 Liquid crystal behavior of PGLU(pNB), at 25°C, showing a late stage of the biphasic region under the polarizing microscope. Solvent is 1-methyl-2-pyrrolidinone. Mag X200. See Color Plate III.

crystalline droplet inside the boundary was 400 μm in diameter, whereas at the boundary the largest liquid crystalline droplet was only 130 μm in diameter. The diameter of the largest liquid crystal spheres, that had not been disrupted or fused, was slightly greater than those observed in the pretransitional region, Figure 3. Although the solution in the cell was filled by the uniform birefringent phase of the solute, the large liquid crystal domains with defined annular lines were still evident in this biphasic region. Very small liquid crystal domains remained dispersed as close packed, slightly distorted, spheres for an appreciable time. In the final stage, the liquid crystalline domains rapidly coalesce until the domains are larger

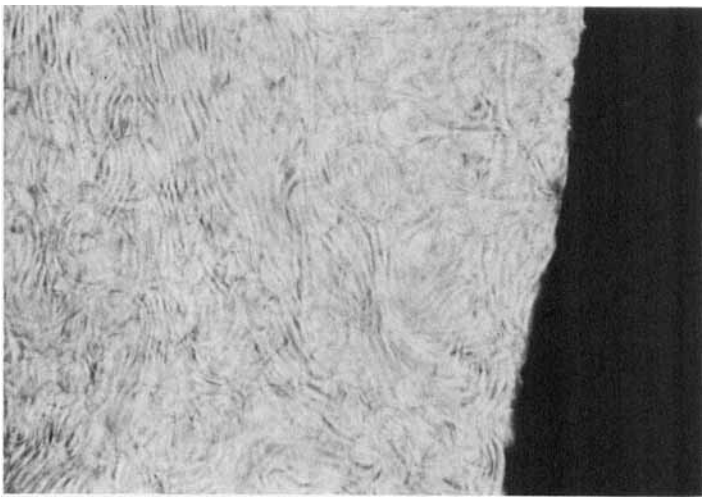

FIGURE 5 Cholesteric liquid crystal behavior of PGLU(pNB), at 25°C, in 1-methyl-2-pyrrolidinone (35% wt/v polymer), under the polarizing microscope. The distance between the equispaced retardation lines is one-half the pitch of the cholesteric structure. The black segment is the teflon cell frame. Mag X200. See Color Plate IV.

than the cell thickness. An alternative explanation for this phenomenon can be attributed to the settling of liquid crystal domains at the boundary of the cell wall. Even when this boundary was some distance from the uniform large birefringent area, small liquid crystal domains were still present (see Figure 3). The droplets at the boundary had a smaller size than the size of the fully developed liquid crystal domain inside the boundary. At a 35% (wt/v) PGLU(pNB) concentration, the continuous birefringent phase covered the entire sample area, as shown in Figure 5. If the cell was filled with the birefringent phase of a polymer and allowed to stand, the groups of equidistant lines were visible either with crossed polarizers or parallel polarizers.

When the biphasic region was well developed, the size of the liquid crystalline droplets at the center of the cell were larger than the liquid crystalline droplets at the boundary of the cell. By contrast, centrally located small liquid crystal domains were more easily disrupted than those at the cell boundary, and very small liquid crystal domains were observed at the cell boundary at all concentrations in the biphasic region. Even at a distance from the uniform birefringent area, the small liquid crystalline droplets remained.

At the boundary of the cell all the liquid crystalline regions exhibited a uniform birefringent phase. When liquid crystalline domains are present at the boundary, their size is much smaller than the domains near the center of the cell. However, given a sufficient period of elapsed time, the liquid crystalline domains displaying a Maltese Cross pattern and annular lines coalesced into a group of large birefringent liquid crystalline droplets (Figure 4). When viewed under the polarizing microscope the internal structure of the droplets had a swirl-like finger print appearance.

A droplet with a diameter of 400 μm showed uniform equidistant lines around

its circumference. When the largest liquid crystalline droplet was magnified, as seen in Figure 4, it showed extensive cholesteric color and equidistant lines attributed to the cholesteric pitch and the optical rotatory power of the system.

In the concentration range from A to B, in which the biphasic region is at equilibrium, a change in the overall concentration of the solution results in a change in the proportion of the two phases. Since the values of "A" and "B" are solvent and molecular weight dependent, the rate of phase separation will be a function of these two parameters. The concentration at which phase separation was discerned for each of the polymers is summarized in Table II.

### Phase Transition of PGLU(pFB) in Chloroform

This polymer also exhibited three well-developed transitional phases when changing from the isotropic to the continuous birefringent phase. When a 19% (wt/v) solution was observed immediately after its preparation, droplets with a defined shape were present in the large isotropic phase (Figure 6). The largest droplet, with a diameter of 65 ~ 70 μm, displayed the Maltese Cross pattern. Raising the concentration to over 20%, reduced the number of uniform liquid crystal patterns relative to the pretransitional state. The equidistant lines appeared to be relatively discontinuous inside the large liquid crystalline domains and within the continuous birefringent

TABLE II

| Polymer | Solvent | Conc. (A) (wgt/vol) | Line Spacing μm | Conc. (>B) (wgt/vol) |
|---|---|---|---|---|
| PBLG | $CH_2Cl_2$ | 15% | 4–6 μm | 35% |
| PGLU(pNB) | NMP | 20% | 11–15 | 35% |
| PGLU(pFB) | $CHCl_3$ | 18% | 6–8 | 38% |
| PGLU(pTFMB) | THF | 23% | 7–9 | 45% |

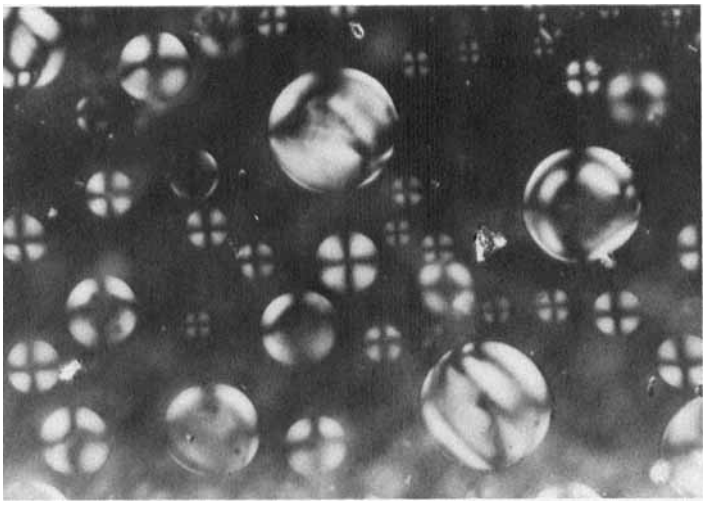

FIGURE 6 Liquid crystal behavior of PGLU(pFB) under the polarizing microscope, at 25°C, showing the formation of spherical particles with a Maltese Cross pattern. Mag X100. See Color Plate V.

area. The isotropic phase was still present inside the boundaries of the closed circular liquid crystalline droplet.

In the continuous birefringent phase, one large liquid crystalline droplet with a 150 μm diameter, retained its structure and showed discrete annular lines and the Maltese Cross pattern in the isotropic-biphasic region (see Figure 7). The unique fingerprint pattern in the surface area just beneath the cover glass was observed at a concentration of 30% (wt/v). Two types of structures are present in Figures 8a and b. These appear to be the focal conic structure indicated by the swirl-like finger print pattern Figure 8a, and the Grandjean layer from the uniform birefringent area surrounded by parallel lines Figure 8b.[8,12,13]

The transition behavior of PGLU(pFB) in chloroform is very similar to that of poly γ-benzyl-L-glutamate [PBLG] and that of poly γ-*p*-nitrobenzyl-L-glutamate [PGLU(pNB)]. In the pretransitional region, liquid crystal domains floated in an extended isotropic phase. In the early stage of the pretransitional region, the alignment of the molecules is parallel to the surface and the helix axis is perpendicular to the direction of view. In the highly compact liquid crystalline region, the frequency of collisions between liquid crystal droplets is greater so that a higher interfacial energy is required to coalesce the liquid crystalline droplets. Once the large droplets in the continuous birefringent area became less mobile, the small liquid crystal droplets in the isotropic region were attracted toward the large birefringent area and coalesced. This behavior appears to be preferred rather than coalescing with each other to form separate liquid crystalline droplets. Increasing the concentration converted the biphasic region into the continuous birefringent phase. The presence of the Grandjean layer did not permit a complete diplay of cholesteric iridescent color. The appearance of these two structures in the continuous birefringent phase was strongly dependent upon the viewer's line of sight

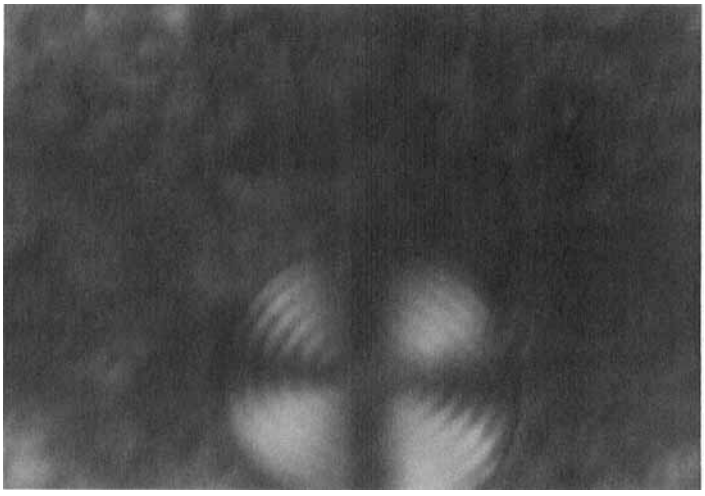

FIGURE 7 Liquid crystal behavior of PGLU(pFB), at 25°C, showing a late stage of the biphasic region under the polarizing microscope. Note the well developed liquid crystalline domain and Maltese Cross pattern with radial lines. Solvent is chloroform and the polymer concentration is 22% (wt/v). Mag X100. See Color Plate VI.

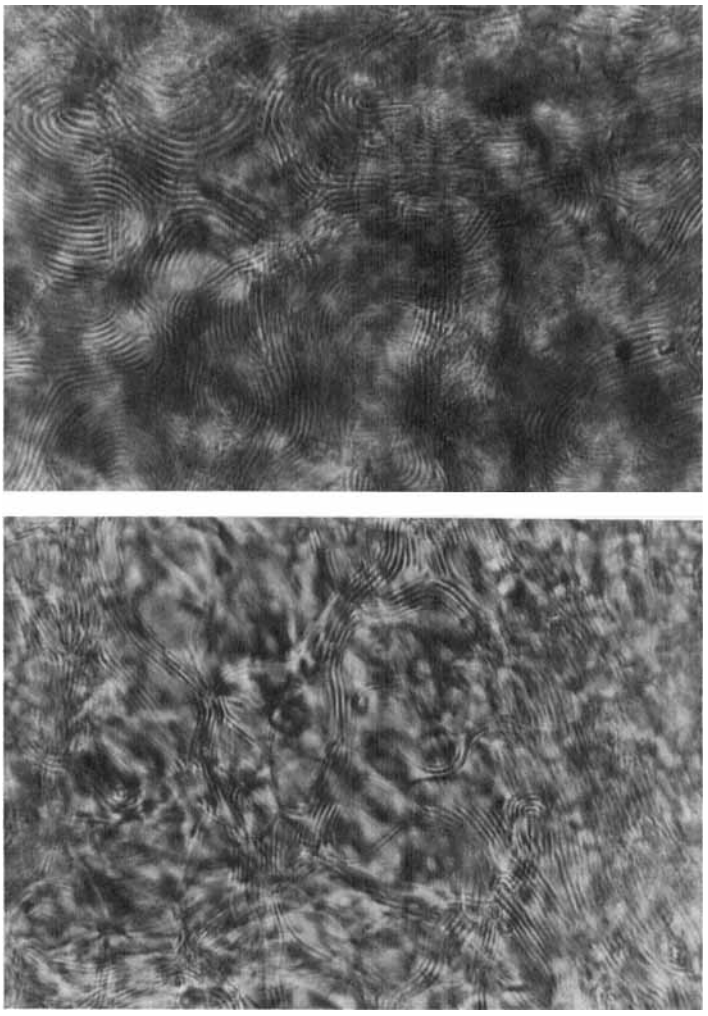


FIGURE 8 Photomicrograph of PGLU(pFB) in chloroform (30% wt/v), at 25°C, showing cholesteric liquid crystal behavior under the polarizing microscope. The distance between the equispaced retardation lines is one-half the pitch of the cholesteric structure: (a) from the focal conic structure, Mag X100, (b) from Grandjean layers. Mag X100. See Color Plate VII.

direction. (Figures 8a and b). The structure seen in Figure 8a is typical for the standard local cholesteric structure. In Figure 8b there are discernible areas where the planes are not perpendicular to the viewing direction and there are interference patterns arising from the cholesteric phase.

### Phase Transition of PGLU(pTFMB) in Tetrahydrofuran

The liquid crystalline characteristics of PGLU(pTFMB) were analyzed with respect to the pretransitional region and the biphasic region using the data obtained from Figure 9. For a 20% (wt/v) concentration of polymer solution, only small liquid

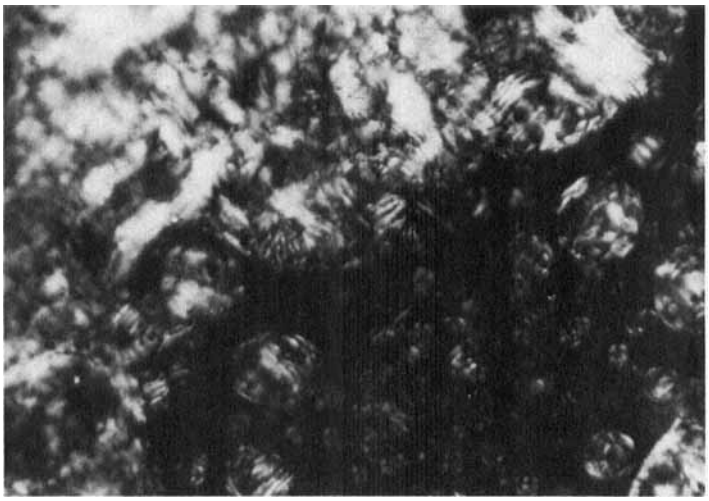

FIGURE 9 Liquid crystal behavior of PGLU(pTFMB), at 25°C, in tetrahydrofuran solvent showing an early stage of the biphasic region under the polarizing microscope. The small liquid crystal domains that are present appear to be less uniform and regular than for the other polymers. See Color Plate VIII.

crystalline particles were observed suspended in the large area of the isotropic phase. Increasing the concentration to 23% (wt/v), transformed the isotropic phase to a different phase. In this phase, liquid crystal domains were visible and were suspended inside the isotropic phase. Comparison of this system to the behavior of PBLG, PGLU(pNB), and PGLU(pFB) revealed a more concentrated swirl type and denser structure within the liquid crystalline droplet. Small liquid crystal units with a diameter of $17 \sim 30$ μm were present in this system. In contrast to the other polymers, when the polymer concentration was increased, a large continuous birefringent area developed from the edge of the cell wall. The other behavior that was observed was very similar to that of the polymers PBLG, PGLU(pNB), and PGLU(pFB).

When the polymer concentration reached 40% (wt/v), most of the liquid crystalline and isotropic domains were filled with the continuous birefringent phase. This was in contrast to the pretransitional and biphasic region behavior, as is shown in Figure 10. The surface structure of the liquid crystalline phase exhibited a uniform color and cholesteric pattern. For this polymer the liquid crystalline phase did not exhibit spherical symmetry so that formation of the Maltese Cross pattern was not observed. Figure 10 shows some classical examples of Frank's currents or disclinations. The arrows indicate specific types of disclinations and are denoted by the letters A and B. The disclination A strength equals $+1/2$, while the strength at B equals $-1/2$.

The behavior can be rationalized in terms of the following argument. The PGLU(pTFMB) that was synthesized had a molecular weight almost half that of the other polymers. When the conformation was assessed by means of the amide bands in its infrared spectrum, the amide I region had two bands, one at 1655.7 $cm^{-1}$ representing the α-helix portion, and one at 1623 $cm^{-1}$ representing the β-sheet contribution. The β-sheet content was determined to be less than the α-helix

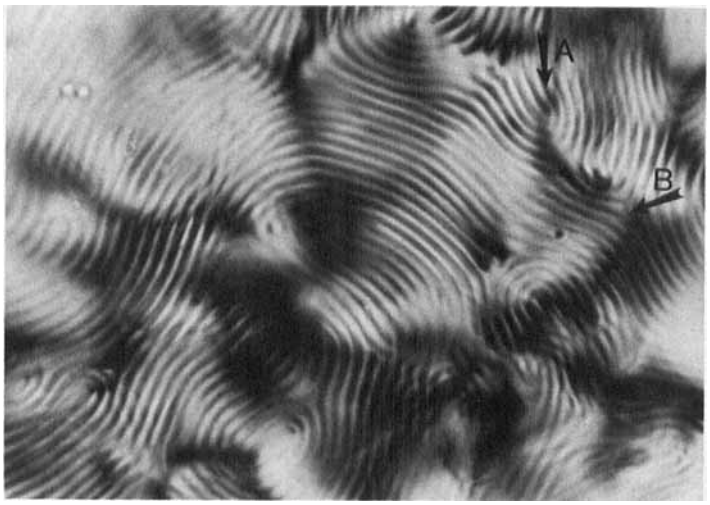


FIGURE 10 Liquid crystal behavior of PGLU(pTFMB), at 25°C, in tetrahydrofuran (40% wt/v polymer) showing cholesteric behavior under the polarizing microscrope. Some of the types of disclinations have been noted by arrows. The letters identify the strengths. (A) = +1/2, (B) = −1/2. See Color Plate IX.

content in both the solid and the solution state. We consider this polymer to consist of a mixture of α- and β-conformations and, therefore the length of extended α-helix conformation is interspersed with regions of β conformation. As a consequence, cholesteric liquid crystal formation in this polymer is not as facile as in the other polymers and an alternative structure consisting of poorly organized liquid crystalline domains is formed. This behavior suggests that low molecular weight polypeptides may not exhibit as well-developed cholesteric liquid crystalline phases as is present in the higher molecular weight polypeptides, Figure 9. In the present instance, the presence of a small amount of β-structure may interfere with the formation of a well defined cholesteric structure in the biphasic region and Figure 9 reveals that the local level of organization is of a low level nematic with a few areas of greater organization. Aside from the irregular and swirl-like finger print pattern in the liquid crystalline domains, the other observed phenomena were very similar to those of the polymers PBLG, PGLU(pNB), and PGLU(pFB).

In the early stage of the continuous birefringent phase, the inner structure showed some boundaries of the liquid crystalline droplets because the continuous birefringent phase was not fully developed. This explanation is supported by the observation that the inner structure was shown to contain some spherical boundaries from the liquid crystaline droplets. Despite the presence of the small amount of β structure, the cholesteric properties are still reasonably well-defined when compared to the cholesteric structures exhibited by other high molecular weight polymers. However, the transition concentration values A and B are higher than those for the high molecular weight polymers. The small interspacings between the equidistant lines in this system, which are equal to half of the pitch of the cholesteric structure, indicates that PGLU(pTFMB) has a reduced optical rotatory power relative to the other polymers. Solutions of this 20 Kd polypeptide do not exhibit the unique order or regularity that are common characteristics of the lyotropic

liquid crystalline phase below concentration B. However, when the concentration was raised above the B concentration, the uniformity of the cholesteric liquid crystalline phase became similar to those of other high molecular weight polymers.

### Retardation Behavior in the Cholesteric Phase

The cholesteric pitches were calculated from the dimension of the equispaced intervals listed in Table II. In the continuous birefringent area of PGLU(pNB), the interspacing (max. 15 μm) was much higher than that of interspacing from other polymers. The data tabulated in Table II shows that the PBLG analogues that have an electronegative group on the benzene ring have higher values for the interspacings than does PBLG. The spacings of PGLU(pNB) are of the order 11 ~ 15 μm at a 35.0% (wt/v) concentration, while a 38% (wt/v) concentration of PGLU(pFB) shows spacings of approximately 6 ~ 8 μm.

The line spacings of these polypeptides in the cholesteric phase are reproducible, although they are dependent on the concentration of the birefringent phase. The spacing did not appear to be very temperature dependent, but did exhibit a strong solvent dependency. It was not possible to observe the line of dislocation and the double spiral in the liquid crystal droplets of *para*-substituted PBLG's. These were observed in the study reported by Frank *et al.*[19]

The results of this study have delineated a number of factors that exert a strong influence on the behavior of a highly helical, polyamino acid liquid crystal system. We have shown that strong electron withdrawing substituents in the para position of poly γ-benzyl-L-glutamate can, under appropriate conditions, enhance the liquid crystal behavior of the substituted polymer system relative to the unsubstituted system. In addition, the nonlinear-optical properties are strongly influenced by such substituents and these studies will be reported in a second paper from this laboratory.

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