Synthesis and Structural Study of the A–B–A Type Block Copolymer Consisting of Poly(γ-benzyl L-glutamate) as the A Component and Polybutadiene as the B Component

Akio Nakajima,*[1a] Toshio Hayashi,[1a] Kouhei Kugo,[1a] and Katsuro Shinoda[1b]

Department of Polymer Chemistry, Kyoto University, Sakyo-ku, Kyoto 606, Japan, and Central Research Laboratory, Sekisui Chemical Co., Shimamoto-cho, Mishima-gun, Osaka 618, Japan. *Received October 10, 1978*

ABSTRACT: A–B–A type triblock copolymers composed of γ-benzyl L-glutamate as the A component and butadiene as the B component were prepared. The method of synthesis consists of the polymerization of the γ-benzyl *N*-carboxy-L-glutamate anhydride initiated by the amine groups capped at both ends of the polybutadiene block which was prepared by bifunctional polymerization followed by a chemical modification of its living ends. From experimental results on the circular dichroism in ethylene dichloride solution, as well as from the infrared spectra measurements in the solid state, it has been found that the polypeptide chain block exists in the α-helical conformation in the same manner as poly(γ-benzyl L-glutamate). By using wide-angle X-ray diffraction and electron microscopy it could be shown that the block copolypeptides exhibit mesophases in different solvents. In this case, polybutadiene chains are in random coil conformation and form domains embedded in the matrix phase formed by the poly(γ-benzyl L-glutamate) chains in the α-helical conformation.

In previous studies,[2-4] one of the authors (T.H.) has synthesized A–B–A type triblock copolypeptides composed of γ-benzyl L-glutamate and L-leucine or L-valine of high molecular weight and characterized the solid-state properties. As expected, the choice of solvent had resulted in significant effect on the solid-state structure of cast films. Films cast from preferential solvents for one component gave evidence of clear phase separation. The solid state morphology also depended on the position of the soluble block, and the properties resembled most closely those of the continuous phase.

In order to increase the information about the triblock copolymers based on the α-amino acid component, we have synthesized A–B–A type triblock copolymers composed of polypeptide blocks and a polydiene block, i.e., A–B–A copolymers composed of poly(γ-benzyl L-glutamate) (A) and polybutadiene (B). Polybutadiene is an interesting block component for the following reasons. The presence of C=C double bonds along the chain allows chemical modifications of the polybutadiene block such as selective fixation of osmium tetroxide. Furthermore, such copolymers may be interesting from a biological point of view. The polybutadiene chains are in random coil conformation, whereas the polypeptide chains are in α-helical conformation. The biological interest is in the preparation of model membranes in which the polybutadiene forms amorphous domains surrounded by the crystalline domains composed of α-helical polypeptide chains. Moreover, the copolymer plays the part of an "amphipatic integral protein" after transformation of γ-benzyl L-glutamate into hydrophilic L-glutamic acid by saponification of the polypeptide component. Until now, Perly et al.[5,6] have synthesized A–B type diblock copolymers composed of polybutadiene (PB) and poly(γ-benzyl L-glutamate) (PBLG), and they concluded that the A–B type diblock copolymers exhibit a lamellar structure in the dry state as well as in concentrated solution in dioxane and in chlorinated solvents.

In this paper, we shall describe the preparation and the characterization of A–B–A type triblock copolymers composed of γ-benzyl L-glutamate (A) and polybutadiene (B). The chain structure and conformation of the block copolymers in the solid state will be studied by X-ray diffraction and electron microscopy. The domain structure and some physical properties will be reported in succeeding papers.

Experimental Section

Synthesis and Purification of Materials. Amine-Terminated Polybutadiene. The middle block, a cycloaliphatic secondary amine-terminated polybutadiene (ATPB), which is high in the trans isomer, was kindly supplied by Drs. K. Rieu and R. Drake of the B. F. Goodrich Chem. Co., Cleveland, Ohio. The molecular weight distribution of the ATPB is very sharp according to their note. The ATPB was purified before use by using benzene, methanol, and water. The amine equivalent weight of the ATPB measured by a potentiometric titration was 1800. Thus, the number average molecular weight, M_n, of the ATPB was 3600.

γ-Benzyl *N*-Carboxy-L-glutamate Anhydride. The monomer, *N*-carboxy-γ-benzyl-L-glutamate anhydride (γ-BLG-NCA) was prepared according to the method proposed by Blout and Karlson[7] and purified by repeated recrystallization from an ethyl acetate solution with the addition of petroleum ether. The Volhard titrimetric method[8] was employed for the determination of chloride content in γ-BLG-NCA present as impurities and certified the chloride content in γ-BLG-NCA as being less than 0.03 mol %.

Synthesis of Block Copolymers. The respective amount of γ-BLG-NCA and ATPB was calculated to obtain the desired degree of polymerization, P_n, of the polypeptide block by P_n = [NCA]/[ATPB], in which [NCA] and [ATPB] are the mole concentration of NCA and ATPB, respectively. The polymerization was carried out in the absence of moisture, at room temperature, in a dioxane–methylene dichloride [1:3 (v/v)] mixture at a total concentration of γ-BLG-NCA and ATPB, 3%. The polymerization was followed by infrared spectroscopy (disappearance of the bands at 1860 and 1790 cm^{-1} characteristic of the NCA[9]). After 48 h, the polymerization was terminated, and the copolymer was precipitated by 5 volumes of pure cold methanol. This method of precipitation allowed the elimination of traces of γ-BLG-NCA still present and of the oligopeptide formed by autopolymerization.[10] Then the copolymer was dried in vacuo.

Selective Extraction and Fractional Precipitation of Block Copolymers. The purification starts with a selective extraction of the homopolymers included. The PB fragment, which was isolated, and a low molecular weight of PBLG are extracted with *n*-hexane. To perform the fractionation, the copolymer was dissolved in a mixture of chloroform and *n*-hexane, and ethanol was used as a precipitant. It was separated into four to five fractions, and the central portions of them were used for physical measurements. The results of all the copolymerizations are listed in Table I.

Measurements. Molecular Weights. The molecular weight of these triblock copolymers was estimated from the limiting viscosity number of the polymer in dichloroacetic acid (DCA) measured by using an Ubbelohde type viscometer, by applying

0024-9297/79/2212-0840$01.00/0

Table I
List of Samples Prepared

sample	[η], dL/g	$M_w \times 10^{-4}$	butadiene mol %	butadiene vol %
GBG-1	0.230	2.7	32.5	14.3
GBG-2	0.280	3.8	28.4	12.0
GBG-3	0.372	5.6	21.9	8.9
GBG-4	0.542	8.6	18.7	7.8
GBG-5	0.625	10.2	16.1	6.6
GBG-6	0.749	12.4	10.5	4.2
GBG-7	0.907	15.4	8.5	3.3
GBG-8	1.079	18.8	7.5	3.0
PBLG	1.420	25.8	0.0	0.0

the [η]-molecular weight relationship proposed by Doty et al.[11] for PBLG. The experimentally determined limiting viscosity numbers [η] and molecular weights are summarized in Table I. To certify the molecular weight heterogeneity of samples, a Water High-Speed GPC Model ALC/GPC 202/R401 (Water Associates, Milford, Mass.) was employed. Four columns (each 7-mm inner diameter and 30-cm length) of microstyrogel R of 10^6, 10^5, 10^4, and 10^3 Å normal pore size were used. The carrier solvent was *N,N'*-dimethylformamide (DMF). The flow rate was 1 mL/min, and the injections were usually 0.3 mL of 0.5% stock solutions. The GPC unit was calibrated for the band-broadening effect together with that for the relation of the V_e, the peak elusion volume, with M_e, the molecular weight corresponding elusion standard, by using several fractionated PBLG homopolymers.

Composition of Copolymers. The percentage of polypeptide, *G* (mol %), in each copolymer was determined by measuring the optical density of the PBLG peak at 259 nm for sample in methylene dichloride using a Hitachi spectrophotometer, Model ESP-3T. Further, the molar composition of some of the GBG block copolymers was also determined from elemental analyses for N, C, and H atoms. These elemental analyses were carried out in the Organic Microanalyses Center in Kyoto University. The volume percentage of butadiene component was obtained by assuming additivity of the densities of both homopolymers, $\rho_A = 1.3$[12] and $\rho_B = 0.9$[13], to hold for the block copolymer. Table I includes the average value of the copolymer composition together with the volume percent of butadiene component for fractionated samples.

Conformation of the Polypeptide Blocks. The circular dichroism (CD) spectra were measured at 25 ± 0.5 °C by a JASCO J-20 CD/ORD spectropolarimeter equipped with a quartz cell having a path length of 1 mm. Infrared spectra (IR) of solid films of the samples cast from chloroform (CF) and from benzene (Bz) solution were measured with a Shimazu Model-30 A IR spectrophotometer in the region of 4000 to 400 cm^{-1}.

X-ray Diffraction and Electron Microscopy. X-ray diagrams were obtained by using Ni-filtered Cu Kα radiation, setting a flat surface of the film parallel to a reflecting surface with an automatic diffractometer. For the electron microscopy measurement, thin films cast from solution were stained with osmium tetroxide and examined by transmission microscopy. All micrographs were taken at an instrumental magnification of 4000.

Results and Discussion

Materials. For the secondary amine-initiated polymerization of the α-amino acid NCA, either the primary-amine mechanism in which the initiator amine undergoes a nucleophilic addition to the C-5 carboxyl group of the NCA or the tertiary-amine mechanism (activated NCA mechanism) in which the initiator amine abstructs the N-3 hydrogen of the NCA is possible.[14,15] In the former case, the polymer contains the initiator fragment, and block copolymers would be formed in this case. In the latter case, the homopolymers will result, because the initiator fragment is not incorporated in the propagating chain. The predominance of one of the two mechanisms is determined by a balance between the nucleophilicity and the basicity of the amine. The former mechanism will be operative in the polymerization initiated by a secondary amine which is sterically less crowded

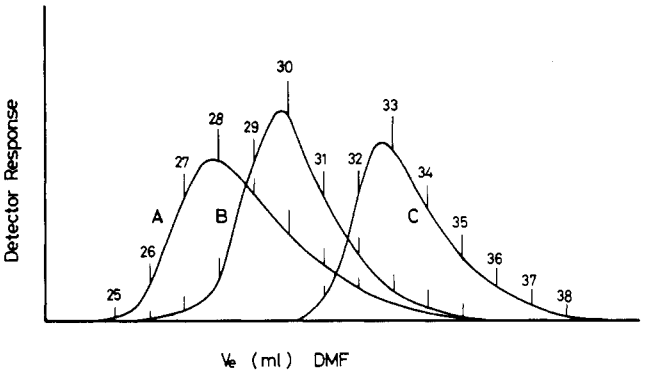

Figure 1. Molecular weight heterogeneity for: (A) PBLG-I (M_w 38.0 × 10^4), (B) GBG-5 (M_w 10.2 × 10^4), and (C) PBLG-II (M_w 7.7 × 10^4), as estimated by GPC in DMF solution.

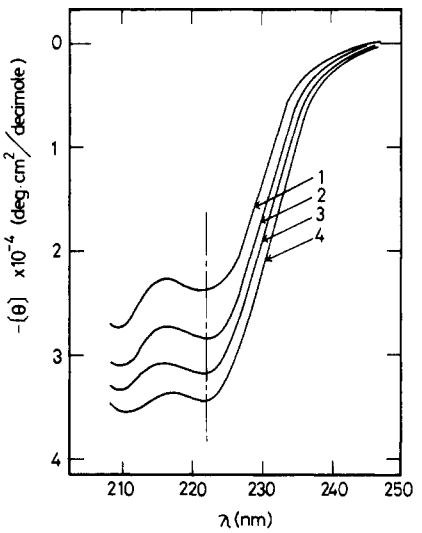

Figure 2. Circular dichroism spectra of GBG block copolymers and PBLG homopolymer in EDC solution at 25 °C: (1) GBG-1, (2) GBG-4, (3) GBG-8, and (4) PBLG.

similar to a primary amine. Recently, Hashimoto et al.[16] suggested that a cyclic secondary amine without bulky substituents would undergo a nucleophilic addition to NCA. Our experimental results, that the amount of the isolated PB and PBLG homopolymer fractions was rather small, should support the former mechanism.

To check the molecular weight heterogeneity of block copolymers, GPC was examined for the samples which were carefully purified and fractionated by fractional precipitation. Figure 1 illustrates the GPC diagram showing corrected UV chromatograms (the amount of the detector response) plotted against elusion count V_e for the GBG-5 sample together with the results on PBLG-I and -II homopolymers, in DMF solution. These samples are all fractionated fractions. The experimental chromatograms for the GBG-5 sample show a very small tailing of the main component toward the larger elusion volume side and a single rather sharp peak similar to those of the PBLG homopolymers. This may mean that the copolymers are sufficiently homologous with respect to both molecular weight and composition.

Circular Dichroism Properties. The circular dichroism (CD) spectra, expressed by the residue ellipticity [θ] of GBG block copolymers and PBLG homopolymer in 1,2-dichloroethane (EDC), are shown in Figure 2. All of these spectra have two negative peaks characteristic of an α-helical conformation with a negative 222-nm band assigned to the n-π* transition, and the second peak, due to the π-π* transition, appears at 209 nm.[17] Table II

Table II
$[\theta]_{222}$ Values of Samples in EDC (25 °C)

copolymer	G, mol %	$-[\theta]_{222}$	$[\theta]^c_{222}/[\theta]^o_{222}$
GBG-1	67.5	23 600	0.681
GBG-2	71.6	24 900	0.719
GBG-3	78.1	27 300	0.788
GBG-4	81.3	28 100	0.812
GBG-5	83.9	29 100	0.838
GBG-6	89.5	31 200	0.900
GBG-7	91.5	31 600	0.912
GBG-8	92.5	31 900	0.919
PBLG	100.0	34 700	1.000

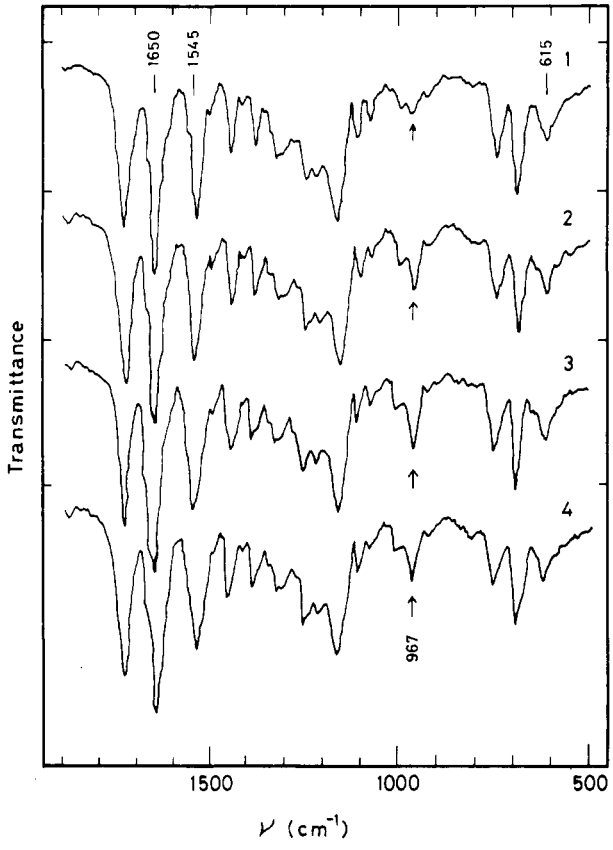

Figure 3. Infrared spectra of unoriented solid films: (1) PBLG; (2) GBG-4; (3) GBG-1, cast from CF; and (4) GBG-4, cast from Bz.

shows the experimental data of $[\theta]_{222}$ for samples in EDC solution at 25 °C. The ratios of the $[\theta]_{222}$ values of GBG block copolymers to that of PBLG homopolymer, $[\theta]^c_{222}/[\theta]^o_{222}$, were shown in the third column of Table II. The value of the ratio for each of the GBG block copolymers is quite in agreement with that of molar composition of the G-block component in the GBG-block copolymers. This indicates that the helical content of the G component in the GBG block copolymers is equal to that of the PBLG homopolymer in EDC solution.

Chain Conformation of Copolypeptides in Solid State. Infrared (IR) spectra were measured with solid films of GBG block copolymers and PBLG homopolymer, both cast from CF and from Bz. These spectra in the region of 1800–500 cm^{-1} were shown in Figure 3. As is obvious from Figure 3, bands of amide I, II, and V of these GBG block copolymers appeared at 1650, 1550, and 615 cm^{-1}, respectively, just at the same wavenumbers for PBLG homopolymer. Such a result means that the G-block component in the GBG block copolymers exists in α-helical conformation, and moreover, the helix content of GBG block copolymers is nearly the same order as that of PBLG homopolymer.

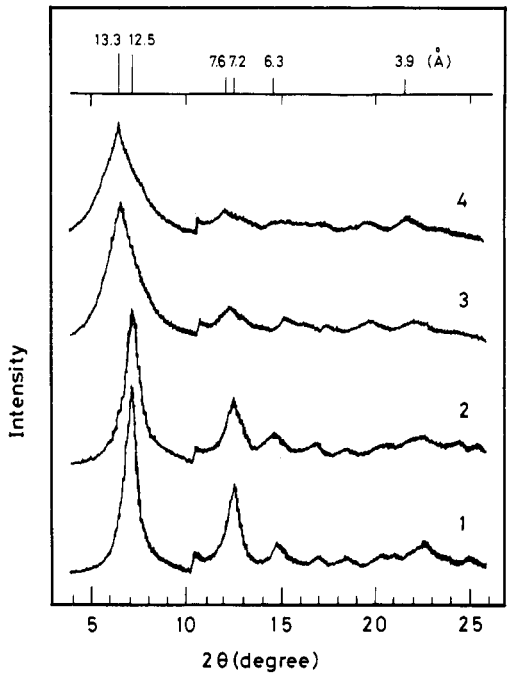

Figure 4. Wide-angle X-ray diffraction profiles of unoriented solid films for: (1) PBLG cast from CF, (2) GBG-4 cast from CF, (3) PBLG cast from Bz, and (4) GBG-4 cast from Bz.

A specific band associated with the C=C tortion and the CH out-of-plane band modes[18] is observed at around 967 cm^{-1}, and the relative intensity of this band increases with an increase in the molar amount of polybutadiene in GBG block copolymers, as expected.

Wide-Angle X-ray Diffraction. The wide-angle X-ray diffraction (WAXD) patterns for the GBG block copolymers and PBLG homopolymer are shown in Figure 4. The pattern observed for PBLG homopolymer depends on the casting solvent and varies from the very sharp, intense reflections of the CF cast film (Figure 4.1) to the diffuse reflections produced by the Bz cast film (Figure 4.3). The effect of the solvents in promoting crystallinity as determined from the WAXD patterns is in the order of CF > Bz. The first main reflection corresponds to an intermolecular spacing of the α-helical chains and has a spacing of 12.5 Å for the film cast from CF and 13.3 Å for film from Bz. Using the notation of McKinnon and Tobolsky,[19] the film cast from CF is assigned the form C solid state modification which has a crystalline structure equivalent to the unit cell described by Bamford.[20] Film cast from Bz has the form A structure, which is a poorly ordered array thought to include super-helical aggregates of PBLG molecules.[21]

Diffraction patterns for the GBG block copolymer show basically similar reflections to those of PBLG homopolymer. The intensity of the diffraction patterns is again higher for the CF cast film, and the corresponding spacing is about 12.5 Å for CF (Figure 4.2) and 13.3 Å for Bz (Figure 4.4). It appears that the G domains assume the same structural modification (A or C) as the PBLG homopolymer.

Electron Microscopy. In order to obtain information on the domain structure of the block copolymer in the solid state, we have also studied the morphology of the GBG block copolymer in the film state by using electron microscopy. Electron micrographs of the GBG-4 film were illustrated in Figure 5. The dark portions in these photographs correspond to the domains composed of polybutadiene block chain portions stained with osmium tetroxide. In these photographs, microheterophase

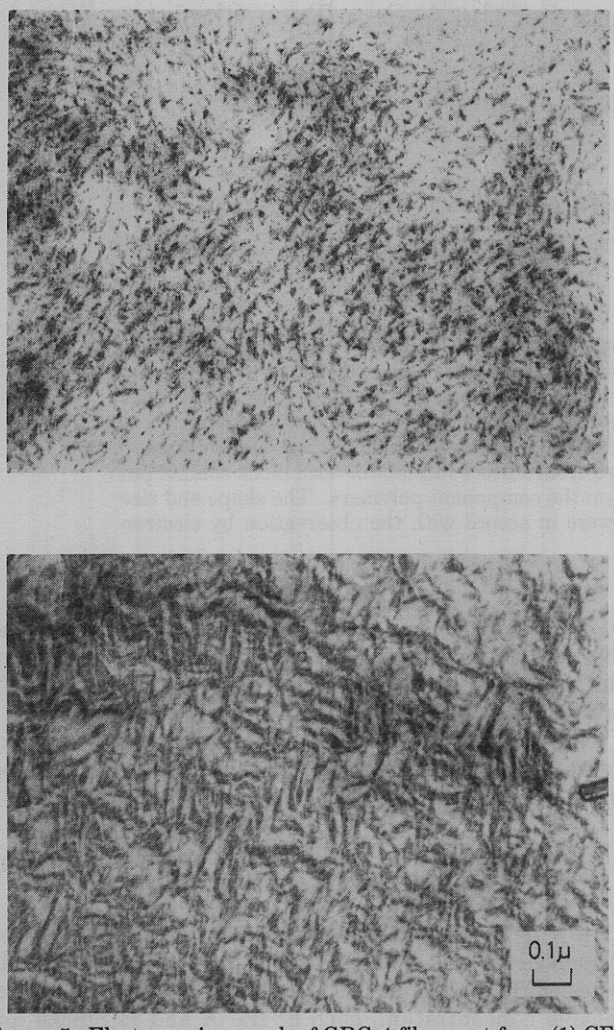

Figure 5. Electron micrograph of GBG-4 films cast from (1) CF and (2) Bz. Cylindrical domains (black portions) correspond to PB domains stained by osmium tetroxide.

structure is observed clearly; the polybutadiene block (B block) domains are cylindrical in the matrix of the G component in the GBG block copolymer. Film cast from CF includes rather fine cylindrical domains (Figure 5.1), while an electron micrograph of GBG-4 film cast from Bz reveals more coarse domains (Figure 5.2). Dimensions of the domains will be discussed in detail in the succeeding paper.

In summary, we have prepared a new type of triblock copolymer composed of polypeptide and polydiene. Characterization of the solid state samples showed that the GBG block copolymer exhibited in the microhetero-phase structure in the cast film resulted from two domains: one formed by the polybutadiene chains in random coil conformation, and the other formed by the polypeptide chains in α-helical conformation. Similar structural studies are now in progress on different copolymers of similar type. By changing the kind of polypeptide block, we may prepare biologically interesting novel block copolymers which exhibit unique structural features owing to these polypeptide components.

Acknowledgment. The authors gratefully acknowledge Drs. K. Rieu and R. Drake, the B. F. Goodrich Chem. Co., for kindly supplying the amine-terminated polybutadiene. They also wish to thank Dr. H. Suzuki, The Chemical Research Institute of Kyoto University, for his help with the GPC measurement. One (T.H.) of the authors is grateful to Professors P. H. Geil, A. Hiltner, and J. M. Anderson, Case Western Reserve University, Cleveland, Ohio, for their encouragement.

References and Notes

(1) (a) Department of Polymer Chemistry, Kyoto University; (b) Central Research Laboratory, Sekisui Chemical Co.
(2) T. Hayashi, A. G. Walton, and J. M. Anderson, *Macromolecules*, **10**, 346 (1977).
(3) T. Hayashi, J. M. Anderson, and A. Hiltner, *Macromolecules*, **10**, 352 (1977).
(4) F. Urallil, T. Hayashi, J. M. Anderson, and A. Hiltner, *Polym. Eng. Sci.*, **17**, 515 (1977).
(5) B. Perly, A. Douy, and B. Gallot, *Makromol. Chem.*, **177**, 2569 (1976).
(6) A. Douy and B. Gallot, *Polym. Eng. Sci.*, **17**, 523 (1977).
(7) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **78**, 941 (1956).
(8) D. A. Shoog and D. M. West, "Fundamentals of Analytical Chemistry", 2nd ed., Holt, Rinehart, and Winston, Inc., New York, 1969, p 232.
(9) J. E. Alter, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, **5**, 739 (1972).
(10) A. Cosani, E. Peggion, E. Scoffone, and A. Portolan, *Biopolymers*, **4**, 695 (1966).
(11) P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956).
(12) T. Kanamori, K. Itoh, and A. Nakajima, *Polym. J.*, **1**, 524 (1970).
(13) T. L. Smith and R. A. Dickie, *J. Polym. Sci., Part C*, **26**, 163 (1969).
(14) M. Goodman and J. Hutchinson, *J. Am. Chem. Soc.*, **88**, 3627 (1966).
(15) M. Terbojenich, G. Pizziolo, E. Peggion, A. Cosani, and E. Scoffone, *J. Am. Chem. Soc.*, **88**, 2733 (1967).
(16) Y. Hashimoto, A. Aoyama, Y. Imanishi, and T. Higashimura, *Biopolymers*, **15**, 2407 (1976).
(17) G. Holtzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).
(18) S. L. Hsu, W. H. Moore, and S. Krimm, *J. Appl. Phys.*, **46**, 4185 (1975).
(19) A. J. McKinnon and A. V. Tobolsky, *J. Phys. Chem.*, **72**, 1157 (1968).
(20) C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides", Academic Press, New York, 1956, Chapter VIII.
(21) T. Fukuzawa, I. Uematsu, and Y. Uematsu, *Polym. J.*, **6**, 431 (1974).