Block Copolypeptides. 2. Viscoelastic Properties

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ABSTRACT: The solid state structure of block copolypeptides of γ-benzyl L-glutamate (G) and L-leucine (L) cast from preferential solvents for G has been investigated by dynamic mechanical and wide angle x-ray diffraction techniques. The WAXD patterns show overlapping reflections characteristic of the individual homopolymers. Further evidence for a phase separated morphology is provided by the viscoelastic behavior. Representation of the dynamic elastic modulus in terms of the equivalent mechanical model suggests that phase separation of GLG-type polymers occurs with G as the matrix phase. In the reverse case, LGL-type polymers, it appears that some phase inversion has taken place.

The preparation of triblock copolypeptides of the ABA type is described in a previous paper.[1] Characterization of the secondary structure, i.e., conformation, in solution and the solid state was also reported. This paper is concerned with the solid state structure and properties of this new class of materials at the tertiary level, particularly as regards the organization of blocks into phase-separated or domain morphologies.

Dynamic mechanical spectroscopy has been extensively used for the characterization of heterogeneous polymer systems such as semicrystalline polymers, block and graft copolymers, polymer blends, and filled polymers. In dynamic mechanical experiments, each region undergoes its own relaxations but these may be altered in temperature and/or intensity depending on the heterphase structure and composition. Theoretical representation of the viscoelastic behavior of heterogeneous polymer systems has been made in terms of the equivalent mechanical model of Takayanagi,[2] and through modifications of the well-known Kerner's equation.[3]

The viscoelastic behavior of many homopolymers of α-amino acids and some copolymers has been reported. The esters of poly-L-glutamic acid have been most extensively characterized, and considerable variation in the loss spectrum has been reported which at least in part appears to be related to solvent dependent structural modifications.[4]

In this paper, the dynamic mechanical method, complimented by wide-angle x-ray diffraction, has been employed to characterize the solid state tertiary structure of block copolymers of L-leucine and γ-benzyl L-glutamate. Several different solvents were used in order to alter both the structure of the γ-benzyl L-glutamate blocks and the domain morphology.

Experimental Section

The preparation and purification of all the polymers, including triblock copolypeptides of L-leucine (L) and γ-benzyl L-glutamate G), are described in a previous paper.[1] The composition of the polymer samples used for solid state characterization is summarized in Table I.

For the mechanical studies, films about 0.1 mm in thickness were cast onto glass plates from approximately 2% solutions in chloroform, dioxane, benzene, or chloroform–TFA mixtures. Any residual solvent in the air-dried films was removed by treatment with ether and methanol. The films were then dried in vacuo for 3 days at room temperature prior to using. All the polymers in Table I were previously shown to have the α-helical conformation.[1] Wide-angle x-ray diffraction (WAXD) profiles were obtained with a GE diffractometer. The dynamic mechanical relaxation behavior was measured with a DDV-II Rheovibron at a frequency of 110 Hz and heating rate of 0.3 °C/min over the temperature range 20 to 220 °C.

Results and Discussion

Wide Angle X-Ray Diffraction. The WAXD patterns for the GL block copolymers, the corresponding homopolymers, and a random copolymer are shown in Figures 1 and 2 and the measured spacings are summarized in Table II. The pattern observed for homopolymer PBLG depends on the casting solvent and varies from the very sharp, intense reflections of the chloroform cast film to the diffuse reflections produced by the benzene film. The effectiveness of the solvents in promoting crystallinity as determined from the WAXD patterns is in the order chloroform > dioxane > benzene. 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 chloroform, and 13.4 and 13.3 Å for films from dioxane and benzene. Using the notation of McKinnon and Tobolsky,[5] the film cast from chloroform is assigned the form C solid state modification which has a crystalline structure equivalent to the unit cell described by Bamford.[6] Films cast from dioxane and benzene have the form A structure, which is a poorly ordered array thought to include superhelical aggregates of PBLG molecules.[4]

The interhelical spacing calculated from the first main reflection of poly-L-leucine is 10.8 Å. The random copolymer of G and L (GL-11) shows a single reflection at an intermediate spacing (Figure 1). The actual interhelical spacing is known to depend on the composition of the copolymer and increases as the amount of G increases.[7] It appears that these two comonomer units cocrystallize in random copolymers and isomorphic structures are obtained.

Diffraction patterns for the GL block copolymers show two main reflections, similar to the superimposed reflections of the two homopolymers observed in the blended film (Figure 1). The diffraction patterns are again sharper for the chloroform cast films, and the corresponding spacings are about 12.5 and 10.8 Å for chloroform and 13.3 and 10.8 Å for dioxane and benzene. It appears that phase separation has occurred, and moreover that the G domains assume the same structural modification (A or C) as the homopolymer. From the width of the diffraction lines the size of the domains is estimated to be at least 100 Å.

Chloroform, dioxane, and benzene are all preferential solvents for the G blocks. Films of the block copolymers were also cast from a nonpreferential solvent, a 1:1 mixture of trifluoroacetic acid and chloroform. The diffraction lines are very diffuse for the copolymer as compared to the blend cast from the same solvent mixture (Figure 2). Apparently in this case some mixing of the blocks has occurred and either the crystallinity is very poor or the crystallites very small.

Dynamic Mechanical Spectroscopy. Solvent-dependent structural modifications of PBLG previously characterized by the WAXD patterns also produce significant differences in the dynamic mechanical behavior. The loss spectrum of PBLG cast from chloroform shows a large peak (β) at 40 °C (110 Hz) with a much weaker peak (α) at 120 °C (Figure 3). As the apparent crystallinity is decreased by changing casting

Table I
Analysis of Amino Acid Polymers

Sample	{η}, dL/g[a]	G, mol %	x[b]	y	z
GLG	0.44	60	130 (G)	120 (L)	50 (G)
GL–LG	0.53	52	130 (G)	250 (L–L)	130 (G)
LGL	0.52	29	300 (L)	150 (G)	60 (L)
LG–GL	0.52	51	115 (L)	270 (G–G)	115 (L)
PBLG	1.07	100			
PLL	1.55[c]	0			
GL-11[d]	1.31	55			
GL-14[d]	1.20	28			

[a] In dichloroacetic acid at 25 °C unless otherwise indicated. [b] Average composition of individual blocks: $A_xB_yA_z$. [c] In trifluoroacetic acid at 25 °C. [d] Random copolymer of G and L. The numerals indicate the monomer ratio in the feed.

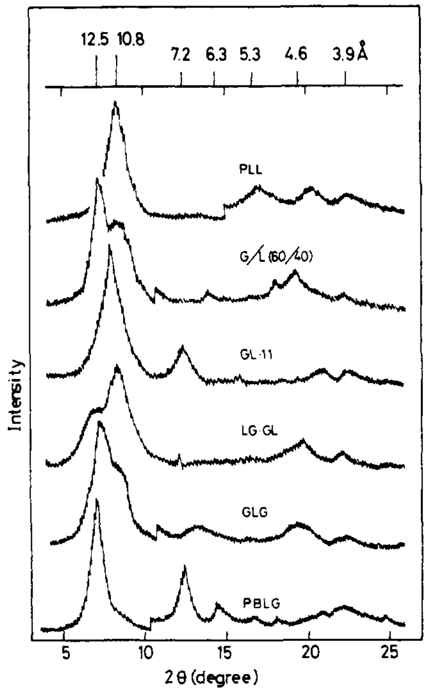

Figure 1. Wide angle x-ray diffraction profiles of G–L polymers cast from chloroform.

solvent in the order chloroform, dioxane, benzene, the intensity of the β peak decreases and that of the α increases (Figures 3-5). Similar results have been observed by Fukuzawa et al.[4] The change in the α peak is considerably more pronounced, and for the benzene cast film the intensities of the β and α peaks are almost comparable. Small temperature shifts are also noted: the β is observed at 40, 42, and 50 °C, and the α at 120, 135, and 140 °C, respectively, for the three solvents.

The accompanying modulus curves (E') show a similar solvent dependency. A fairly large modulus drop occurs at the β process for the chloroform cast film followed by a smaller drop at the α temperature. The magnitude of the former decreases and the latter increases as the crystallinity decreases, and for the benzene cast film the largest drop accompanies the α process.

Table II
First Equatorial Spacings for Polypeptide Films

Sample	Solvent[a]	Spacings, Å	
PBLG	CF	12.6 vs	
PBLG	Bz	13.3 s	
PBLG	DO	13.4 s	
PLL	CF–TFA (1:1)		10.8 vs
GLG	CF	12.5 vs	10.9 s
GL–LG	CF–TFA (19:1)	12.5 vs	10.8 vs
LGL	CF–TFA (19:1)	12.5 s	10.9 vs
LG–GL	CF–TFA (19:1)	12.5 s	10.9 vs
GLG	Bz	13.2 s	10.9 m
GL–LG	Bz–TFA (9:1)	13.3 m	10.9 s
LGL	Bz–TFA (9:1)	13.2 m	10.8 s
LG–GL	Bz–TFA (9:1)	13.0 m	10.7 s
GLG	DO	13.3 s	10.8 m
LG–GL	DO–TFA (19:1)	13.2 m	10.9 s
LG–GL	CF–TFA (1:1)	13.0 m	10.8 m
G/L (60:40)[b]	CF–TFA (1:1)	12.5 vs	10.8 s
GL-11	CF	11.0 vs	

[a] CF = chloroform; Bz = benzene; DO = dioxane; TFA = trifluoroacetic acid. [b] Blend of homopolymers PBLG and PLL.

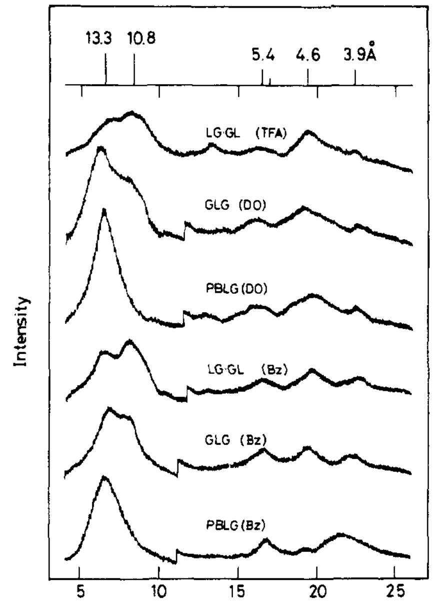

Figure 2. Wide-angle x-ray diffraction profiles of G–L polymers cast from benzene and dioxane.

The α process is generally attributed to the onset of motion of chain segments which are in a disordered conformation.[7] The increase in α intensity with decreasing crystallinity is consistent with this interpretation. The β process, which is associated with motion of the side chains, appears to include a strong crystalline contribution since the intensity decreases significantly in the less crystalline specimens. The effect of crystallinity is particularly apparent in the modulus drop which accompanies the β process where comparison of Figures 3 and 5 shows that in the plateau region between the β and α relaxations, the modulus of the chloroform cast film lies well below that of the benzene cast film. If the side chains assumed ordered positions in the crystalline lattice as suggested by Fukuzawa et al.,[4] the β process should be interpreted as a side chain melting. However, it is evident from DSC[9] and dielectric relaxation studies[10] that the β process more closely resembles a glass–rubber transition. A consistent hypothesis is that the side chains do not crystallize in the solid state but constitute a relatively disordered material filling the lattice space between ordered, helical backbones. However, it is not clear why the β process should have a larger effect on the crystalline properties. Possibly, intermolecular effects are important, in which case the extended and ordered arrangement of the rigid

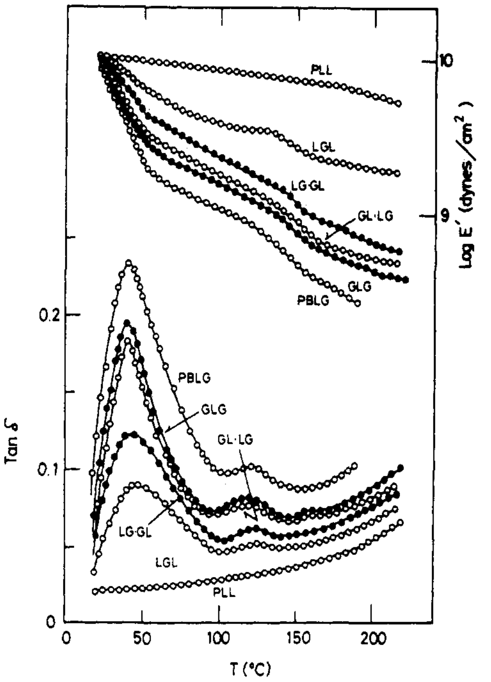

Figure 3. Temperature dependence of tan δ and E' for the G–L polymers cast from chloroform.

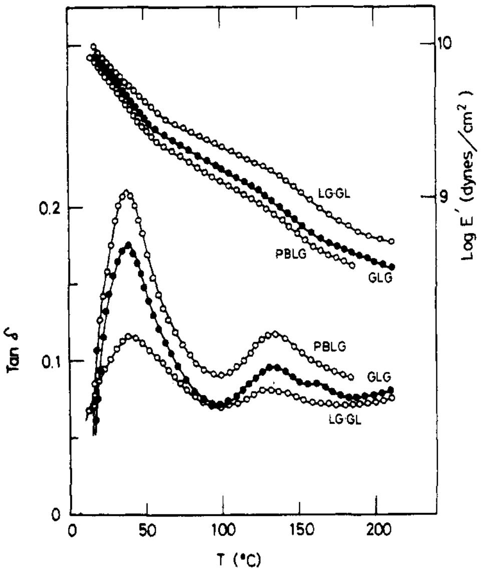

Figure 4. Temperature dependence of tan δ and E' for the G–L polymers cast from dioxane.

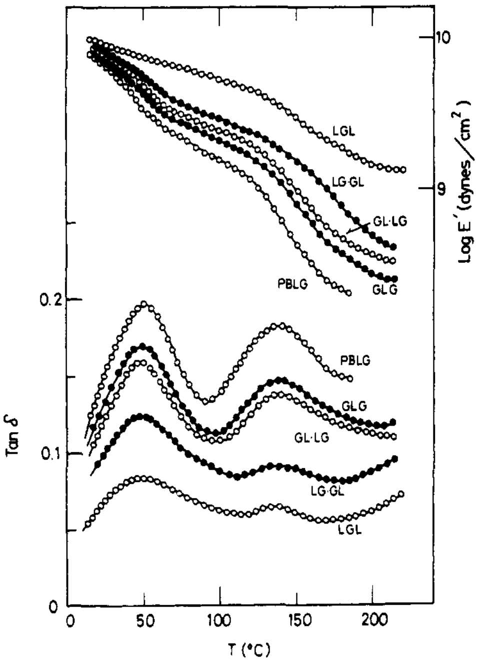

Figure 5. Temperature dependence of tan δ and E' for the G–L polymers cast from benzene.

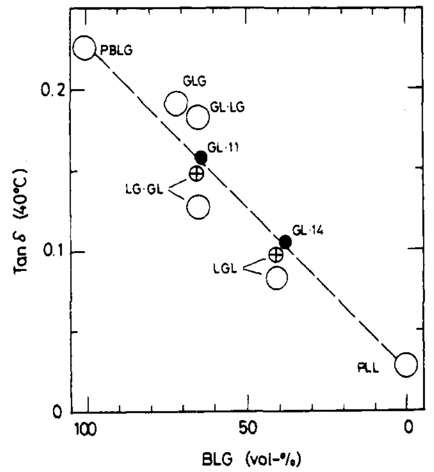

Figure 6. Intensity of the β peak as a function of composition. Polymers were cast from chloroform except PLL and block polymers designated by ⊕ which were cast from 1:1 CF–TFA mixtures. The dashed line is taken from Anderson et al.[11] and represents random copolymers of G and L.

backbones in the crystal could facilitate cooperative motion of the side chains and provide less resistance to intermolecular slip.

It is known that the intensity of the β relaxation for GL random copolymers is proportional to the volume fraction of G. The dotted line in Figure 6 is reproduced from the work of Anderson et al.[11] on GL random copolymers cast from dioxane. The loss spectra obtained in the present study for two random copolymers cast from chloroform (Figure 7) are essentially identical with those reported in the earlier study and show no evidence of the structural modifications which characterize the homopolymer.

Loss spectra of the GL block copolymers cast from chloroform, dioxane, and benzene are also shown in Figures 3–5. PLL is viscoelastically inactive over the temperature range investigated and the peak profiles of the block copolymers resemble those of the corresponding PBLG film with only composition-dependent variations in peak intensity. Some of the block copolymers also have a small but reproduceable peak at 155 °C. For all the block copolymers the intensity of the β peak is found to deviate significantly from the proportional line in Figure 6. GLG and GL–LG which have G as the outer blocks lie above the proportional line, while LGL and LG–GL with G as the midblock lie below. This difference is most dramatically seen by comparing the loss spectra of GL–LG and LG–GL which have the same molar composition but the blocks in reverse order. The tan δ curves for the two LGL-type copolymers cast from the nonpreferential solvent 1:1 CF–TFA are shown in Figure 8. When plotted as a function of composition, the intensity of the β peak lies considerably closer to the proportional line (Figure 6) than is the case for the films cast from preferential solvents.

Since phase separation is thought to occur, especially in the films cast from preferential solvents, it can be assumed that

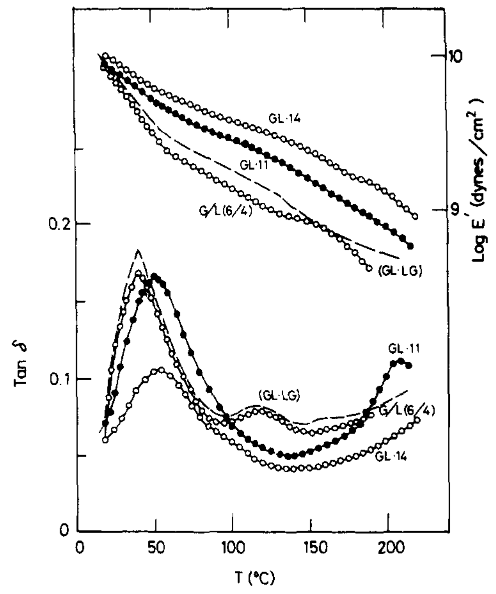

Figure 7. Temperature dependence of tan δ and E' for a block copolymer, random copolymer, and blend of PBLG and PLL all of approximately the same composition.

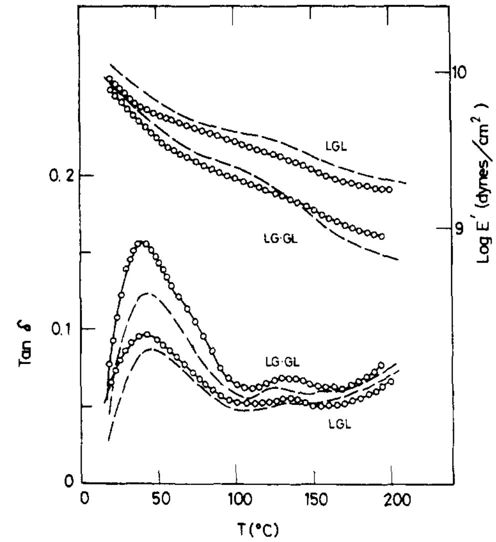

Figure 8. Temperature dependence of tan δ and E' for LGL-type block copolymers cast from 1:1 CF–TFA. Dashed curves represent the same polymers cast from chloroform.

morphological differences account for the effect of G placement on the β process. Unfortunately efforts to determine the nature of the phase separation by direct microscopic examination have not been successful. However, in principle the dynamic mechanical behavior can also provide insight in this area.[12]

Model Calculations. A qualitative discussion of the expected morphological differences between GLG- and LGL-type polymers depends on the fact that a preferential solvent for G is used in the film preparation. The L blocks being insoluble are assumed to aggregate in some fashion. In the case of GLG-type polymers, these aggregates can be small with a core of L segments surrounded by a solvated layer of G segments. When cast as a film the aggregates coalesce to produce L inclusions in a G matrix. The viscoelastic properties of these materials would more closely resemble those of PBLG than PLL. In the case of LGL-type polymers the outer blocks are insoluble. Here larger aggregates might be formed, in fact, solutions of these polymers are characteristically cloudy.

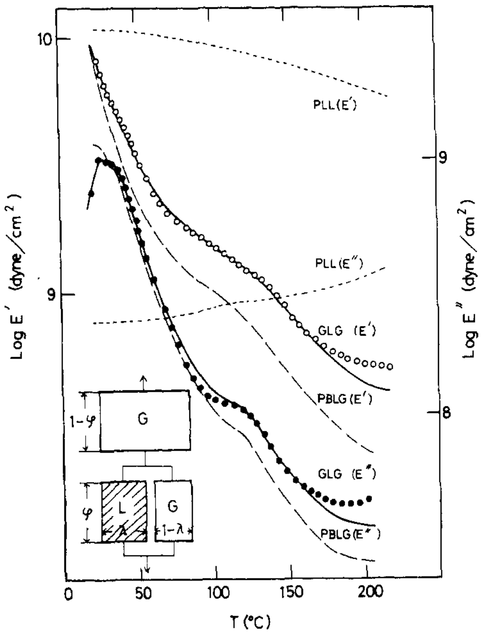

Figure 9. Temperature dependence of E'' and E' for GLG cast from chloroform. The solid line is calculated from eq 1.

Depending upon the size of the aggregates, dried films may contain many small regions in which L is the matrix material. In this case, the L would have considerably more effect on the viscoelastic behavior.

Takayanagi[2] has reported considerable success in representing the viscoelastic properties of heterogeneous materials in terms of simple mechanical models comprised of elements connected partly in series and partly in parallel. The individual elements are assumed to have the viscoelastic properties of the constituent phases. One such model is illustrated in Figure 9. The relative magnitudes of ϕ and λ can be interpreted as representing the extent of series and parallel character of the observed behavior. The dynamic Youngs modulus E^* for this model is given by

$$E^* = \left[\frac{\phi}{\lambda E_2^* + (1-\lambda)E_m^*} + \frac{1-\phi}{E_m^*}\right]^{-1} \tag{1}$$

if the assumption is made that the elastic Poisson's ratio $\nu = \nu_m = \nu_i = 0.5$ (the subscripts m and i refer to the matrix and inclusion phases, respectively). Equation 1 is also equivalent to the modified Kerner's equation used by Dickie.[3] The expression for E^* can be separated into real and imaginary parts to give explicit expressions for E' and E''

$$E' = |E^*| \cos \delta \tag{2a}$$

$$E'' = |E^*| \sin \delta \tag{2b}$$

Equation 1 is applicable to the block copolypeptide system since the WAXD results indicate that the G blocks take the same structural modification as the homopolymer. The analysis was carried out for the films cast from chloroform using data for the homopolymers from Figure 3. Figure 9 presents the calculated curves of E' and E'' for the copolymer GLG. In this case L is assumed to be the dispersed phase. The solid lines represent the best fit of the experimental data and correspond to ϕ and λ values of 0.49 and 0.56.

Efforts to fit the data of all four copolymers are shown in Figure 10. Calculations were made assuming inclusions of the minor component. A good fit was obtained with the materials having G outer blocks. For the LGL-type materials the agreement is less satisfactory and the curves presented are those which best fit the intermediate modulus region between

Table III
Values for Parameters in Equation 1 Used to Fit Experimental Data

Sample	v_i	ϕ	λ	λ_{sphere}
GLG	0.28 (L)	0.49	0.56	0.57
GL-LG	0.35 (L)	0.51	0.68	0.61
LGL	0.40 (G)	0.53	0.76	0.64
LG-GL	0.36 (L)	0.72	0.50	0.62

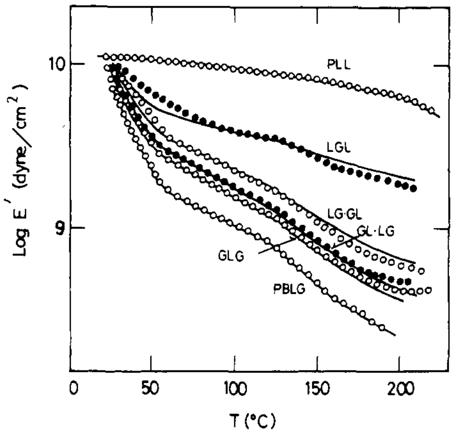

Figure 10. Temperature dependence of E' for block copolymers cast from chloroform. The solid lines through the data points for the block copolymers were calculated using eq 1 with the parameters in Table III.

relaxations. The values of ϕ and λ for the "best fit" calculated curves are summarized in Table III.

For the special case of spherical inclusions the values of ϕ and λ are given by

$$\phi = 5v_i/(2 + 3v_i) \tag{3a}$$

$$\lambda = (2 + 3v_i)/5 \tag{3b}$$

where $v_i = \phi\lambda$ is the volume fraction of the included phase.

The values of λ which would correspond to spherical inclusions, λ_{sphere}, are also included in Table III. Only GLG conforms to this ideal case. GL–LG shows more series character than expected for spherical inclusions. The modulus curves lie closer to those of the continous phase as would be the case if the inclusions were elongated in shape.[13] This shape change is consistent with the known relationship between inclusion shape and volume fraction for SBS block copolymer systems.[14] The modulus curves for LG–GL (L inclusions) and LGL (G inclusions) deviate in the other direction and lie closer to those of the included phase than predicted by the sphere model. Since the model has predicted that the modulus curves of the composite be closest to those of the matrix phase, the conclusion drawn is that partial phase inversion has occurred in the case of the LGL-type polymers.

Conclusions

In this and the preceding paper in this series, the preparation and solid state structure of block copolypeptides have been investigated. The unique feature of this new class of materials lies in the potential for controlling structure on the molecular level–chain conformation and organization, as well as at the level of phase separation. As regards the latter, these materials were found to behave similarly to other block polymers. As expected, the choice of solvent had a significant effect on the solid state structure of cast films. Films cast from preferential solvents for one phase 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. Phase separation was not as distinct in films cast from a nonpreferential solvent, and in this case the properties were closer to those of random copolymers.

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