small molecules, several can have slightly nonplanar amide groups (as deduced from an examination of space-filling models) as in PBIC. Since electron delocalization effects and exciton effects do not seem to be significant in PBIC, we would have expected some of these low-DP model compounds to have similar optical properties as PBIC. The reason seems to be (again, from an examination of space-filling molecular models) that these low-DP model compounds lack one important feature of the PBIC structure, which may account for the lack of similarity of their spectra and that of PBIC. Because of the helical structure of PBIC, the chain is folded in such a manner that the lone-pair electrons of N_{i+1} are close to the C=O group of the ith residue shown in Figure 6. Such a conformation might give rise to a new transition on forming the helix, especially since nitrogen–carbonyl transitions (from remote groups in the chain) have been observed previously; for example, Leonard and Oki[63] have observed an apparent charge-transfer transition from the nitrogen lone pair to the antibonding π orbital of the carbonyl group in 6-methyl-6-aza-2-hydroxycyclononanone, with log ϵ_{max} = 3.77 at 228 mμ.

Certainly, more investigation is required before the <203- and 249-mμ bands can be assigned to specific transitions in the electronically complicated PBIC molecule. Perhaps the molecular orbital calculations on models of PBIC, which Hoffmann[64] is currently carrying out, can show us the origin of the spectral properties, and also whether a charge-transfer transition contributes to the spectrum. Clearly, the quantitative ED polarization data provide important new evidence on the spectral properties of PBIC, which should be a helpful guide in theoretical investigations.

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V. Conclusion

A study of the molecular weight dependence of the ED of PBIC has shown that the molecule behaves as a rigid rod up to DP $\sim$ 600. The use of ED to detect the onset of flexibility has been demonstrated.

Three striking features of the ED spectrum are (1) the absence of negative dichroism, (2) the small variation in g with wavelength, and (3) the observation that g_{max} = 0.27. These lead to the following conclusions: (1) the residual double bond character of the N—C bond, which is nonplanar in PBIC, is too weak to cause electron delocalization over the whole polymer; (2) there appears to be at most weak exciton coupling in the spectral region studied; (3) the polarization directions 53 and 44° for the <203- and 249-mμ bands, respectively, seem to correspond to transitions which are primarily polarized along the C=O bond, and in the plane of the N—C=O group but perpendicular to the C=O bond, respectively. Although enough information is not available to be able to make definite assignments for these transitions, it would be worth considering the possibility of a nonneighboring nitrogen–carbonyl transition.

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Laser Raman Spectroscopy of Polypeptides. I. Water-Soluble Block Copolymers of L-Alanine and D,L-Lysine[1]

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ABSTRACT: Laser Raman spectra of block copolymers of L-alanine and D,L-lysine in the solid state, in salt-free water, and in aqueous salt solutions were obtained. Under all these conditions, the spectra were sensitive to the conformational states of the polymers. In addition, the spectra in aqueous salt solutions indicated the presence of interactions not present in salt-free water and were consistent with an earlier suggestion that, in the presence of salt, the shielding of the charges of the poly(D,L-lysine) end blocks permits the central poly(L-alanine) block to adopt a hairpin-like conformation (with the α helices folded back and forth) which is stabilized by side-chain to side-chain methyl–methyl hydrophobic bonding.

In the continuing search for experimental methods to provide information about protein conformation in solution, attention has turned recently to laser Raman spectroscopy of solutions of polypeptides. This technique has been applied, for example, to monomeric L-alanine and L-lysine[3] in water, to oligomers of alanine[4] in the solid state and in water, and to homopolymers of L-lysine[5,6] and of L-alanine.[7,8] The interpretation of the results from such experimental work was aided materially by theoretical normal-coordinate analyses on poly(L-alanine) by Miyazawa, *et al.*,[9] and by the publication of polarized Raman spectra of poly(L-alanine) by Peticolas, *et al.*[7]

These investigations are of direct applicability to our studies of various water-soluble block copolymers[10–12] to elucidate

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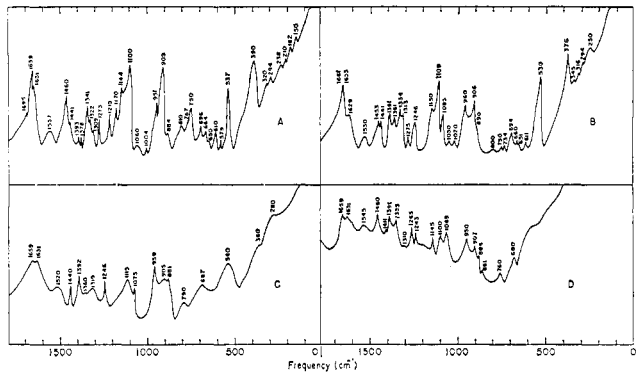

Figure 1. Raman spectrum of (D,L-Lys)$_{80}$(L-Ala)$_{160}$(D,L-Lys)$_{80}$ in (A) the solid state, (B) salt-free water at 3 g/100 cm^3, (C) aqueous 0.15 *M* KCl at 3 g/100 cm^3; (D) Raman spectrum of (D,L-Lys)$_9$-(L-Ala)$_{10}$(D,L-Lys)$_9$gly at 3 g/100 cm^3 in salt-free water.

the nature of hydrophobic bonding in proteins. Therefore, it appeared desirable to apply laser Raman spectroscopy to the block copolymers in order to obtain independent experimental evidence for their conformations. Hence, in this paper, we have obtained the solid-state and aqueous-solution spectra of the water-soluble copolymer in which a block of poly(L-alanine) is flanked on each side by blocks of poly-(D,L-lysine). These fractionated and characterized materials are the same ones used in an earlier investigation[10] of the helix–coil transition in poly(L-alanine) in water and in salt solutions. In contrast to the earlier Raman work on poly-(L-alanine),[7,8] the present results pertain to aqueous solutions of this poly(amino acid), since the block copolymer is water soluble.

Experimental Section

(A) Materials. The synthesis and characterization of the block copolymers (D,L-Lys)$_m$(L-Ala)$_n$(D,L-Lys)$_m$ were described earlier.[10] The average values of *m* and *n*, respectively, for the fractions are: (A) 9, 10; (B) 80, 160; (C) 100, 450; and (D) 210, 1000. Sample A has one glycine residue at the C terminus of the chain.

(B) Preparation of Samples. The solid materials were pressed into disks using a pellet press. The aqueous solutions were prepared at various concentrations from 3 to 8% and clarified on a column of activated charcoal. They were then passed through a 0.45-μ Millipore filter directly into the quartz vial used to obtain the spectra. A number of aqueous salt solutions were made from the filtered solutions, using NaCl, KCl, and KBr, and these solutions once again were passed through the Millipore filter directly into the quartz vial.

(C) Apparatus. The spectrometer was a homemade one, using a Coherent Radiation Laboratory Model 52A argon-ion laser. The monochromatic exciting lines used to obtain the Raman spectra were at 4880 and 5145 Å, respectively, with a laser light intensity of 400 mW. At this power level, optimum Raman scattering was obtained, without sample decomposition in the laser beam (as judged by the time-independent character of the spectrum).

The solid sample disk was placed in the laser beam at an angle of incidence of 60°, with the scattered light collected off the illuminated face. Solutions were held in quartz vials with planar quartz end plates; the laser light traversed the vertical axis of the vial, with observation of the scattered light made at right angles to the incident beam. In both cases, the scattered light was focused with a lens onto the entrance slit of a Spex 1400 double-grating monochromator. The scattering intensity at the exit slit was measured with an EMI 6256s phototube cooled with Dry Ice. The photoelectron pulses from the phototube were counted by a scaler and recorded in the memory of a Nuclear Data multichannel analyzer, Model 1100, at wavelength intervals of both 1 and 0.1 Å, with integration times of up to 60 sec at each interval. The spectra recorded in the memory of the multichannel analyzer were read out by teletype.

The spectrometer was calibrated against the discharge lines of neon, with a reproducibility of ± 1 cm^{-1}. Because of the high intensity of the signals from the polymers in all cases except sample A, slits of 20 mm (allowing a resolution of 1 cm^{-1}) were used; an 80-mm slit was used for sample A.

All of the samples exhibited fluorescence, which could be minimized by proper choice of the exciting wavelength. By saturating the excited electronic states by prolonged exposure to the laser beam, the fluorescence was allowed to decay, a technique which worked for all samples except sample A. Neither this saturation technique nor a number of purification methods worked for this sample, and the resulting spectrum has a high background because of the fluorescence, but the Raman lines are still observable.

Results

The spectra of sample B in the solid state, in salt-free water, and in aqueous 0.15 *M* KCl are shown in Figures 1A, 1B, and 1C, respectively, and the data presented in Table I. Similar spectra (not shown here) were obtained with samples C and D, from which we conclude that, in this range of chain length, the vibrational frequencies are independent of the lengths of the poly(L-alanine) and poly(D,L-lysine) blocks.

Without discussing all the frequencies in Table I, we comment here on a few pertinent ones. For this purpose, we list the assignments[3,13] of the amide bands in Table II. Because of excessive Rayleigh scattering from the samples, the lowest vibrational frequency observable in the Raman spectrum was the 150-cm^{-1} band. The band at 210 cm^{-1} remains unassigned, since this frequency was not reproduced in any normal-coordinate treatment.[9,14] The spectra of poly(L-lysine) in water[5] could not be extended below 400 cm^{-1}; however, since the band at 238 cm^{-1} is a new one, not observed in the spectra of poly(L-alanine) in the solid state,[7,8] we assign it to a conformationally dependent mode arising from the D,L-lysine blocks. The band at 390 cm^{-1} is of considerably higher frequency than that observed[7,8] (375 cm^{-1}) in the spectrum of poly(L-alanine) in the solid state, but, since both of these bands are of similar intensity, it is suggested that they both arise from a helix-deformation mode of poly(L-alanine). The vibration at 579 cm^{-1} could possibly be a mode associated with some ordered backbone structure of poly(L-lysine) since it does not seem to appear in the salt-free solution spectrum. However, in the solution, it could happen that the 570-cm^{-1} band is obscured by the envelope of the 537-cm^{-1} band (see Figure 1B). Neither homopolymer has a band at 650 cm^{-1} in the solid state;[5,7,8] however, this frequency (observed in Figures 1A and 1B) is in the amide IV region[15] and, since the corresponding vibration has been observed for poly(L-alanine)[7,8] at 662 cm^{-1} and we observe a similar band at 664 cm^{-1}, we have assigned the 650-cm^{-1} band to the amide IV vibration of poly(D,L-lysine) (its absence from the poly(L-lysine) spectrum[5] is probably due to the high background). The frequencies at 951 cm^{-1} (arising from poly(L-lysine)[5]) and at 1004 cm^{-1} (arising from poly(L-alanine)[7,8]) could not be assigned to specific modes. From a comparison of Figures 1A, 1B, and 1C, it appears that the solid-state vibration at 1170 cm^{-1} (which appears at 1085 and 1075 cm^{-1} in the solutions) arises from an unspecified

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TABLE I
RAMAN FREQUENCIES FOR (D,L-Lys)$_{80}$(L-Ala)$_{160}$(D,L-Lys)$_{80}$ IN THE SOLID STATE, IN SALT-FREE WATER, AND IN AQUEOUS 0.15 *M* KCl SOLUTION

Frequency, cm^{-1}			Approximate group modes	
Solid state	Aqueous salt-free solution	Aqueous salt solution	Lys[b]	Ala[c]
150 (w)[a]				Helix def
182 (w)				Helix def
210 (vw)				
238 (vw)				
	250 (w)	280 (vw)		
294 (w)	294 (vw)			C^{α}–C^{β} bend
320 (vw)	316 (vw)			Skeletal def
	345 (vw)			
		360 (vw)		
390 (vs)	376 (vs)			Helix def
537 (s)	530 (vs)	540 (m)		C=O in-plane bend
579 (vw)				
610 (w)	611 (vw)			Amide VI
650 (vw)	651 (vw)		Amide IV	
664 (w)	660 (vw)			Amide IV
		687 (w)		
696 (w)	694 (w)			Backbone def
750 (m)	750 (vw)			Amide V
767 (w)	734 (vw)		ϵ amino group N—H wag	
		790 (vw)		
810 (w)	800 (vw)		Residue	
884 (w)	890 (m)	881 (m)	Residue	
909 (vs)	906 (s)	905 (m)		C^{α}–C^{β} stretch + skeletal
951 (m)	960 (s)	959 (s)		
1004 (vw)	1020 (vw)			
1060 (vw)	1050 (vw)		In-plane C—H bend	
1100 (vs)	1109 (vs)	1115 (m)		C^{α}–C^{β} stretch
1144 (s)	1150 (s)		Stretch NH_3^+	
1170 (m)	1085 (m)	1075 (m)		
1215 (w)	1246 (m)	1246 (m)	CH_2 int rot.	
1273 (w)	1275 (w)			
1309 (w)	1313 (s)			Amide III
		1319 (w)		
1322 (w)			Amide III	
1341 (m)	1334 (s)		C—H bend	C—H bend
		1360 (w)		
1378 (vw)	1361 (m)			Me sym def
1393 (vw)	1391 (s)	1392 (m)	Amide III	
1441 (w)	1441 (m)	1440 (m)	CH_2 bend	
1460 (m)	1453 (m)			Me asym def
1557 (w)	1530 (w)	1520 (w)	Amide II	Amide II
1651 (s)	1661 (s)		Amide I	
1659 (vs)	1653 (s)	1631 (s)		Amide I
1695 (m)	1629 (m)	1659 (s)		Amide I

[a] Qualitative estimates of relative intensity: vw = very weak, w = weak, m = medium, s = strong, vs = very strong. [b] The poly(D,L-lysine) group modes were obtained from ref 5. [c] The poly(L-alanine) group modes were obtained from ref 8 and K. Itoh and T. Shimanouchi, *Biopolymers*, **9**, 383 (1970).

mode of poly(D,L-lysine). This conclusion is based on the fact that poly(L-alanine) bands generally undergo small changes in frequency upon dissolution, whereas the 1170-cm^{-1} band undergoes a large change in frequency; further, in salt solutions (Figure 1C), poly(L-alanine) bands are broadened, whereas poly(D,L-lysine) bands remain fairly sharp, and the 1075-cm^{-1} band is fairly sharp. The bands observed in other regions of the spectrum, which have been well characterized by previous workers, are given in Table I together with their assignments.

When the copolymer is dissolved in salt-free water, the low-frequency band at 238 cm^{-1} disappears, with new bands appearing at 250 and 345 cm^{-1}. The helix-deformation mode at 390 cm^{-1} shifts to 376 cm^{-1}, where it was observed in previous work on solid-state poly(L-alanine).[7,8] The side-chain wag of the ϵ amino group of D,L-lysine (observed at 764 cm^{-1} in solid-state poly(L-lysine)[5]) shifts from 767 to 734 cm^{-1} (probably because of hydration of the amino group), and the lysine side-chain vibration at 1215 cm^{-1} shifts to 1246 cm^{-1}. The poly(D,L-lysine) amide III band at 1322

TABLE II
ASSIGNMENTS OF AMIDE BANDS[a]

Amide band	Approximate frequency, cm^{-1}	Assignment
I	1650	C=O stretch
II	1550	N—H in-plane def
III	1300	Coupled C—N stretch and N–H in-plane bend
IV	630	O=C—N in-plane bend
V	725	N—H out-of-plane bend
VI	600	C=O out-of-plane bend
VII	200	Torsion about C—N bond

[a] R. C. Lord and N. Yu, *J. Mol. Biol.*, **50**, 509 (1970); ref 13.

cm^{-1} vanishes, and a large increase in the intensity of the 1393-cm^{-1} mode takes place. Also, the intensities and frequencies of bands in the amide I region shift upon dissolution.

As an aid in the interpretation of the results, the spectrum of sample A (with one glycine residue and a very short alanine chain) in salt-free water was also obtained. The data for this sample are shown in Figure 1D and Table III; despite the presence of a large amount of fluorescence from this sample (as seen from the appearance of Figure 1D), the Raman frequencies are still detectable.

Finally, the spectra of samples B, C, and D in aqueous 0.15 *M* salt (using a number of different salts including NaCl, KCl, and KBr) differed from those in salt-free water (*cf.* Figures 1B and 1C). In essence, the poly(D,L-lysine) bands, for the most part, have comparable widths in the solid state, in salt-free water, and in 0.15 *M* (or higher) salt. However, *every* poly(L-alanine) frequency exhibited an unusually large breadth and decrease in intensity in the presence of salt. This width increased very suddenly in the range of salt concentration between 0.10 and 0.15 *M*, and did not change at higher salt concentrations.

TABLE III
RAMAN FREQUENCIES FOR
$(D,L\text{-Lys})_9(L\text{-Ala})_{10}(D,L\text{-Lys})_9Gly$ IN SALT-FREE WATER

Frequency, cm^{-1}	Approximate group modes		
	Gly[b]	Lys[c]	Ala[d]
680 (w)[a]			Backbone def
760 (w)		ϵ amino group N–H wag	
861 (vw)	Residue		
884 (m)		Residue	
907 (s)			C^{α}–C^{β} stretch + skeletal
950 (m)			
1069 (s)		In-plane C–H bend	
1100 (s)			C^{α}–C^{β} stretch
1145 (m)		Stretch NH_3^+	
1243 (m)		CH_2 int rot.	
1265 (m)			
1310 (vw)	Residue		
1355 (s)		C–H bend	C–H bend
1391 (s)		Amide III	
1411 (vw)			
1460 (m)			Me asym def
1545 (w)		Amide II	Amide II
1631 (m)			Amide I
1659 (s)		Amide I	

[a] Qualitative estimates of relative intensity: vw = very weak, w = weak, m = medium, s = strong, vs = very strong. [b] The glycine group modes were obtained from M. Smith, A. G. Walton, and J. L. Koenig, *Biopolymers*, **8**, 29 (1969). [c] The lysine group modes were obtained from ref 5. [d] The alanine group modes were obtained from ref 8 and 14.

Discussion

The bands which are most sensitive to changes of conformation of the backbone are the low- and intermediate-frequency ones. By comparing the spectrum in the solid state with that in solution, it is possible to observe the variation in frequency which accompanies the change of conformation upon dissolution.

Some of the low frequencies of Figure 1A are similar to those of the homopolymer α-helical poly(L-alanine),[7,8] and indicate that the poly(L-alanine) block of the copolymer in the solid state is α helical. Low-frequency poly(D,L-lysine) bands are interspersed among the poly(L-alanine) bands. Unfortunately, these bands were not observed in earlier studies of poly(L-lysine)[5] because of experimental limitations and cannot be identified. In the intermediate-frequency region, the bands at 909, 1100, and 1273 cm^{-1} (observed previously[7,8]) confirm the conclusion that the poly(L-alanine) is α helical, and the band at 1215 cm^{-1} (which has been suggested[5] as being an indication that poly(L-lysine) is in the α-helical form) indicates that the poly(D,L-lysine) may be α helical in the solid state. This indication is supported by the poly(D,L-lysine) band at 1322 cm^{-1}, which is very close to the amide III band at 1323 cm^{-1} of α-helical poly(L-lysine).[5] The suggestion that poly(D,L-lysine) may be α helical in the solid state is surprising, since it may indicate that the D and L residues are not randomly distributed but may occur in separate runs of D and of L residues. Alternatively, the question arises as to whether a random D,L-lysine copolymer can form an α-helical structure in the solid state. Perhaps conformational energy calculations can ultimately provide an answer to this question. The conformational consequences of the splitting of the amide III band [from both poly(D,L-lysine) and poly(L-alanine)] into a number of components (1309, 1322, 1341, and 1393 cm^{-1}) are the following. The 1309-cm^{-1} band (in the region of the amide III band of α-helical poly(L-alanine)[7,8]) also implies that the poly(L-alanine) block is α-helical in the solid state. The one at 1393 cm^{-1} indicates the coexistence of random-coil and α-helical conformations among the lysines. This interpretation is consistent with previous studies of poly(L-lysine) in water at neutral pH[5] and also with the appearance of a similar band in salt-free water solutions of sample A, where the lysines are known[10,11] to be in a random-coil state. Finally, in the high-frequency region, the amide I band is split into three components. The band at 1651 cm^{-1} coincides with that observed[5] for α-helical poly(L-lysine), that at 1659 cm^{-1} is in close agreement with that found[7,8] for the amide I band in poly(L-alanine), and the weaker component at 1695 cm^{-1} corresponds to a non-hydrogen-bonded C=O stretching mode (as obtained in studies of oligomers of L-alanine[4]). This indicates that the L-alanine residues are distributed among both α-helical and random-coil conformations.

In salt-free water, the poly(D,L-lysine) blocks are known to be devoid of α-helical structure[10,11] and the poly(L-alanine) blocks to be mixtures of α-helical and random-coil conformations,[10] on the basis of optical rotatory dispersion (ORD) measurements. This is confirmed by the Raman spectra of these copolymers in salt-free water. The 1695-cm^{-1} frequency of the solid-state spectrum shifts to 1629 cm^{-1}, indicating that the C=O groups of random-coil poly(L-alanine)

residues now extend into water and bind water (to lower their frequency). The 1661-cm^{-1} band (appearing in aqueous poly(L-lysine)[5] and in copolymer samples A, B, C, and D) may be attributed to random-coil poly(D,L-lysine); hence, the 1653-cm^{-1} band must arise from α-helical poly(L-alanine). The Raman scattering of pure water produces only one (weak) band in the region of the spectra of Figure 1. Unfortunately, this band occurs at 1640 cm^{-1} and could cast doubts on our assignments of the amide I band. However, the consistency in amide I frequencies of poly(L-lysine)[5,6] and of samples A, B, C, and D in salt-free water gives us confidence in the above assignments. In the amide III region, the 1322-cm^{-1} band [from α-helical poly(D,L-lysine)] disappears and that at 1391 cm^{-1} [from random-coil poly(D,L-lysine)] increases in intensity in water. The band at 1313 cm^{-1}, which persists in water, and other bands in the intermediate and low-frequency range indicate the presence of α-helical poly(L-alanine) residues. The existence of a complete random-coil conformation of poly(D,L-lysine) is indicated[5] by a shift of the 1215-cm^{-1} band to 1246 cm^{-1}; the appearance of two new bands at 250 cm^{-1} and 345 cm^{-1} is also probably attributable to random-coil poly(D,L-lysine).

In salt solution, the charges on the poly(D,L-lysine) blocks are partially shielded; as a result of the reduced electrostatic repulsions, the poly(L-alanine) block is thought[10] to fold into a hairpin-like structure with interhelical side-chain to side-chain hydrophobic bonds. The laser Raman spectrum of Figure 1C tends to support this view; *i.e.*, the vibrational frequencies of poly(L-alanine) [but not of poly(D,L-lysine)] are spread out into considerably broader bands. It was shown earlier[16] that formation of a hydrophobic bond leads to changes in the force constants (for torsional oscillations about carbon–carbon single bonds) which depend on the strength of the hydrophobic bond. Presumably, the broad peaks arise from a distribution of hydrophobic bonds of variable strength (arising from variable locations of the hairpin bends) mixed in with a certain amount of nonbonded methyl side chains; this variable degree of bonding would be expected to influence all of the poly(L-alanine) frequencies, not only those of the side-chain methyl groups. While one might have argued that the change in the nature of the environment (*i.e.*, addition of salt) would have a similar broadening effect on the spectrum, this argument is not valid since the effect is quite discrete, *i.e.*, only the alanine bands are broadened. In addition, other poly(amino acids) in salt solutions studied by us[17] and others[18] show no comparable broadening effects.

In conclusion, the laser Raman spectra of the copolymers studied here indicate that both the poly(D,L-lysine) and poly(L-alanine) blocks consist of mixtures of α helix and random coil in the solid state. In aqueous solution, the poly(D,L-lysine) is exclusively in the random-coil form, but the poly(L-alanine) remains as a mixture of helical and coil conformations. In the presence of salt, the spreading of the vibrational frequencies indicates the presence of alanine–alanine hydrophobic bonds (postulated earlier[10] as a feature of hairpinlike structures to account for the melting behavior in salt solutions). Since the Raman spectra are in agreement with the earlier ORD studies, we may use the laser Raman technique (which provides detailed information about vibrational modes in these biopolymers) as a probe of internal interactions.

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Light-Scattering Studies on Polystyrene–Polyisoprene Block Copolymers

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ABSTRACT: Two-block (AB) copolymers of styrene and isoprene have been prepared by an anionic method. Three samples were studied by light-scattering techniques in a number of solvents. With solvents having a refractive index far removed from those of polystyrene or polyisoprene, normal Zimm plots are obtained. Where the solvent refractive index approaches that of either component, the Zimm plots become distorted as expected if intermolecular interference is an important phenomenon. Under these conditions, the intermolecular excluded volume is a function of the size of the whole molecule, whereas only a smaller part of the molecule actually scatters light. It is shown that this can produce the results observed. Some separation of the two types of segments is implied.

Theoretical predictions[2] and experimental observations[3] show that chemically dissimilar polymers are generally incompatible as evidenced by a general phenomenon of phase separation in ternary systems consisting of two polymeric species and a common solvent. The exceptions to this incompatibility rule occur only in a limited number of cases, principally among pairs of polar molecules which interact favorably, *i.e.*, with negative heat of mixing. A peculiar situation arises, however, when dissimilar and incompatible homopolymers are combined in a block or graft copolymer. The repulsions between unlike segments of chain are expected to give rise to unusual structures, and this has been shown to be so for the structures of block copolymers of styrene and

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