Solution Properties of Poly(D-β-hydroxybutyrate). 1. Biosynthesis and Characterization

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ABSTRACT: Dilute solution properties of poly(D-β-hydroxybutyrate) (PHB), extracted from Azotobacter *vinerandii* and fractionated with the system dichloromethane–propylene glycol, were investigated by viscometry, osmometry, light scattering, and optical rotatory dispersion (ORD) with samples ranging in molecular weight from 11.5×10^4 to 339×10^4. The shape of ORD curves of PHB did not vary appreciably with solvents studied, and specific rotation exhibited no abrupt change with temperature or solvent composition in mixtures of ethylene dichloride (EDC) and dichloroacetic acid (DCA), differing from the report of Marchessault et al. The indices for molecular weight dependence of intrinsic viscosity and radius of gyration in trifluoroethanol (TFE), chloroform, and EDC approached the limits expected for flexible linear polymers whose expansion factor obeys the fifth-power law. The values of the second virial coefficient for the PHB–TFE system were quite large, ranging from 7.25×10^{-4} to 16.4×10^{-4}. We conclude that the molecular conformation of PHB in what Marchessault et al. called helicogenic solvents is essentially random coil and the molecule is expanded by an unusually large volume exclusion.

Poly(D-β-hydroxybutyrate) (PHB) is a chiral, optically active, and crystallizable polymer (Figure 1), which is biologically synthesized by some bacteria. Morphorogy of native PHB grannules was first investigated by Merrick et al.[1] and then more extensively by Eller et al.[2] The crystal structure of solid PHB was examined by Alper et al.,[3] Okamura et al.,[4] Cornibert et al.,[5] and Yokouchi et al.[6] It was shown that the PHB molecule in crystalline regions assumes a right-handed 2_1 helix. The bond angles and bond lengths of the helical molecular chain as determined by Yokouchi et al.[6] are indicated in Figure 1. The question then arises as to whether the helical structure of PHB is maintained in solution.

Dilute solutions of this polyester were first investigated by Marchessault et al.[7] and then by Cornibert et al.[8] Marchessault et al. found that optical rotatory dispersion (ORD) of PHB in solution depended on the solvent and that specific rotation underwent a sharp change, similar to helix–coil transitions of polypeptides, when the temperature was varied at a fixed solvent composition or the solvent composition was varied at a fixed temperature, in a binary mixture of dichloroacetic acid (DCA) and ethylene dichloride (EDC). From these observations they concluded that the molecular conformation of PHB in such solvents as EDC, trifluoroethanol (TFE), and chloroform is predominantly helical and becomes randomly coiled in such solvents as DCA and dimethyformamide (DMF).

Marchessault et al.[7] carried out measurements of intrinsic viscosity $[\eta]$ and limiting sedimentation coefficient s_0 on chloroform solutions of unfractionated PHB. However, their results did not conform to the expectations for rigid rods with constant diameter, as can be understood from the following relations they reported:

$$[\eta] = 7.7 \times 10^{-5} \overline{M}_w^{0.82} \quad \text{(chloroform at 30 °C)} \quad (1)$$

$$s_0 = -0.02 \times \overline{M}_w^{0.46} \quad \text{(chloroform at 20 °C)} \quad (2)$$

Here $\overline{M}_w$ is the weight-average molecular weight, and $[\eta]$ and s_0 are measured in dl/g and Svedbergs, respectively. Marchessault et al.[7] offered an interrupted helix model for PHB in chloroform in order to compromise these different results from optical and hydrodynamic measurements.

Cornibert et al.[8] aimed at establishing a more definite model of PHB in solution by light-scattering measurements, this time using TFE as solvent. The analysis of the data was undertaken on the presumption that the overall shape of PHB in this "helicogenic" solvent be essentially rodlike, and it was found that the values of the mass per length varied appreciably with the chain length, the behavior apparently inconsistent with rigid rods of constant diameter. On this finding Cornibert et al. proposed a new model that they called the folded chain model. From the observed values of Marchessault et al.[7] for $[\eta]$ of PHB in TFE, they derived an empirical relation

$$[\eta] = 2.51 \times 10^{-4} \overline{M}_w^{0.74} \quad \text{(TFE at 30 °C)} \quad (3)$$

The exponent 0.74 is typical of randomly coiled polymers in good solvents. However, these authors interpreted the viscosity data in terms of the prolate ellipsoid model and showed that the results were compatible with the hypothesis of chain folding.

Except for polypeptides which are capable of forming the β structure, this is certainly the first time that the folded chain model was invoked to explain light-scattering and hydrodynamic data of a macromolecule in dilute solution. Cornibert et al.[8] reasoned the possibility for this model, but it does not seem that their proposed mechanism is very much persuasive. It is apparent that before the final acceptance of it one must reexamine the reproducibility and accuracy of their original experimental results.

With this idea we have performed light-scattering and viscosity measurements on a number of fractionated samples of PHB covering a broad range of molecular weight, with TFE as the solvent. This paper describes the observed data and their interpretations. It is shown that, contrary to the expectations from the polarimetric data of Marchessault et al., our dilute solution data can be explained in terms of the conventional random coil model if excluded-volume effects are taken into account. Separate polarimetric experiments have failed to confirm the results of Marchessault et al. The data obtained are also included in the present article.

Experimental Section

Samples. Biosynthesis of PHB. The microorganism used was an Azotobacter *vinerandii* ATCC 12837, which had been known to accumulate PHB more than 30% of the weight of dry cells under suitable culture conditions.[9] The liquid reported by Stockdale et al.[10] was prepared for culturing, excepting that glucose in their prescription was replaced by sucrose.

First, after the stock-culture medium had been inoculated, the liquid medium was maintained at about 30 °C for about 30–40 h in shaker flasks. This precultured medium was then transferred into the main culture medium in shaker flasks at a ratio of 1:40, and the microorganism was allowed to grow under similar conditions. The growth was followed by measuring 610 nm optical density of the liquid medium. The incubation was terminated at the maximum growth, because previous work had indicated that a maximum

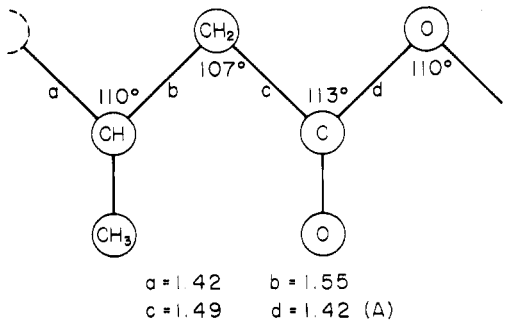

Figure 1. Schematic projection of the PHB chain.

polymer production can be obtained then.[11] The bacterial cells were separated from the culture medium by centrifugation at 5000 rpm for 25 min, washed with water to remove water-soluble components, and again centrifuged at the same angular speed. They were then lyophylized from suspensions in water.

The polymer was extracted from the dry cells with chloroform or dichloromethane (DCM). After the bacterial residues had been removed by filtration, the polymer was precipitated by pouring the filtrate into *n*-hexane and dried under reduced pressure. The entire process was undertaken several times to accumulate the polymer. Yields of individual operations ranged from 8 to 30% of the weight of dry cells. NMR and ir spectra taken on the product checked the reported results of PHB.[12,13] The ratio of the numbers of methyne, methylene, and methyl protons computed from the NMR spectrum agreed closely with the value theoretically expected for this polymer. Thus we concluded that there was no contamination from poly(β-hydroxyvalerate).[14] The intrinsic viscosity of the product in chloroform at 30 °C was about 9 dl/g, which, according to eq 1, suggested a very high molecular weight.

Alcoholysis. The native PHB thus obtained was subjected to alcoholytic cleavage in order to prepare samples of lower molecular weight. With methanol and *p*-toluene sulfonic acid (*p*-Ts) as alcohol and catalyst, respectively, the alcoholysis was performed in the following way. A methanol solution of *p*-Ts, which had been adjusted to a proper composition, was added to a DCM solution of the polymer, and the mixture was allowed to stand at room temperature. The flow time of the mixture was measured at suitable intervals of time by a capillary viscometer of the Ubbelohde type to see the progress in alcoholytic cleavage. The flow time decreased rapidly at the beginning and then gradually. When it reached a desired level, the mixture was poured into methanol to precipitate the polymer. *p*-Ts was removed from the precipitate by thorough washing with methanol. By repeating this process we prepared eight samples, which had intrinsic viscosities in chloroform at 30 °C ranging 1.66 to 8.12 dl/g.

Fractionation. The most serious point relating to the solution studies by Marchessault et al.[7] and Cornibert et al.[8] is that the PHB samples they used were too broad in molecular weight distribution. For example, the $\overline{M}_w/\overline{M}_n$ ($\overline{M}_n$ is the number-average molecular weight) ratios for the samples E and K of Cornibert et al. were about 6 and 4.5, respectively. Apparently, the data obtained with such samples are of less value for an accurate evaluation of the behavior of a polymer in dilute solution unless proper corrections for polydispersity are made.

Marchessault et al.[7] state that low molecular weight samples of PHB can be fractionated by extraction with hot ethanol or ether, or by passing a chloroform solution through a Sephadex column, but these techniques fail for high molecular weight samples. Therefore, we sought a solvent–precipitant pair which could be utilized for the fractionation of our high molecular weight samples, and after a series of extensive solubility tests, we found that fractional precipitation with chloroform or DCM as solvent and propylene glycol as precipitant allowed fairly effective separation of such samples of PHB to be made. It is worth noting that very recently de Mola et al.[15] have reported the use of the system chloroform–acetone for precipitation fractionation of this polymer.

We carried out the fractionation in the following way. A given sample was dissolved in DCM to a concentration ranging from 0.2 to 1.0%, depending on its intrinsic viscosity. The solution was filtered through a sintered glass disk to remove insoluble materials. Then the filtrate was thermostated at 25 °C in a fractionation vessel, and propylene glycol was added until it remained turbid upon vigorous stirring. The precipitate disappeared completely when the temperature was raised to 30 °C. Then the solution was cooled to 25 °C and allowed to stand. The resulting turbid solution separated quite rapidly into distinct sol and gel phases. The gel phase was taken out of the vessel, diluted with DCM to a suitable concentration, and poured into methanol to precipitate the polymer, while the sol phase was directly subjected to further fractionation in the vessel. After these operations had been repeated on the eight samples, fractions having nearly identical intrinsic viscosities (in chloroform at 30 °C) were combined and refractionated. This process was repeated several times, and ten samples were selected from the final fractions for the present study.

Light-Scattering Measurement. Light-scattering experiments were done on TFE solutions at 25 °C by use of a Fica 50 automatic light-scattering apparatus. The procedures described by Tsuji et al.[16] were followed. Test solutions and the solvent were centrifuged at about 40 000 g for 2 h in a Sorvall RC2-B centrifuge and directly pipeted into the cell. Data were taken in the range of scattering angles from 30 to 150°, in most cases, with vertically polarized light of 436 nm as the incident beam. Values of $\overline{M}_w$, $\langle S^2 \rangle$ (mean-square radius of gyration), and A_2 (second virial coefficient) were determined by applying Berry's square-root plot,[17] except for the samples of $\overline{M}_w$ higher than 10^6, for which Fujita's plot[18] was used, since preliminary measurements had indicated very large dimensions of these samples.

Specific refractive index increment dn/dc of PHB in TFE was determined by use of a differential refractometer of the modified Schulz-Cantow type. The values for the wavelengths of 436 and 546 nm at 25 °C were 0.152_5 and 0.149_1 ml/g, respectively.

Osmometry. Osmotic pressure measurements were made on samples B-32, B-42, B-5, B-62, and B-72 in chloroform at 35 °C and on sample B-23 in EDC at 30 °C. Use was made of a Knauer membrane osmometer, fitted with Superdense Sartrius Ultrafine filters conditioned carefully.

Viscometry. Except for samples A-12, A-22, and AB-12 in TFE, viscosity was measured by capillary viscometers of the Ubbelohde type designed to need no kinetic energy correction. For these three samples in TFE use was made of a rotational viscometer of the Zimm–Crothers design, because non-Newtonian behavior was anticipated. For all the ten samples the data were obtained in TFE at 25 °C, in chloroform at 30 °C, and in EDC at 30 °C.

Optical Measurement. Sample F-232, which had an intrinsic viscosity of 7.92 dl/g in chloroform at 30 °C, was used to study specific rotation and optical rotatory dispersion (ORD) of PHB in TFE, chloroform, EDC, DCA, DMF, and so forth.

ORD measurements were performed on a JASCO ORD/UV-5 autorecording spectropolarimeter. For wavelengths from 270 to 650 nm use was made of a cell 10 cm long and about 5 ml in capacity. The cell used for wavelengths between 190 and 300 nm was 0.5 mm long. Data with the former cell were taken at polymer concentrations between 0.7 and 1.0 g/dl and those with the latter cell at polymer concentrations between 1.3 and 3.0 g/dl. No extrapolation of the data to infinite dilution was attempted.

Specific rotations in mixed solvents of EDC and DCA as a function of temperature and solvent composition were measured by the JASCO polarimeter mentioned above and also by a JASCO Model DIP-SL automatic polarimeter. A 2-cm cell was used for the former apparatus and a 10-cm cell for the latter.

From flow time measurements, slight and gradual degradation of the polymer was detected in DCA, DMF, and 2-chloroethanol. In order to minimize this effect, each polarimetric measurement was completed within about 20 min after the preparation of a test solution.

Results and Discussion

Molecular Weights and Second Virial Coefficient. Figure 2 shows the concentration dependence of reduced scattering intensities extrapolated to zero scattering angle on Berry's square-root plot. The set of data points for each sample may be extrapolated to infinite dilution as indicated by the solid lines. The data points, except for samples A-12 and A-22, fit closely the indicated straight line and can be extrapolated accurately to infinite dilution. For these two samples whose data points reveal a slight downward curvature, the ordinate intercepts were determined by Fujita's method (see Figure 5). The values of $\overline{M}_w$ and A_2 determined in this way are summarized in Table I. This table also gives the values of $\overline{M}_n$ and A_2 obtained from the osmotic pressure data displayed in Figure 3.

Table I
Results of Light Scattering and Osmometry on PHB Fractions

Sample code	Light scattering in TFE at 25 °C			Osmotic pressure in $CHCl_3$ at 35 °C		
	$\overline{M}_w \times 10^{-4}$	$A_2 \times 10^4$ [c]	$\langle S^2 \rangle^{1/2}$, A	$\overline{M}_n \times 10^{-4}$	$A_2 \times 10^4$ [c]	$\overline{M}_w/\overline{M}_n$
A-12	339[a]	8.45	1350[a]			
A-22	164[a]	8.12	886[a]			
AB-12	135[a]	8.53	783[a]			
AB-22	85.7	9.76	569			
B-23	63.0	10.6	493	28.0[b]	6.07[b]	2.25
B-32	53.3	10.6	430	27.7	8.40	1.92
B-42	37.4	12.4	355	22.5	8.72	1.66
B-5	23.6	13.5	273	19.1	9.34	1.24
B-62	22.9	12.9	279	16.0	10.3	1.43
B-72	11.5	16.4	189	8.55	12.5	1.35

[a] Calculated from the Fujita plot. [b] Estimated from the data in EDC at 30 °C. [c] Second virial coefficient A_2 is given in mol ml/g^2.

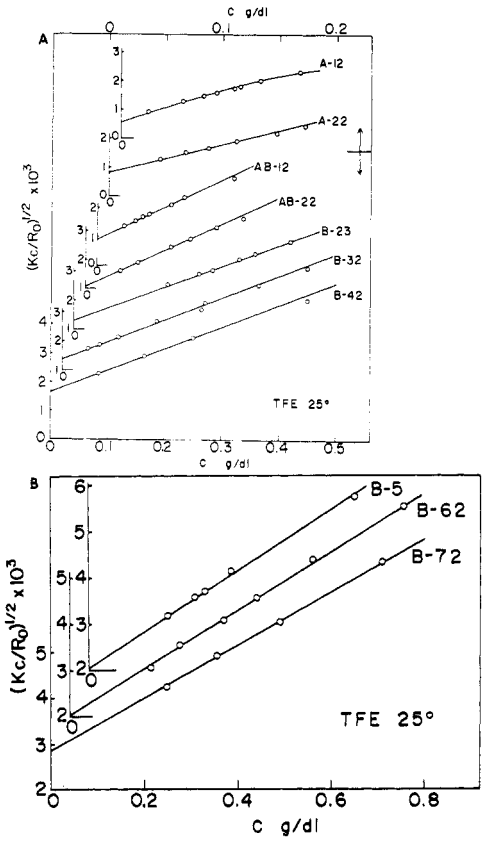

Figure 2. Concentration dependence of excess scattering intensity at zero angle plotted as $(Kc/R_0)^{1/2}$ vs. polymer concentration c: (a) for samples with molecular weights higher than 37.4×10^4; (b) for samples with molecular weights lower than 23.6×10^4.

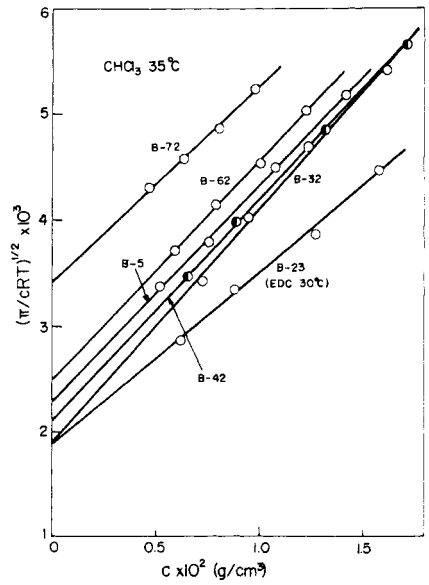

Figure 3. Square roots of reduced osmotic pressure $(\pi/cRT)^{1/2}$ plotted against polymer concentration c.

It is seen that the A_2 values from light-scattering and osmotic pressure measurements, though differing by a factor of about 1.3 due to the difference in the solvents used, are quite large, being of the order of magnitude expected for flexible polymers in very good solvents. The $\overline{M}_w/\overline{M}_n$ ratios indicated in the seventh column of Table I range from 1.24 to 2.25, indicating that the samples at least subjected to osmotic pressure measurements were still considerably polydisperse in molecular weight. But they are not as much polydisperse as those studied by Marchessault et al.[7] and Cornibert et al.[8] A more extensive fractionation of PHB is being carried out in this laboratory for acquiring less polydisperse samples which hopefully will allow a more accurate molecular characterization than made in the present preliminary study.

Particle Scattering Function and Radius of Gyration. Figure 4 shows the particle scattering functions $P(\theta)$ (θ is the angle of scattering) for the ten samples studied on Berry's square-root plot. Except for the three higher molecular weight samples, the data points may be fitted by a straight line as indicated and allow $\langle S^2 \rangle$ to be determined with accuracy. The plots for the remaining samples display so pronounced a downward curvature that their initial tangents, and hence $\langle S^2 \rangle$, can be estimated only with uncertainty. This behavior is attributed, if not all, to very large radii of gyrations of the samples, expectable from their high molecular weights as well as the large A_2 values in the solvent used. We therefore undertook to analyze their data in terms of the Fujita plot,[18] which had been known to be useful in such circumstances. Figure 5 shows the resulting plots, and one finds that the initial slopes, and hence $\langle S^2 \rangle$, can be determined without much uncertainty. The values of $\langle S^2 \rangle^{1/2}$ derived in these ways for the ten samples are also summarized in Table I.

The molecular weight dependence of $\langle S^2 \rangle$ is shown on a conventional log–log plot in Figure 6. The thick straight line drawn on the graph is represented by

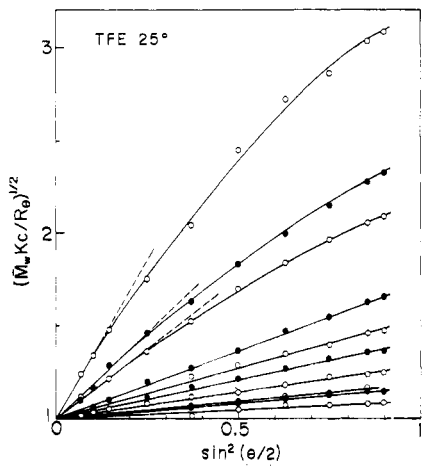

Figure 4. Angular dependence of particle scattering function $P(\theta)$ plotted as $(\bar{M}_w Kc/R_\theta)^{1/2}$ vs. $\sin^2(\theta/2)$. Here $(\bar{M}_w Kc/R_\theta) = P(\theta)^{-1}$. The lines correspond to samples A-12, A-22, AB-12, AB-22, B-23, B-32, B-42, B-62, B-5, and B-72, from top to bottom.

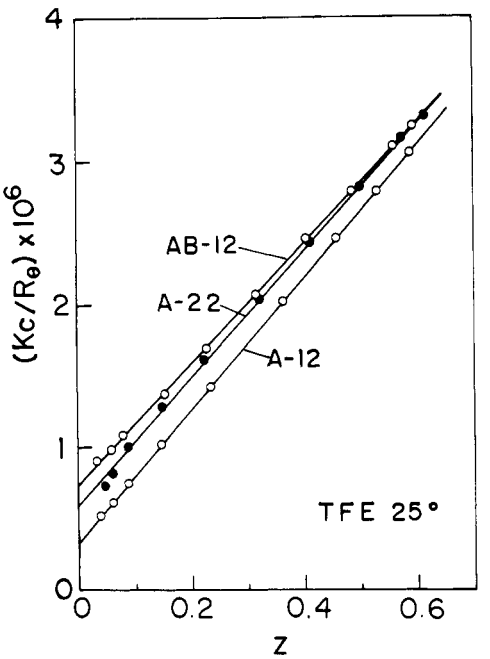

Figure 5. Fujita's plots for samples A-12, A-22, and AB-12. Here z is given by $z = \int_0^u yvdv/yu^{4/3}$, where $y = \lim_{c\to 0} R_\theta/Kc$ and $u = \sin^3(\theta/2)$.

$$\langle S^2\rangle = 3.55 \times 10^{-18}\bar{M}_w^{1.18} \quad (4)$$

where $\langle S^2\rangle$ is given in cm^2. The exponent 1.18 is quite close to the limiting value of 1.20 expected from the fifth-power theories for the expansion factor of randomly coiled polymers in good solvents.[19] In this connection, we note that the A_2 values in Table I suggest TFE to be a very good solvent for PHB. Thus our light-scattering results may be interpreted if the molecule of PHB in TFE is visualized as a random coil expaned pronouncedly by volume exclusion.

The thin line in Figure 6 has been drawn to fit the data point of Cornibert et al.[8] for the three higher molecular weight samples of PHB in TFE at about 22 °C. Its slope is nearly the same as that of the thick line, but there is a great gap between the locations of the two lines. In fact, when compared at the same $\bar{M}_w$, the $\langle S^2\rangle$ values of Cornibert et al. are about four times larger than the values from the present measurements. The discrepancy seems to be too

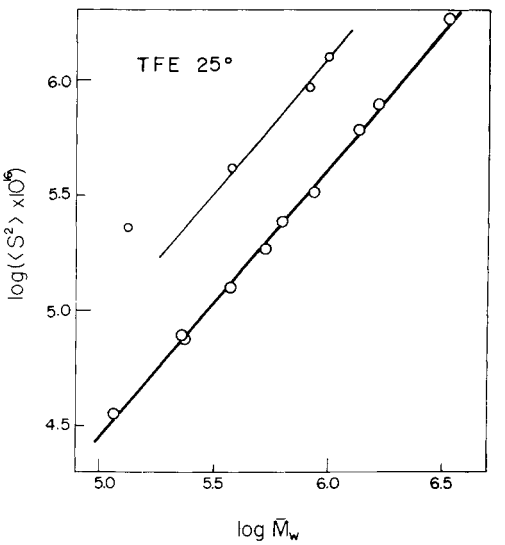

Figure 6. Molecular weight dependence of radius of gyration $\langle S^2\rangle$ in TFE. Large circles, present data at 25 °C; Small circles, data of Cornibert et al. at about 22 °C.

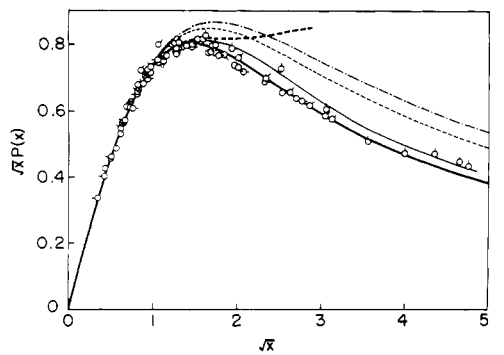

Figure 7. Angular dependence of particle scattering function $P(\theta)$ represented as a plot of $x^{1/2}P(x)$ vs. $x^{1/2}$. Various marks represent difference of samples: pip up, A-12; successive 45° rotations clockwise correspond to A-22, AB-12, AB-22, B-23, B-32, B-42, B-5, respectively; circles with cross, B-62; no pip, B-72; (—) monodisperse Gaussian coil; (- - -) monodisperse rod; (- . -) Schulz–Zimm distribution Gaussian coil with $h = 1$; (- - -) Schulz–Zimm distribution Gaussian coil with $h = 2$; (—) experimental curve for A-12. h is defined by $\bar{M}_w/\bar{M}_n = (h + 1)/h$.

large to be explained simply in terms of the difference in polydispersity between the samples used by the two groups of workers.

It is probably fair to examine, as was done by Cornibert et al., whether our data for $P(\theta)$ are interpretable in terms of the rigid rod model. In Figure 7, the values of $x^{1/2}P(x)$ computed from the present data are plotted against $x^{1/2}$, where $x^{1/2}$ denotes $(4\pi/\lambda)\langle S^2\rangle^{1/2} \sin(\theta/2)$, with λ being the wavelength of light in the solvent. The thick solid curve in the figure shows the theoretical values expected for monodisperse Gaussian coils. It has been shown recently[20,21] that this type of plot for $P(\theta)$ is unexpectedly insensitive to excluded volume effects when the polymer assumes randomly coiled conformation. Furthermore, calculations with the Schulz–Zimm distribution of molecular weights disclosed that polydispersity effects on the plot for such polymers manifest themselves mostly in the region beyond the maximum. The thin curves in Figure 7 illustrate this fact. The data points plotted in this graph by and large conform to the predictions from these observed and computed results for randomly coiled polymers. However, it must be pointed

Table II
Intrinsic Viscosities of PHB Fractions

Sample code	[η], dl/g, TFE at 25 °C	$CHCl_3$ at 30 °C	EDC at 30 °C
A-12	17.3[a]	10.9	8.91
A-22	12.1[a]	8.11	6.46
AB-12	9.39	6.84	5.26
AB-22	7.09	4.96	3.96
B-23	5.63	3.99	3.14
B-32	4.79	3.28	2.61
B-42	3.70	2.58	2.02
B-5	2.52	1.84	1.41
B-62	2.43	1.75	1.35
B-72	1.39	1.04	0.802

[a] Obtained by using a rotational viscometer.

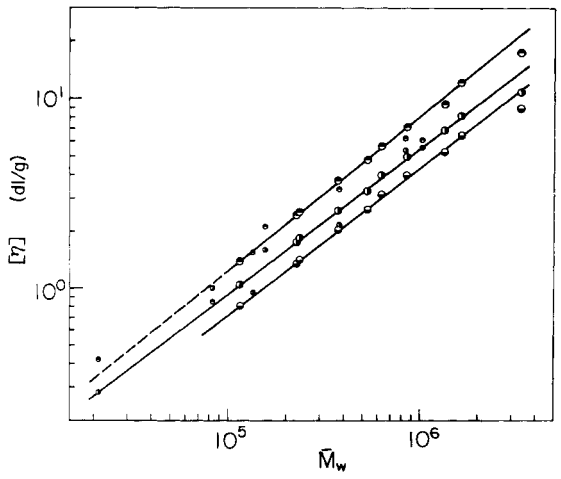

Figure 8. Molecular weight dependence of intrinsic viscosity [η]. Large circles, present data: ◓, TFE 25 °C; ◑, chloroform 30 °C; ◒, EDC 30 °C. Small circles, data of Marchessault et al.: ◓, TFE 30 °C; ◑, chloroform 30 °C.

out that our data for higher molecular weight samples appear too closely to the curve for monodisperse Gaussian coils, dispite the fact that they are presumably quite polydisperse. The thick dashed line in Figure 7 shows the behavior expected for monodisperse rigid rods. Apparently, it is hard to say that our data are compatible with it. Thus, even though some points still remain to be clarified, we may conclude that the overall feature of the results from the present light-scattering experiments substantiate random coil conformation of PHB in TFE. This conclusion, however, does not rule out the possibility that the chain holds helical conformation locally.

Cornibert, et al.,[8] state that Figure 2 of their paper, which shows $x^{1/2}P(x)$ vs. $x^{1/2}$ for their four samples in TFE, demonstrates the asymptotic behavior of rodlike molecules. But it is difficult to accept this statement, none of the plots for all the four samples exhibiting evidence for the approach to a horizontal asymptote. Rather, the plots for the two highest molecular weights display maxima, thus being more or less similar in shape to the curve for random coils. However, these maxima appear at a $x^{1/2}$ of about 2.0, while the solid curve in Figure 7 has its maximum at $x^{1/2} = 1.4$. In sum, Cornibert et al. had no definitive evidence to interpret their light-scattering data in terms of rodlike molecules, and the folded chain model that they invoked to account for the variation of the mass per length ratios with molecular weight seems to be a mere ad hoc structure.

Intrinsic Viscosity. Data obtained for the intrinsic viscosities in TFE, chloroform, and EDC are collected in Table II and double-logarithmically plotted against $\overline{M}_w$ in Figure 8. The data of Marchessault et al.[7] and Cornibert et al.[8] are also included for comparison. The lines indicated have been drawn to fit our data, except for the highest molecular weight sample. They are expressed by the relations:

$$[\eta] = 1.25 \times 10^{-4}\overline{M}_w^{0.80} \quad \text{(TFE, 25 °C)} \quad (5)$$

$$[\eta] = 1.18 \times 10^{-4}\overline{M}_w^{0.78} \quad \text{(chloroform, 30 °C)} \quad (6)$$

$$[\eta] = 9.18 \times 10^{-5}\overline{M}_w^{0.78} \quad \text{(EDC, 30 °C)} \quad (7)$$

where [η] is expressed in dl/g. Equations 5 and 6 differ from eq 2 and 1, respectively, but the disagreement should not be taken seriously, because, as seen in Figure 8, the data points of previous investigators scatter around the corresponding straight lines which fit our less scattered points.

The exponents in eq 4 to 6 are equal or close to the limiting value expected for nondraining linear flexible polymers in good solvents, provided that the expansion factor obey the fifth-power law.[19] This result is in conformity with the conclusion deduced above from the molecular weight dependence of $\langle S^2 \rangle$, the particle scattering function, and the magnitudes of A_2 that the molecule of PHB in TFE assumes a randomly coiled form expanded greatly by excluded-volume effect.

The Flory Viscosity Constant. One of the ways to assure the pertinence of the random coil model for PHB in TFE is to compute the Flory viscosity constant Φ from the experimental values of $\overline{M}_w$, [η], and $\langle S^2 \rangle$. Table III shows the results of computation from the present data and those of Cornibert et al. The Φ values from our measurements, excepting those for the two lowest molecular weights, scatter about 2.0×10^{21} with ±10% deviations. This mean value is close to 2.1×10^{21} accepted empirically for moderately polydisperse random coils in good solvents. Our random coil model for PHB in TFE is thus substantiated also from the Flory viscosity constant. On the other hand, the values of Φ computed from the measurements of Cornibert et al. are about one-eighth of the present ones. If the random coil model is accepted, this consequence is primarily attributable to the fact that for the same $\overline{M}_w$ these authors obtained values of $\langle S^2 \rangle$ which were about four times larger than those we found. In fact, we have pointed out this similar discrepancy in the discussion of Figure 6.

Table III
Flory Viscosity Constant

Present data in TFE at 25 °C		Cornibert et al.[1] in TFE	
Sample code	$\Phi \times 10^{-21}$	Sample code	$\Phi \times 10^{-21}$
A-12	1.95	M	0.299
A-22	1.94	E	0.39
AB-12	1.80	K	0.31
AB-22	2.24	J	0.13
B-23	2.01		
B-32	2.18		
B-42	2.10		
B-5	1.99		
B-62	1.74		
B-72	1.61		

[a] Values of [η] and $\langle S^2 \rangle$ are obtained at 30 °C and about 22 °C, respectively.

Polarimetric Data. When the present work was undertaken, we had little doubt about the polarimetric data of Marchessault et al.[7] which suggested helical structure for PHB in such solvents as EDC, TFE, and chloroform, and hoped that accurate measurements of light scattering and viscosity would reveal rodlike behavior of PHB compatible with such molecular conformation. As described above, however, our actual data failed to confirm this. Hence we

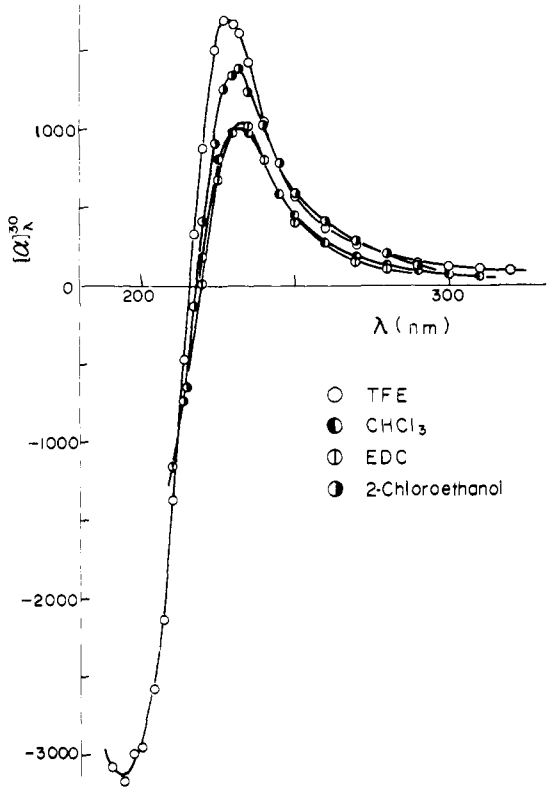

Figure 9. ORD curves at wavelengths below 300 nm in the solvents indicated and at 30 °C.

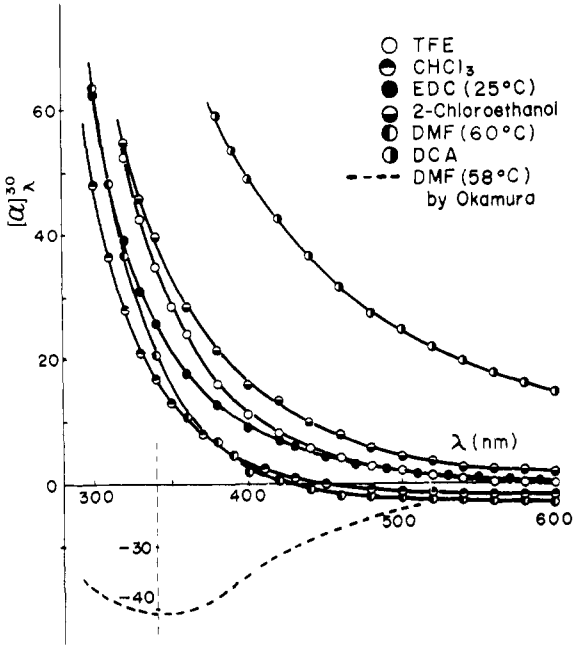

Figure 10. ORD curves at wavelengths above 300 nm in the solvents indicated and at 30 °C. Dashed line, Marchessault et al.

thought it worthwhile to check the reproducibility of the findings of Marchessault et al. and conducted the polarimetric measurements as described in the Experimental Section.

Figure 9 shows that PHB in TFE, EDC, chloroform, and 2-chloroethanol exhibits a single Cotton effect in the uv region. The characteristic wavelengths for these curves are about 213 nm. These results show no difference from those obtained by Marchessault et al.[7] and Delsarte et al.[22] for the system PHB–TFE.

Figure 10 illustrates our ORD curves of PHB in the above-mentioned four solvents as well as in two other solvents DCA and DMF over the wavelength region above 300 nm. The data of Marchessault et al. for a DMF solution in the corresponding region display different features as indicated by a dashed line in Figure 10. These authors interpreted this difference as implying that the molecular conformation of PHB in DCA or DMF differs from those in TFE, EDC, and chloroform. Our ORD curves, however, show no characteristic difference which allows DCA and DMF to be distinguished from the four other solvents indicated and hence lead to the conclusion that the molecular chain of PHB assumes an essentially identical conformation in any of these solvents. No one can say any more than this.

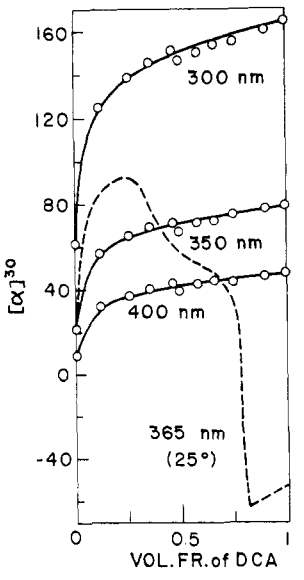

Figure 11. Dependence of specific rotation on solvent composition in DCA–EDC mixtures. Dashed line, Marchessault et al.

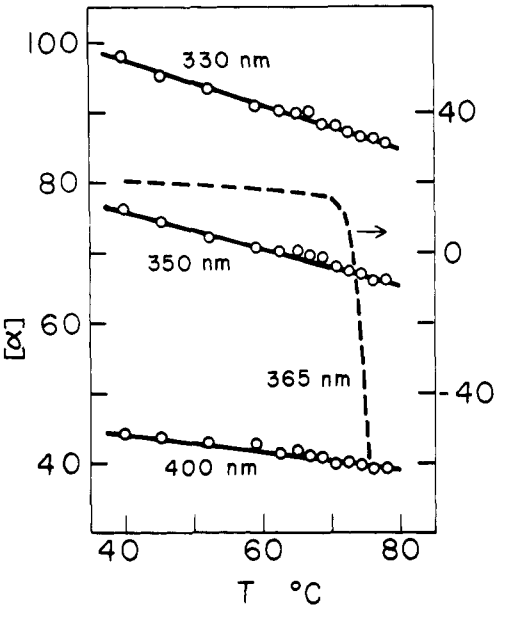

Figure 12. Temperature dependence of specific rotation in a DCA–EDC mixture containing 76 vol % DCA. Dashed line, Marchessault et al.

Though not illustrated here, our ORD curves in all these solvents changed only gradually with temperature; there were gradual decreases of specific rotations at fixed wavelengths with the increase in temperature. This suggests that, in the solvents studied, no abrupt change in the molecular conformation of PHB, something like a helix–coil transition, is induced thermally.

Specific rotations of PHB in mixtures of DCA and EDC at 30 °C are plotted against the volume fraction of DCA in Figure 11. The solid curves from the present measurements show a decidedly different behavior from the dashed curve of Marchessault et al. which demonstrates an abrupt decrease in specific rotation at about 80 vol % DCA.

In Figure 12, specific rotations of PHB in a DCA–EDC mixture of 76 vol % DCA are shown as a function of temperature. The data from our measurements decrease almost linearly with the increase in temperature, whereas the dashed curve of Marchessault et al. undergoes a sharp drop at about 74 °C.

It was from the characteristic features of the dashed curves in Figures 10, 11, and 12 that these authors supposed that PHB assumes a helical conformation in such solvents as EDC, TFE, and chloroform and becomes randomly coiled in such solvents as DCA and DMF. Our polarimetric data display nothing definitive to substantiate their supposition nor give evidence that allows one to determine the actual conformation of PHB in these solvents. In such circumstances, it is reasonable to try analyzing given light-scattering and hydrodynamic data in terms of the conventional theories for nonideal solutions of random coil polymers, as has been done by a great many previous authors. In fact, we have followed this idea in the present article and have shown that, as far as light-scattering and viscosity data are concerned, the behavior of PHB in TFE is consistent with the random coil model. In the forthcoming article, we shall show that the dilute solution properties of this polymer–solvent system can be described adequately by the two-parameter theory of random coils with volume exclusion.

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Compatibility in Mixtures of Poly(vinylidene fluoride) and Poly(ethyl methacrylate)

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ABSTRACT: Thermal analysis of mixtures of poly(vinylidene fluoride) and poly(ethyl methacrylate) has been carried out under a variety of controlled heating and cooling conditions. From the depression of the melting point of PVF_2 in the mixture, the binary interaction parameter is found to be −0.34, comparable to the value obtained for PVF_2–PMMA systems. Mixtures containing 80% or more PEMA are amorphous and exhibit single glass transitions. When the PEMA content is low, crystallization of PVF_2 is observed even with rapid quenching. The crystalline region coexists with two conjugate amorphous phases which contain ~100 and ~45% PVF_2, respectively. The microstructures of the blends are influenced strongly by the kinetics of phase separation and crystallization.

Mixtures of several alkyl methacrylate polymers and poly(vinylidene fluoride) have been reported to be compatible over a wide range of composition.[1-6] These compatible blends have elicited a great deal of interest from both practical and fundamental viewpoints. First, the compositions of the blends can be tailored to combine the excellent chemical resistance of polyvinylidene fluoride with the transparency of the methacrylate polymers. Second, these mixtures offer a rare opportunity for the study of the crystallization of one polymer in the presence of another.

In previous studies by several investigations, single glass transition temperatures were observed for mixtures of PVF_2 and poly(methyl methacrylate). The crystallization behavior of PVF_2 is found to be influenced significantly by the composition of the mixture as well as the rate of cooling.[3] From the melting point depression which increases with increasing PMMA content the interaction parameter between the two polymers was calculated to be −0.30 at 160 °C.[3]

An extension of the above study to mixtures of PVF_2 with poly(ethyl methacrylate) appears to be natural because it would allow us to assess the dependence of the interaction parameter on chemical structure. We also wish to reexamine the glass transition characteristics of the blends because the data of Paul and co-workers[7] indicate a single transition for each composition but the glass temperature remains nearly constant for blends containing more than 30% PVF_2. Ex-