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*Received March 22, 1984*

## Physical Properties of a Naturally Occurring Polyester: Poly($\beta$-hydroxyvalerate)/Poly($\beta$-hydroxybutyrate)

The chiral optical polyester poly($\beta$-hydroxybutyrate) (PHB) is the first example of a true thermoplastic from biotechnology. Although it has been synthesized[1] it is clearly much more readily prepared by processes related to industrial microbiology.[2] In addition to being a thermoplastic with interesting biodegradable properties, it is also serving as a source of starting materials that serve as synthetic building blocks in both sophisticated and straightforward organic syntheses.[3] Recently, the availability of a family of polyesters of this type based on $\beta$-substituted $\beta$-hydroxypropionate derivatives had been reported. It was not clear whether true copolyesters were being obtained from the natural synthesis. The present communication is a detailed analysis of one such system containing PHB and poly($\beta$-hydroxyvalerate) (PHV).

The heteropolymer sample was kindly provided by Dr. L. L. Wallen, Northern Regional Research Laboratory, Peoria, IL. This sample was used either "as received" or after partial fractionation in the following manner. The polymer was first purified by dissolving in boiling ethanol (0.05%), which dissolves only low molecular weight PHB (DP < 30), followed by crystallization at room temperature for 24 h. The recrystallized polymer was dissolved again in boiling ethanol, the solution was kept at 65 °C for 24 h, and the precipitate formed (65 °C fraction) was collected by filtration. From the resulting filtration lower temperature fractions (50 °C and room-temperature fractions) were prepared in a like manner.

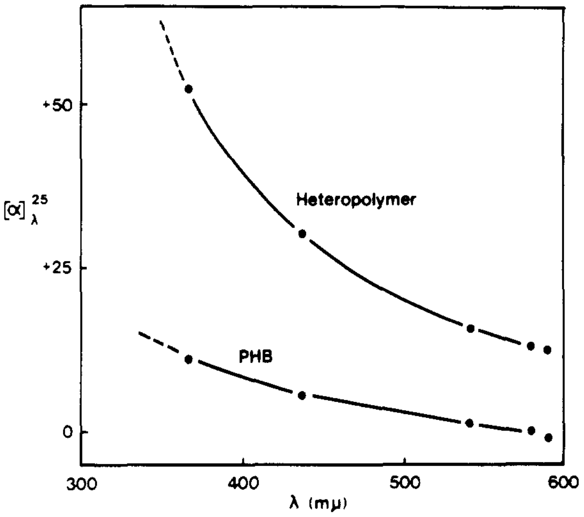

Figure 1. Optical rotatory dispersion curve for HV/HB heteropolymer and PHB in chloroform. The optical rotation of the heteropolymer (65 °C fraction) was recorded on a Perkin-Elmer 241 instrument. The results for PHB ($M_n$ = 127 000) were taken from ref 9.

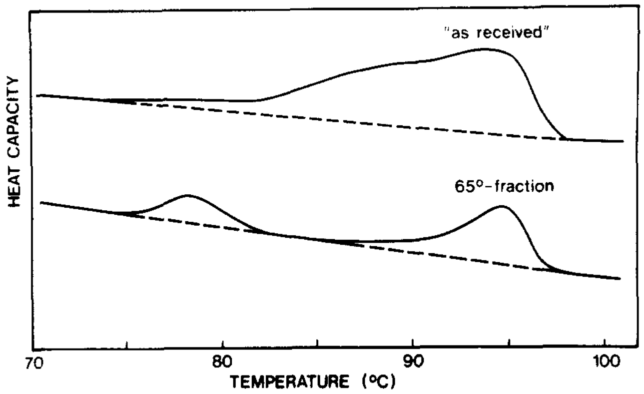

Figure 2. Differential scanning calorimeter trace for HV/HB heteropolymer. The heteropolymer "as received" and the 65 °C fraction were measured with a Perkin-Elmer 1B instrument at a scan rate of 10 °C/min.

On the basis of gas chromatography–mass spectroscopy of saponified heteropolymer, Wallen and Rohwedder[4] have shown that the ratio of $\beta$-hydroxyvalerate (HV) to $\beta$-hydroxybutyrate (HB) is 5:1. Pyrolysis studies[5] have shown that the heteropolymer is composed mainly of HV but contains a significant amount of HB. It seems, however, still open to question whether $\beta$-hydroxybutyrate is present as a comonomer or physically mixed as PHB in the "heteropolymer".

Infrared spectra of the heteropolymer[4] showed a strong band at 1465 cm$^{-1}$, which is absent in the spectrum of PHB. This band is assigned to the bending mode of the $CH_2$ (bonded to $CH_3$) group of the HV polymer. In the proton NMR spectrum,[4] signals characteristic of the protons on the methyl groups of HV and HB were observed at 0.89 and 1.27 ppm, respectively. In this study it was observed that the ratio of integrated intensities of these signals (HV/HB) change as a function of sample preparation. The ratios were 2.5, 4.9, 5.1, and 1.4 for the "as received", 65 °C, 50 °C, and room-temperature fractions, respectively.

The optical rotatory dispersion curve for the heteropolymer is shown in Figure 1. The heteropolymer has a higher specific rotation in chloroform than PHB. The $[\alpha]^{25}$ value was +12.5° for the former while a very small negative value is reported for the latter.[2,6]

In the differential scanning calorimeter trace (Figure 2)

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**Table I**
**Comparison of Observed and Calculated X-ray *d* Spacings for Unit Cell of Optically Active PHV with $a = 9.52$ Å, $b = 10.08$ Å, and *c* (Fiber Axis) = 5.56 Å**

| *d* spacing, Å | | | intensity[a] | |
|---|---|---|---|---|
| obsd | calcd | index | X-ray | electron |
| | | PHV | | |
| 6.93 | 6.92 | 110 | VS | S |
| 5.02 | 5.04 | 020 | S | S |
| 4.75 | 4.76 | 200 | W | W |
| 4.44 | 4.45 | 120 | S | S |
| 3.15 | 3.17 | 130 | W | M |
| 3.01 | 3.03 | 310 | M | M |
| 2.73 | 2.75 | 230 | W | W |
| 4.81 | 4.80 | 101 | W | |
| 4.38 | 4.33 | 111 | S | |
| 3.61 | 3.62 | 201 | VW | |
| 3.37 | 3.40 | 211 | S | |
| 2.86 | 2.88 | 031 | M | |
| 2.73 | 2.75 | 131 | W | |
| 2.64 | 2.66 | 311 | W | |
| 2.42 | 2.41 | 321 | VW | |
| 2.77 | 2.78 | 002 | W | |
| 2.57 | 2.58 | 112 | M | |
| 2.32 | 2.34 | 212 | M | |
| 2.07 | 2.09 | 302 | M | |
| | | PHB[b] | | |
| 6.66 | 6.60 | 020 | W | |
| 5.33 | 5.28 | 110 | M | |
| 4.15 | 4.14 | 101 | M | |
| 3.75 | 3.95 | 111 | M | |

[a] S = strong, M = medium, W = weak, and V = very. [b] The *d* spacings for PHB were calculated from the reported unit cell parameters of PHB.[9,10]

the heteropolymer "as received" showed a broad peak ranging from 75 to 95 °C, which suggests a wide distribution of melting points. Wallen and Rohwedder[4] have reported that the heteropolymer preparation melts at 100–105 °C and that repeated washing of the preparation with hexane reduced this value to 97–100 °C. With the 65 °C fraction of the polymer two peaks centered at 78 and 95 °C were observed (Figure 2). The latter peak probably corresponds to the high molecular weight PHV polymer in the fraction while the former peak could be due to a low molecular weight component (perhaps a PHB phase) that is either physically mixed or copolymerized in the polymer.[7]

When heat-molded films of the heteropolymer (as received) were stretched to about 300% followed by annealing at 70 °C under tension in a nitrogen atmosphere for 1 day, an X-ray fiber diagram with 23 separate reflections was recorded (Table I). Among them, 19 reflections can be attributed to PHV. A unit cell fitting these reflections was determined by initially indexing the reflections with the reported unit cell parameters for the racemic PHV: $a = 9.32$ Å, $b = 10.02$ Å, and $c = 5.56$ Å (fiber repeat).[8] The least-squares refined parameters for the naturally occurring PHV polymer were found to be $a = 9.52$ Å, $b = 10.08$ Å, $c = 5.56$ Å. These parameters are in good agreement with those reported for the racemic polymer except for the $a$ parameter. Systematic absence of reflections indicates that the same space group ($P2_12_12_1$) as proposed for the racemic PHV[8] is assignable to the natural PHV.

The observed and calculated *d* spacings and the intensities are listed in Table I. It should be noted that in the diffractogram weak but distinct arcs due to the (002) reflection are observed see Table I) whereas this reflection is not observed in the racemic polymer.[8]

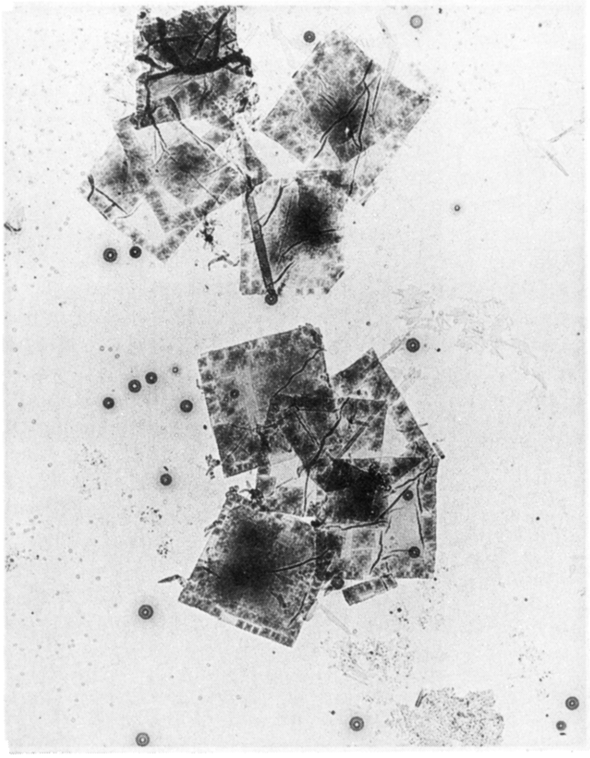

**Figure 3.** Electron micrograph of single crystals of HV/HB heteropolymer. The large, square crystals are PHV while the thin, rectangular crystals are PHB.

The four reflections in the fiber diagram that are not attributed to PHV are almost complete "rings". They are attributed to the (020), (110), (101), and (111) reflections of PHB present in the heteropolymer (Table I) which have characteristically strong intensities in the X-ray pattern of PHB.[9,10] The (110) reflection of PHB showed a slight "thickening" normal to the direction of the fiber axis of the PHV, suggesting a perpendicular orientation of the PHB chains with respect to the PHV molecular axes. The relatively unoriented nature of the PHB reflections after stretching suggests that a majority of these molecules are present as a separate crystalline phase. The PHB reflections are also observed in the powder diagram of each fraction (65 °C, 50 °C, and room temperature) of the heteropolymer.

Finally, single-crystal electron diffraction studies of the heteropolymer were undertaken. One milligram of the 65 °C fraction of the polymer was dissolved in 4 mL of boiling ethanol in a screw-capped tube and then kept at 65 °C for 24 h. The single crystals thus obtained were subjected to electron microscopy and electron diffraction. The large single crystals in Figure 3 are characteristic of PHV as shown by electron diffraction studies.[11] Also observable in the electron micrograph shown in Figure 4 are extremely thin rectangular-shaped crystals similar to those previously observed for PHB.[12] Due to the thermal instability of these crystals, it was not possible to obtain electron diffractograms from them.

A total of 23 reflections was observed in one quadrant of the electron diffractogram obtained from PHV single crystals. From these data the PHV unit cell base plane parameters $a$ and $b$ were found to be 9.52 and 10.10 Å, respectively , which agrees, within experimental error, with those obtained by the X-ray analysis as described above. The observed intensities for the equatorial reflections in the X-ray diagram and the observed intensities in the electron diffraction pattern are in good agreement (see Table I). Since the electron diffractogram is an (*hk0*) section of the PHV reciprocal lattice, the molecular chains in the crystal are perpendicular to the surface of the crystalline lamellae. The diffractogram also confirmed the *pgg* symmetry since only even-order reflections are observed along (*h*00) and (0*k*0) directions. Furthermore, this confirms the orthorhombic $P2_12_12_1$ space group for the chiral PHV, the same as proposed by the X-ray analysis[8] for the racemic polymer.

In conclusion, the crystallographic results for the naturally occurring optically active PHV by X-ray diffraction and electron diffraction are in good agreement. The unit cell parameters for the optically active and racemic PHV are the same except for a significant disagreement in the *a* parameter. From the results presented here, it also appears that the heteropolymer is a physical mixture of PHV and PHB. Since activated sewage sludge was the source of the heteropolymer,[13] it may be assumed that different bacterial species were present, some of which produced PHV and others PHB. A true copolyester of β-hydroxyvalerate/β-hydroxybutyrate from a homogeneous bacterial culture has been reported.[14]

**Registry No.** PHV, 67291-18-3; PHB, 26744-04-7; β-hydroxyvaleric acid homopolymer, 83120-66-5; β-hydroxybutyric acid homopolymer, 26063-00-3.

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*Received March 20, 1984*

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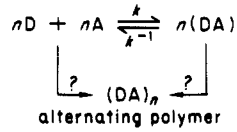
## Monomer Orientation Control by Donor–Acceptor Complex Participation in Alternating Copolymerization

Alternating copolymerization of certain pairs of vinyl monomers has been known for many years, and debate and disagreement about the mechanism of this process have been extensive.[1,2] The probability of formation of such regularly alternating copolymers via a random process is very low. However, one set of properties invariably associated with monomer pairs that exhibit this alternating tendency is the complementary electron-donor/electron-acceptor character of the pair. A strongly electron-donating monomer (D) tends to undergo alternating copolymerization with a strongly electron-accepting monomer (A). Thus, two independent theories have been advanced to account for this result. The first postulate[3] was that polarity differences between the radical chain end and the incoming monomer would lower the energy of activation of cross-propagation, as opposed to a homopropagation reaction, thus producing strictly alternating copolymers when the comonomers have widely different polarities. The second postulate[4] was that an electron-donating/electron-accepting monomer pair formed a donor–acceptor complex (DA) that, due to an inherently higher reactivity of the complex relative to the free monomers, preferentially added to the chain end:[5–9]



$$nD + nA \underset{k_{-1}}{\overset{k}{\rightleftharpoons}} n(DA) \qquad (1)$$

The most convincing evidence for participation of DA is that the overall rate of polymerization ($R_p$) is maximum at a monomer composition ratio of nearly 1:1, where the concentration of DA ([DA]) is greatest. However, the rate maximum seldom occurs at exactly 1:1. Also, $R_p$ at constant monomer ratio is generally greater than first order in total monomer concentration, $[M]_T$, inconsistent with cross-propagation. In spite of the large amount of data available, there is still disagreement regarding the exact mechanism. The actual [DA] in systems that show an alternating tendency is invariably low (*K*'s are generally 0.01–0.5). Thus, if DA is involved in the propagation step, it must have high reactivity relative to the free monomers. This point is still open to debate as well.

In previous studies, predominantly kinetic and polymer composition data have been used to draw conclusions. Both techniques are subject to considerable error. Thus, it is important to have reliable data to distinguish between such mechanistic subtleties as DA formation prior to addition and complex formation with the chain end.

We considered that some innovative source of data was needed to resolve the question. According to Mulliken,[10] a DA is expected to have a preferred geometry; thus, a DA may add to the chain end in a concerted manner (as opposed to stepwise addition of D and A), introducing controlled stereoregularity. If the "free" monomers add to the chain end, random stereochemistry would be expected.[11] A study of copolymer structure and stereochemistry as a function of copolymerization conditions was thus undertaken. Conditions were used so as to shift the DA equilibrium one way or the other. The system *N*-phenylmaleimide (NPM)–2-chloroethyl vinyl ether (CEVE) was selected. NPM is an acceptor monomer with an *e* value of +3.24.[12] CEVE is a donor monomer with an *e* value of −1.58.[12] The objective was to determine whether or not DA participates significantly in the propagation step. $^{13}C$ NMR spectroscopy was used extensively for the determination of the stereoregularity. Copolymer epimerization studies and comparison of copolymer $^{13}C$ NMR chemical shifts with those of stereospecific model compounds indicated that the stereochemistry at the succinimide units in the copolymer was predominantly cis. Copolymerization conditions that were expected to enhance the fraction of

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