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Co-crystallization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

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A combined study of X-ray diffraction, differential scanning calorimetry and density measurements in copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate is presented. It is shown that the unit cell volume expands linearly with the comonomer content in the polymer. The reduction in the enthalpy of fusion is interpreted in terms of limited co-crystallization of the two monomers. The amount of 3-hydroxyvalerate in the crystals has been estimated to be approximately equal to two-thirds of the total content in the copolymer.

1. Introduction

Poly(3-hydroxybutyrate) (PHB) and its copolymers with 3-hydroxyvalerate P(HB/HV) are bacterially produced polymers which show optical activity [1, 2]. These thermoplastics are stored by a wide range of bacteria as a carbon source; they can be isolated from the cells by solvent extraction [2]. This interesting class of biodegradable and biocompatible polymers is now produced in a large-scale fermentation process by the Imperial Chemical Industries (ICI) [3] from *Alcaligenes eutrophus* grown in a medium containing propionic acid and glucose. PHB is a highly crystalline polymer having an orthorhombic crystal unit cell. There are two molecules per unit cell adopting a conformational left-handed 2_1helix [4, 5]. It has been demonstrated by nuclear magnetic resonance analysis that the HV and HB units are randomly distributed along the copolymer chains [6, 7]. While copolymers with a molar content of HV smaller than 30% exhibit an expanded PHB crystal lattice [6], those having a molar content greater than 40% crystallize with a different unit cell [8]. It has been suggested that the lower values of brittleness in the copolymers with an HV content greater than 12% are due to a reduction in the difference between radial and circumferential thermal expansion coefficients of the spherulites [9]. In this work we are restricted to samples with molar HV concentration values between 0 and 30%. The analysis of the unit cell expansion observed in this region is one of the purposes of this paper. It has been established that the increasing content of HV units provokes a decrease in the experimental enthalpy of fusion [7, 8]. However, the crystallinity as measured by X-ray decreases only slightly [8]. It is our aim to discuss these observations by combining X-ray, calorimetry and density measurements with special reference to the possible accommodation of HV units into the PHB lattice.

2. Experimental results

2.1. Sample characterization

The PHB and P(HB/HV) samples used in this work were supplied by ICI Biological Products Division from their "Biopol" range. In order to remove cell debris all the samples were dissolved in chloroform and filtered through a 5 μm filter. The total HV content was determined using the optical rotation technique [10]. For this study the samples were dissolved in chloroform at concentrations of ~ 2% (wt/vol). Optical rotations were measured at 365.5 nm on a Perkin–Elmer 241 MC polarimeter using a 10 cm glass microcell thermostated to 29 °C. The total mass concentration of HV units was calculated using the expression [10]

$$X(\%) = \frac{86[a] - 748.2}{4150 - 14[a]} \tag{1}$$

where $[a]$ is the specific rotation obtained from

$$[a] = \frac{r}{lc} \tag{2}$$

r is the angle of rotation, l the number of decimetres of solution and c the sample concentration (g cm^{-3}). The calculated HV content values are collected in Table I.

Compression-moulded samples 1 mm thick were crystallized at 50 °C. The crystallization times were chosen by crystallizing thin samples in a hot stage at 50 °C and observing through an optical microscope the spherulites growing until their final impingement.

2.2. X-ray crystallinity and unit cell

Wide-angle X-ray diffractograms have been obtained using a Rigaku X-ray powder diffractometer attached to a conventional Philips generator with a copper tube operating at 40 kV and 40 mA. Nickel filtered $K_{\bar{\alpha}}$ radiation was detected using a scintillation counter

TABLE I Values of total HV content, X, crystallinity α (measured using the Ruland method), enthalpy of fusion, ΔH, and macroscopic density, ρ

X (%)	α	ΔH (J g^{-1})	ρ (g cm^{-3})
0	0.62 ± 0.02	82 ± 2	1.241 ± 0.001
6	0.59	75	1.230
13	0.57	62	1.220
19	0.52	52	1.212
26	0.47	41	1.203

equipped with a pulse-height analyser unit. All samples had well-defined flat surfaces and were carefully aligned on the axis of the diffractometer by means of a thin layer of silicon spread on to the surface. The position of the different diffraction lines were corrected for sample absorption and thickness, slit divergence and sample planarity [11]. All the above-mentioned experimental details have to be considered if one has to deal with unit cell variations which are smaller than 0.002 nm. Basal unit cell parameters, a and b, were obtained from the 1 1 0 and 0 2 0 main diffraction lines. The remaining diffraction lines were neglected because the error introduced by separating them masks the observed unit cell variations calculated from the 1 1 0 and 0 2 0 lines. In order to calculate the unit cell volume, V, one needs a value for the c-axis. The accurate determination of this parameter is a difficult matter, due to the low intensity of $h k l$ ($l \neq 0$) reflections. As far as we can tell there is no discernible variation in the c-spacing of samples with different HV contents. Indeed we do not expect significant changes in c because in both PHB and PHV, c is similar and there is no change in the monomer length on replacing an HB unit with an HV unit. Accordingly we have used the standard value of 0.596 nm [4, 12] for the c-axis. The results of a, b and V are shown in Fig. 1.

Crystallinity, α, of the samples was measured using the Ruland method [13] with the help of a computer program FF-CRYST described by Vonk [14]. The program was adapted to run in a normal PC computer and corrected for use with these polymers. Scans between 8° and 150° (2θ) were carried out in the step mode using the Ross balanced filter method, with nickel and cobalt, for the monochromatization of the beam [11]. In order to account for intensity loss due to the penetration of the beam, three different recording 2θ intervals were chosen: 8–35°, 32–60° and 55°–150° using divergence and scattering slits of $\frac{1}{2}$°, 1° and 2°, respectively. The calculated crystallinities are collected in Table I.

2.3. Density and enthalpy measurements

Macroscopic densities, ρ, were measured at 25 °C by the flotation method in aqueous KBr solutions. The obtained values are presented in Table I. Melting endotherms of the samples were obtained with a DSC-4 Perkin–Elmer differential scanning calorimeter at a heating rate of 10 °C min^{-1}. Experimental enthalpies of fusion were determined from the area of the thermograms using a data station. All these values are collected in Table I.

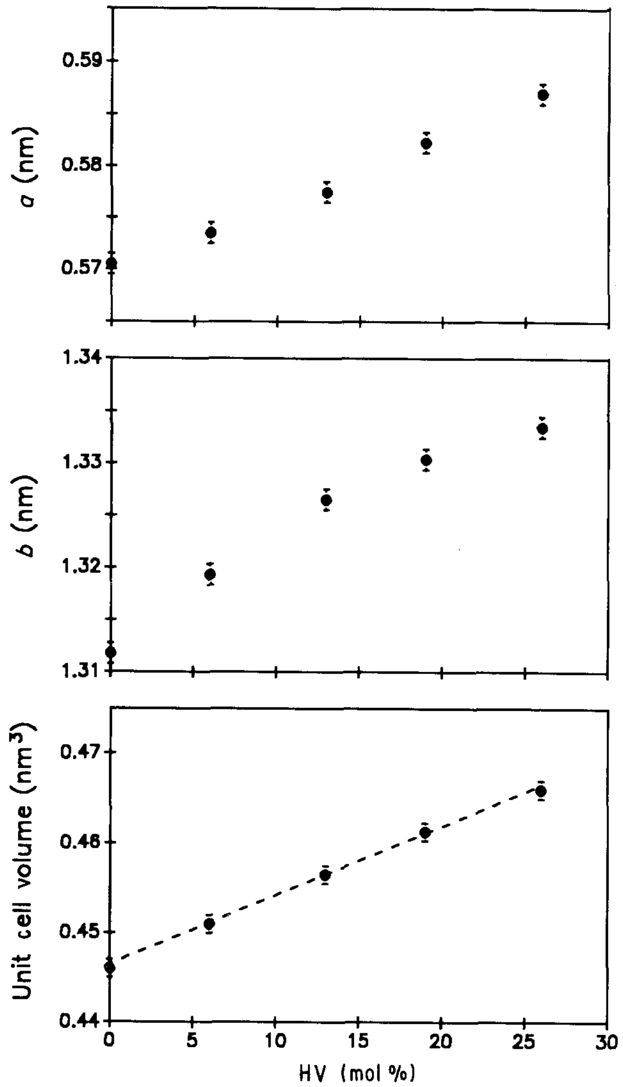

Figure 1 Basal unit cell parameters, a and b, and unit cell volume, V, as a function of the total amount of HV comonomers in the polymer.

3. Discussion

Perhaps the most striking feature of the P(HB/HV) system is the fact that it exhibits a high crystallinity at high comonomer content [8]. This behaviour which is confirmed by the data in Table I clearly implies a high degree of incorporations of comonomer within the lattice (always provided that the copolymers are truly random). The argument is strengthened by our observation of a significant linear decrease in the ΔH values with the increasing HV content which is, of course, only to be expected when there is a high degree of disorder present in the crystals. Thus it is very important to know the comonomer content of the crystals themselves and not just of the whole polymer. A similar problem has been addressed in the past by one of us (J.M.S.) in isothermally melt-crystallized polyethylene (PE) containing alkyl branches [15–18]. The unit cell expansion [15] and the parallel increase in lattice distortions [16] with the increasing number of branches were interpreted in terms of an accommodation of a fraction of branches within the crystals. The calculations led to an inclusion of branches close to 20% of the total amount of branches in the polymer

[15]. Further calculations based on statistical concepts confirmed this result [17]. Later studies on quenched PE proved again the increase in lattice distortions [19] with branching, and the parallel linear decrease of the enthalpy of fusion of the crystals [20].

In this work we will follow a similar approach. First of all we must consider in more detail the unit cell behaviour of the different copolymers. Fig. 1 shows the variation of the unit cell parameters with the HV content. It can be clearly seen that the increase of the total HV content produces a linear expansion of the unit cell volume. The *b*-axis expands more rapidly than the *a*-axis at HV content lower than 15%. In this region both axes show a nearly linear expansion. However, for higher HV values the *b*-axis expansion becomes less pronounced. The absolute values of the *a*- and *b*-axes are close to those reported by Kunioka *et al.* [8] although the systematic increase of the unit cell axis shown in our paper is not clearly observed by these authors. This discrepancy might be due either to the HV determination or to a proper calculation of the crystallographic axis. A direct method to estimate the amount, X_c, of HV units which are incorporated into the PHB lattice is to assume that the copolymer lattice has a mean volume given by averaging between the extreme values corresponding to the PHB and PHV unit cells (data for the PHV unit cell are available elsewhere [8]). This approach is also based on the crystallographic structure of solid solutions where the unit cell varies linearly with the atomic percentage of the solute (Végard's law) [21]. Thus the percentage value of HV units included in the lattice can be estimated from the expression

$$X_c^X = \frac{V_{BV} - V_B}{V_V - V_B} \qquad (3)$$

where V_{BV}, V_B and V_V are the unit cell volumes of the copolymer and the PHB and PHV homopolymers, respectively. The calculated X_c^X are collected in Table II.

A relationship between molar crystallinity and molar mass of HV units in the crystal, for cases where the average molar mass of HV units in the crystal and amorphous phases differs, has been developed by some of us previously [22]. Using this approach and taking from this reference the experimental values of amorphous density for the samples used in this work, we have obtained the molar mass of HV comonomers included in the crystal phase, X_c^d, with the specific crystallization conditions employed in this work. The X_c^d values are collected in Table II.

TABLE II Values of total HV content and crystal HV content calculated from density, X_c^d, and X-ray, X_c^x, measurements as described in the text. We estimate the error in measurement in each case to be of the order of 5%.

X(%)	X_c^d(%)	X_c^X(%)
0	–	–
6	3.9	4.2
13	8.2	9.2
19	13.5	13.3
26	18.4	16.7

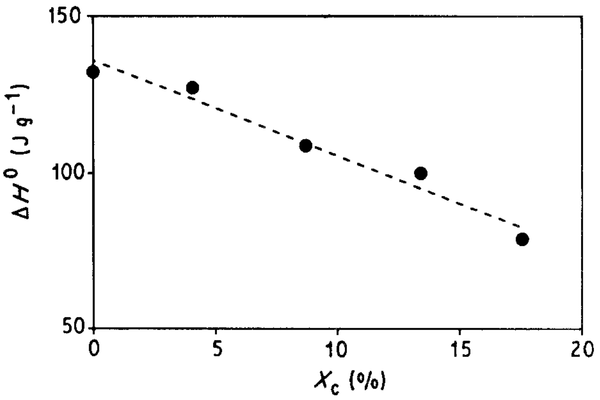

Figure 2 Enthalphy of fusion of P(HB/HV) crystals as a function of HV content.

One can clearly see the excellent agreement between the values of X_c obtained from both methods. It is noteworthy to indicate that the ratio X_c/X is approximately constant for all the samples. This means that the percentage amount of the HV units which are accommodated into the PHB lattice is constant and equal to two-thirds of the total HV content in the polymer.

We are now in a position to see how ΔH^0 varies with the HV content in the crystals. From the experimental values obtained for the enthalpy of fusion and the crystallinity data derived from X-rays, one can calculate the enthalpies of fusion for infinite crystals, ΔH^0. In Fig. 2 these values are plotted against the average values of X_c calculated by the two methods described above. Such data are readily interpreted using the Sanchez–Eby model [23]. In this model they derived the following expression for the enthalpy of fusion as a function of the comonomer content, X_c

$$\Delta H^0(X_c) = \Delta H^0 - \varepsilon X_c \qquad (4)$$

where ΔH^0 is the enthalpy of fusion of the pure homopolymer. This leads us to a ΔH^0 decrement of 2.642 J g^{-1} per defect. An interesting point to raise here is that if we were to extrapolate the $\Delta H^0(X_c)$ to an HV content of $\approx 50\%$ we could observe that ΔH^0 would take a zero value. This means that there is a very high penalty incurred by the incorporation of an HV unit in the HB lattice which nevertheless does not prevent the occurrence of high crystallinities, as shown in Table I.

4. Conclusions

Two independent methods based on sample density and crystallinity and on the expansion of the crystal's unit cell with increasing HV content have been used to determine the HV content of copolymer crystals. The excellent agreement between these two methods gives us confidence in the conclusion that the concentration of HV in the crystals is only two-thirds that of the whole sample.

The inclusion of HV units as defects in the PHB lattice causes the observed unit cell expansion and a large reduction in the enthalpy of fusion of the crystals. The energy excess per defect calculated using the Sanchez–Eby model is $2.642\ \mathrm{J\ g^{-1}}$. This is a large energy and suggests that the enthalpy of fusion of a PHB lattice crystal containing 50% (corresponding to a sample HV content of 75%) would tend to zero, thus rendering it unstable. In practice, at sample HV contents greater than ~ 45% the copolymers preferentially crystallize into the PHV lattice.

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References

1. H. G. SCHLEGEL, G. GOTTSCHALK and R. V. BASTHA, *Nature* **191** (1961) 463.
2. D. G. LUNDGEN, R. ALPER, C. SCHNAITMAN and R. H. MARCHESSAULT, *J. Bacteriol.* **89** (1965) 245.
3. P. A. HOLMES, *Phys. Technol.* **16** (1985) 32.
4. M. YOKOUCHI, Y. CHATANI, H. TADOKORO, K. TERANISHI and H. TANI, *Polymer* **14** (1973) 267.
5. J. CORNIBERT and R. H. MARCHESSAULT, *J. Mol. Biol.* **71** (1972) 735.
6. T. L. BLUHM, G. K. HAMER, R. H. MARCHESSAULT, C. A. FYFE and R. P. VEREGIN, *Macromolecules* **19** (1986) 2871.
7. Y. DOI, M. KUNIOKA, Y. NUKAMURA and K. SOGA, *ibid.* **19** (1986) 2860.
8. M. KUNIOKA, A. TAMAKI and Y. DOI, *ibid.* **22** (1989) 694.
9. J. MARTÍNEZ-SALAZAR, M. SÁNCHEZ-CUESTA, P. J. BARHAM and A. KELLER, *J. Mater. Sci. Lett.* **8** (1989) 490.
10. P. A. BARKER and P. J. BARHAM, *ibid.* **8** (1989) 1049.
11. J. MARTÍNEZ-SALAZAR and F. J. BALTÁ CALLEJA, *Coll. Polym. Sci.* **262** (1984) 361.
12. K. OKAMURA and R. H. MARCHESSAULT, "Conformation of Biopolymers", Vol. 2, edited by G. N. Ramachandran (Academic Press, New York, 1967) pp. 709–20.
13. W. RULAND, *Acta Crystallogr.* **14** (1961) 1180.
14. C. G. VONK, *J. Appl. Crystallogr.* **6** (1973) 148.
15. F. J. BALTÁ CALLEJA, J. C. GONZÁLEZ ORTEGA and J. MARTÍNEZ-SALAZAR, *Polymer* **19** (1978) 1094.
16. J. MARTÍNEZ-SALAZAR and F. J. BALTÁ CALLEJA, *J. Crystal Growth* **48** (1980) 283.
17. *Idem, Polym. Bull.* **2** (1980) 163.
18. J. MARTÍNEZ-SALAZAR, A. KELLER, M. E. CAGIAO, D. R. RUEDA and F. J. BALTÁ CALLEJA, *Coll. Polym. Sci.* **261** (1983) 412.
19. M. SÁNCHEZ-CUESTA, J. MARTÍNEZ-SALAZAR and F. J. BALTÁ CALLEJA, *Polym. Bull.* **17** (1987) 23.
20. J. MARTÍNEZ-SALAZAR, M. SÁNCHEZ-CUESTA and F. J. BALTÁ CALLEJA, *Coll. Polym. Sci.* **265** (1987) 239.
21. A. F. WELLS, "Structural Inorganic Chemistry" (Clarendon Press, Oxford, 1975) pp. 1028–9.
22. P. A. BARKER, F. MASON and F. J. BARHAM, *J. Mater. Sci.* **25** (1990) 1952.
23. I. C. SANCHEZ and R. K. EBY, *Macromolecules* **8** (1975) 628.

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