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Morphology of single crystals of poly(hydroxybutyrate) and copolymers of hydroxybutyrate and hydroxyvalerate

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Single crystals of poly(hydroxybutyrate), and copolymers of hydroxybutyrate and hydroxyvalerate, have been grown from a variety of solvents and their morphology studied. In all cases the crystals appear elongated, with the crystallographic *a* axis along the long axis of the crystals. Screw dislocations (which act as branch points and can lead to large crystal aggregates) have been observed, and solvents, e.g. octanol in which more of these dislocations occur, have been identified. Decoration of the crystals with polyethylene shows that the fold plane is the (0 1 0) plane, while crystallographic and crystallinity arguments require successive adjacent folds to be along ⟨1 1 0⟩ directions. It is argued that folding is along [1 1 0] on the top, and [1 $\bar{1}$ 0] on the bottom surface of a crystal.

1. Introduction

Polyhydroxybutyrate, PHB, and copolymers of hydroxybutyrate and hydroxyvalerate, PHB/HV, are thermoplastics which can be produced by bacterial fermentation. Currently there is widespread interest in these materials as they are readily biodegradable [1, 2]. A range of homopolymers and copolymers are now being produced commercially by Zeneca Bioproducts Business, under the trade name BIOPOL™. These materials have already established several uses in the biomedical and commodity plastic fields [3]. PHB and PHB/HV copolymers have also proved to be useful as model materials for studies of the physical properties of polymers. For example, PHB has been found to be an excellent model material for studies of polymer nucleation due to the lack of catalyst residues and the perfect tacticity which is a result of its biological origin [4]. Furthermore the polymer is available with a wide range of molecular weights and with a (relatively) low polydispersity (~ 2), also the fact that the chain has a direction in the crystal makes it a more useful model than a comparatively featureless molecule such as polyethylene. Data on the crystallization of PHB from the melt [5] can be particularly useful in testing theories of polymer crystallization because PHB crystallizes over an unusually wide temperature range. Further, PHB has been used to study fracture mechanisms in polymer spherulites [6] as large spherulites may readily be prepared. Single crystals of PHB have been used in conjunction with etching techniques to study the degree of regularity of folding in polymer single crystals [7].

PHB single crystals can be grown from a number of solvent systems: propylene carbonate [5]; triacetin [8]; polyethylene glycol [9]; and ethanol–chloroform mixtures [10] have all been used. However, apart from crystals grown from propylene carbonate solutions, little is known about the morphologies of these crystals. The single crystals are very thin (typically 4–6 nm) and spear shaped with irregular edges. Studies of single crystals of PHB/HV copolymers grown from propylene carbonate showed similar morphologies, but with generally smaller crystals with rougher edges [11, 12]. In all these studies it has been very difficult to obtain individual isolated single crystals; usually only parts of isolated crystals could be seen. Most of the polymer crystallized in large aggregates and the isolated lamellae were only found around the edges of these aggregates.

We wished to obtain large quantities of isolated mono-layer crystals to continue the earlier etching studies of Welland *et al.* [7]. Accordingly we decided to investigate crystals grown from alternative solvents, in the hope that we could find a more suitable solvent than propylene carbonate. We also decided to investigate the fold-surface structure and direction of folding using the polyethylene decoration technique, developed by Wittman and Lotz [13], to reveal fold-

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surface structure in polymer single crystals, by evaporating a thin layer of polyethylene on to their surfaces. In studies of polyethylene decoration of polypropylene [14] and paraffins [15] it has been deduced that vapour-deposited polyethylene crystals are nucleated so that their long axes lie along the troughs formed by regular folds in the crystal surface of the other polymer. It was found that the direction of the long axes of the polyethylene crystals was perpendicular to the direction of folding.

In the case of crystallization of PHB from propylene carbonate [5], the crystallographic *a* axis lies along the long direction of the crystals. It has been deduced from fracture studies [11] that the molecular chains are predominantly folded along the [1 0 0] direction. However, folding along the [1 0 0] direction leads directly to a contradiction. The PHB chain has a direction, either up, or down, in the crystal. Obviously when a chain folds it must change from up to down; successive stems must therefore alternate between up and down sites in the crystal structure. The [1 0 0] direction consists of stems all of which are up, or down. The [1 1 0] direction is the most closely packed direction along which successive sites alternate between up and down.

The purpose of this paper is firstly to report the findings of a survey of morphology of single crystals grown from a variety of solvents, secondly to report our data on the direction of chain folding, and finally to highlight and discuss the problem of the fold direction.

2. Experimental procedure

The polymers we used were kindly supplied by Zeneca Bioproducts Business, the code names, molecular weights and HV contents are summarized in Table I. The solvents used were propylene carbonate, toluene, triacetin, and octanol, all of which were normal laboratory grade chemicals. The dissolution, seeding and crystallization temperatures and the polymer concentrations used are summarized in Table II.

TABLE I Details of the polymers used in this work

Polymer	Weight average molecular weight, M_w	Polydispersity M_w/M_n	HV content (%)
PHB (G04)	700 000	≈ 3	0.0
P(HB/HV)	494 000	≈ 3	8.1

Samples were prepared for electron microscopy in the usual manner (see, for example, [5]) and the transmission electron micrographs were taken using a Philips EM301 microscope operating at 80 kV. The decoration of crystals with polyethylene was performed by evaporating polyethylene on to the crystals under vacuum [13, 15].

3. Results and discussion

3.1. Morphology of crystals grown from different solvents

Fig. 1 shows typical examples of the morphology of crystals grown from all the solvents. In general, little difference was observed between the appearance of homopolymer and copolymer crystals grown from the same solvent. Crystals grown from all solvents were elongated; however, the ends of the crystals grown from octanol (Fig. 1a) were more rounded than those grown from other solvents. These crystals also showed some evidence of a flat surface at the growing tip. In all the preparations there was considerable aggregation of crystals, and the examples shown in Fig. 1 came from the edges of these aggregates. Fig. 2 shows a typical, small aggregate of crystals grown from triacetin. In some cases, especially the copolymer crystals grown from octanol, where the problem of aggregation was least pronounced, the actual branching of the crystals from screw dislocations was readily observed, as seen in Fig. 3. It is likely that the aggregation was caused by arrays of such screw dislocations. The importance of branching of screw dislocations has been highlighted in recent studies [16] which suggest that screw dislocations may play a role in the regular twisting of polymer crystals which could lead to banded spherulites.

The appearance of the crystals varied with the crystallization temperature: in general higher crystallization temperatures led to crystals with smoother and better defined edges. This is best illustrated by the crystals grown in propylene carbonate which are shown in the series of micrographs in Fig. 4 of PHB crystallized at three different temperatures.

We performed diffraction experiments on the crystals to check the orientation of the crystal axes. In all cases we found, as has been previously reported for the

TABLE II Details of the dissolution and crystallization conditions

Solvent	Concentration (wt/vol) (%)	Dissolution temperature (°C)	Seeding temperature (°C)	Crystallization temperatures (°C)
Octanol	0.01	129	133.0	70, 97
Triacetin	0.01	133	135.0	42, 68, 95
Toluene	0.01	110.6[a]	–	41, 56, 69, 86
Propylene Carbonate	0.01	101	102.5	35, 65, 68, 82

[a] Boiling point of toluene.

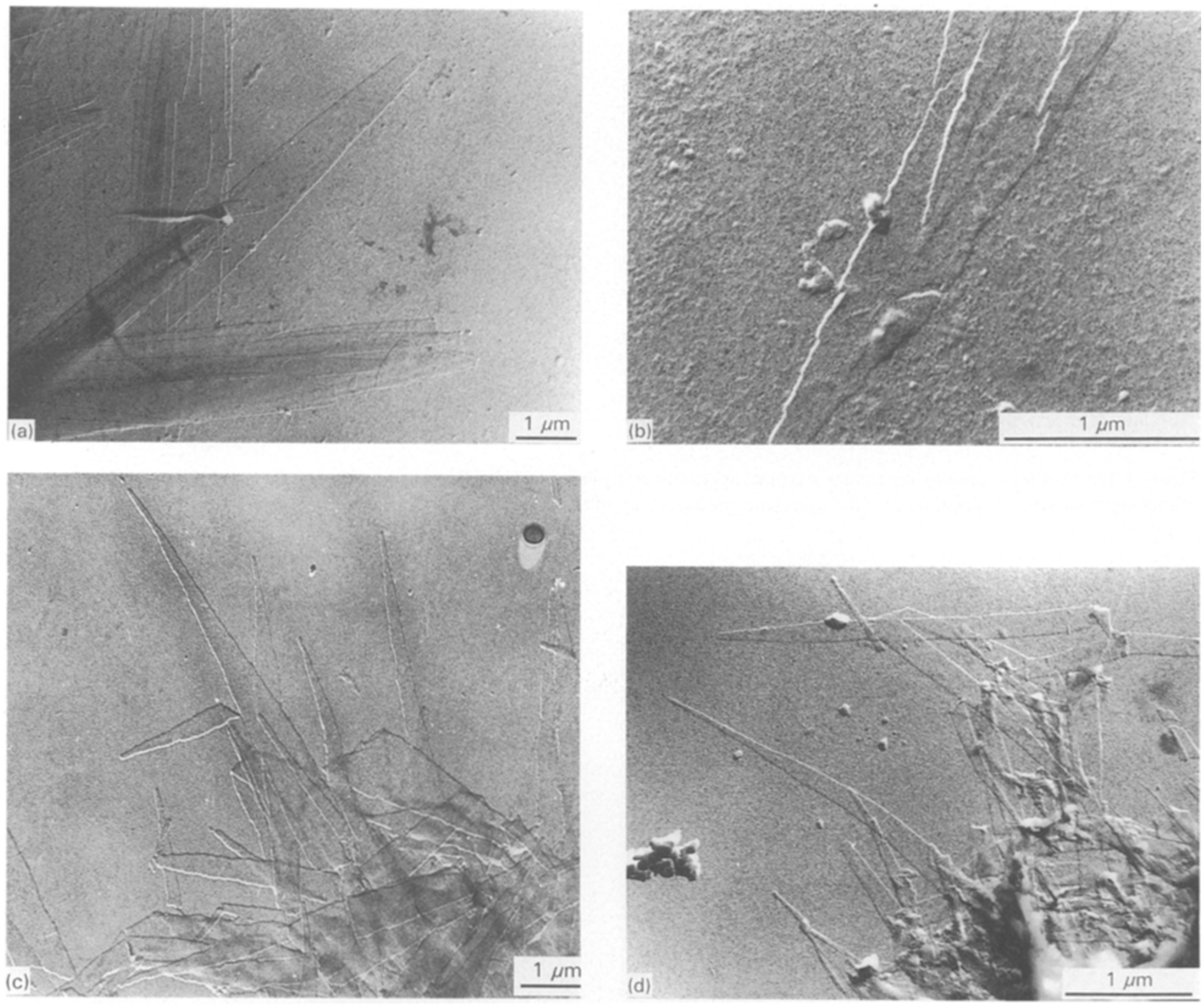


Figure 1 A series of electron micrographs illustrating the morphology of PHB/HV crystals grown from a variety of solvents: (a) crystals grown at 97 °C from octanol solution; (b) crystals grown at 68 °C from triacetin solution; (c) crystals grown at 68 °C from propylene carbonate solution; (d) crystals grown at 86 °C from toluene solution.

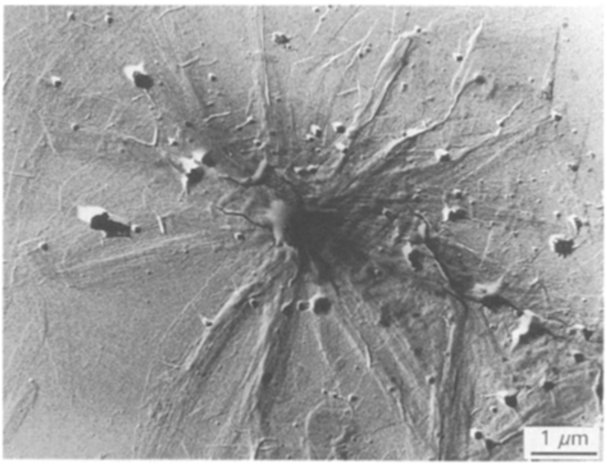


Figure 2 An electron micrograph illustrating a typical small aggregate of PHB/HV crystals grown at 42 °C from triacetin solution.

homopolymer crystals [5], that the crystallographic *a* axis lies along the long axis of the crystals and that the sides do not correspond to any low index plane. We should also note that the screw dislocations (growth spirals) are aligned along the [1 0 0] direction.

We also note that along the edges of many of the crystals in the sample shown in Fig. 3, there are several small "nicks". The origin of these "nicks" is not known; however, because they probably correspond to sharp re-entrant corners, they might have been expected to act as sites for the growth of crystals, due to the lower surface free energy penalty at such sites.

3.2. Decoration of crystals, chain trajectories and chain folding

We show in Fig. 5 examples of decorated isolated mono-layer crystals of both the homopolymer grown from propylene carbonate solution and the copolymer grown from octanol solution. Note that in all cases the polyethylene decoration appears as crystals that have their long axes perpendicular to the long axes of the PHB crystals (the PHB [1 0 0] direction) and the polyethylene crystals run more or less continuously across the PHB and PHB/HV crystals. We may, therefore, deduce that the net, overall, direction of folding is indeed along the [1 0 0] direction. We may

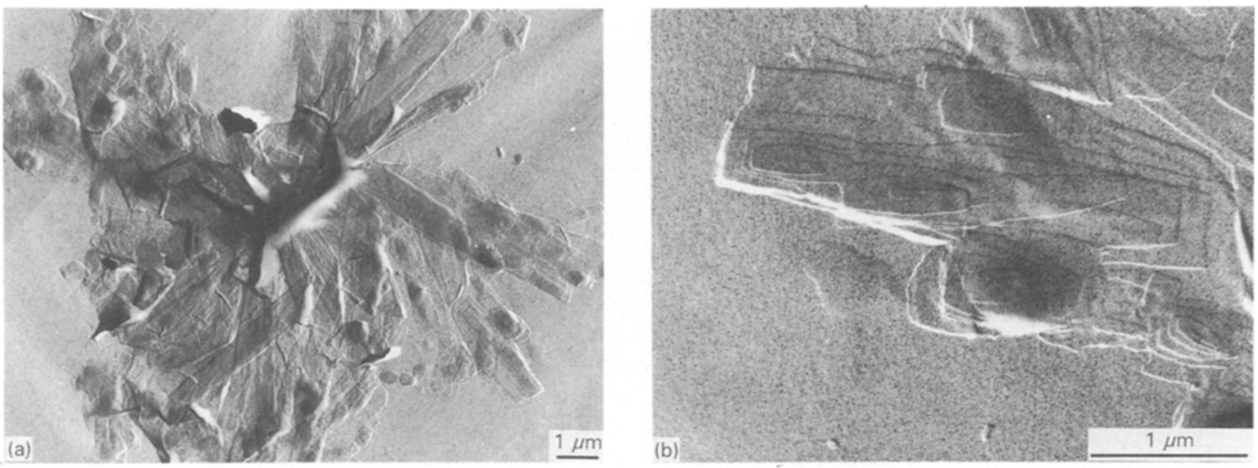
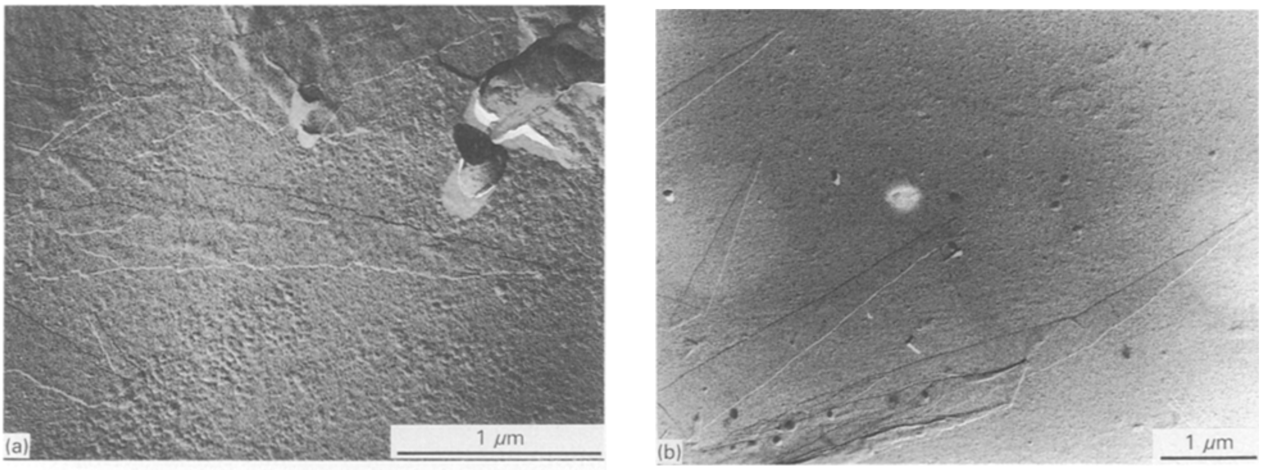


Figure 3 Electron micrographs illustrating a typical aggregate of crystals of PHB/HV grown at 70 °C from octanol solution showing the branching from screw dislocations: (a) the aggregate; (b) a close up of the aggregate.



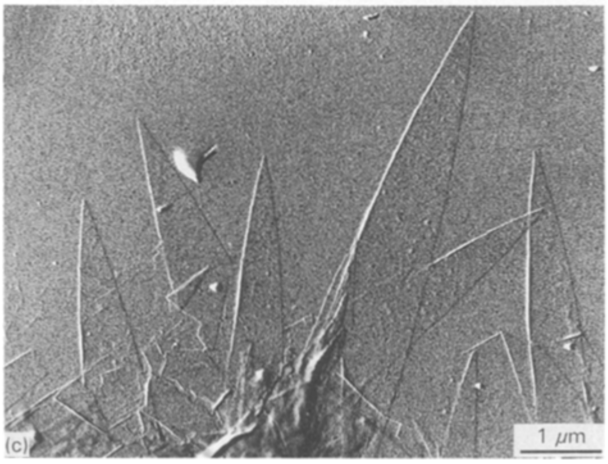


Figure 4 A series of electron micrographs illustrating the variation in morphology with crystallization temperature of PHB crystals grown from propylene carbonate solution grown at (a) 35 °C; (b) 65 °C; (c) 82 °C.

also note that the polyethylene decoration shows that the fold direction is the same in all parts of the crystal and therefore there is no sectorization in the PHB and PHB/HV crystals. These deductions require further discussion.

First, in discussing our results, we consider the lack of evidence for any sectorization in PHB and PHB/HV crystals. If there really are no differences in folding between the two halves of a crystal each side of the central long axis, then we may immediately deduce that the crystals grow as flat objects and that the chains are accurately arranged perpendicular to the crystal surfaces. If the chains were not normal to the surface as the crystal grows, the crystal would have had to have collapsed on sedimentation and we would expect to see a different direction of tilt in the chains in the two halves of the crystal, which would be reflected in the polyethylene decoration pattern. In addition, the electron diffraction studies on individual crystals indicate that, in the sedimented state, the chains are always accurately perpendicular to the crystal surface.

Next we consider the implications of the observation that the length of the polyethylene decorating crystals is rather large. In most of the PHB crystals we have decorated, the polyethylene decorating crystals extend across the PHB crystals in straight lines. Such long decorating crystals could arise from a particularly low nucleation density on the surface of the PHB crystals, or it is possible that there is some long-range order in the fold surface. The decoration in the homopolymer seems to be a little more regular, with longer

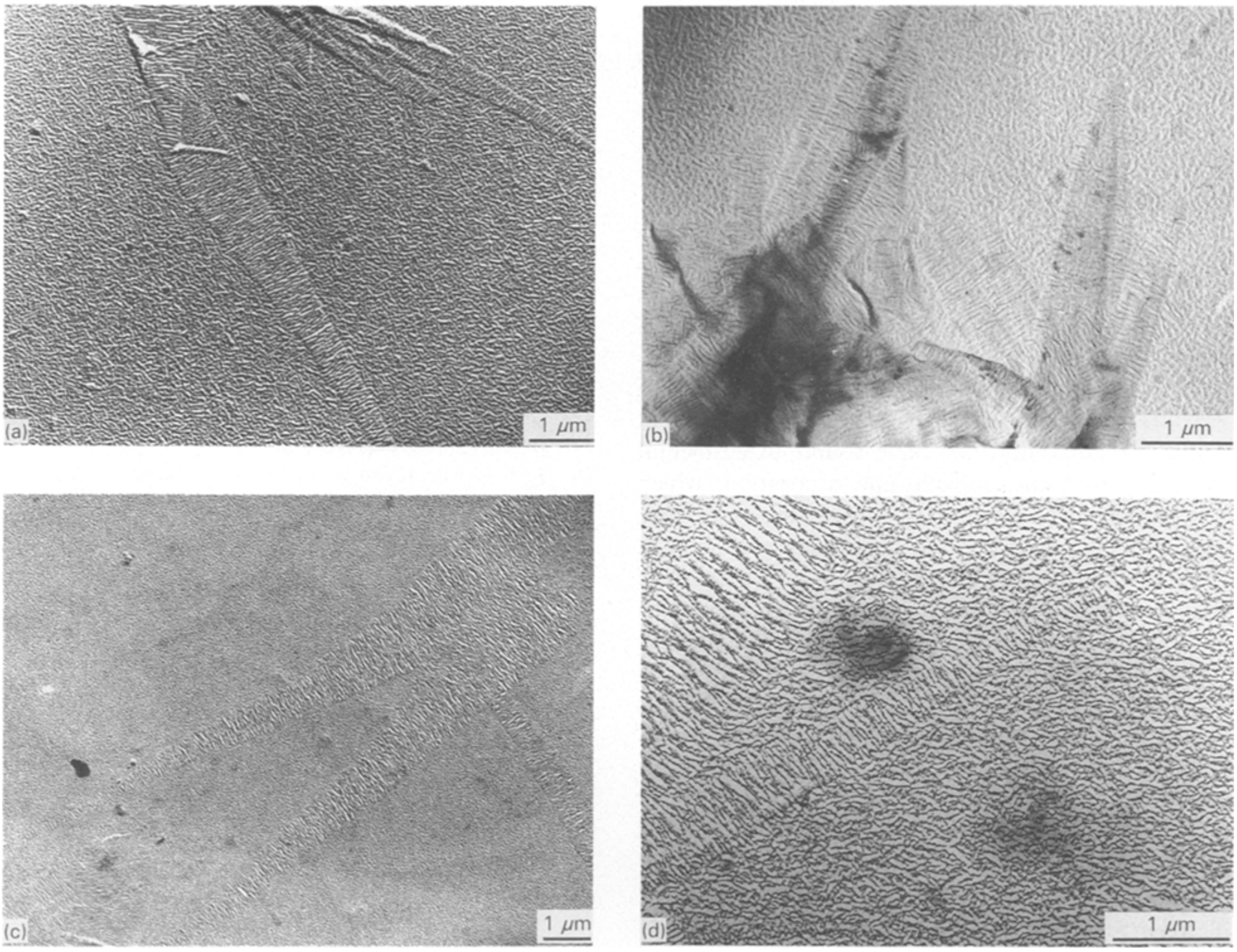


Figure 5 A series of electron micrographs illustrating the decoration of PHB, and PHB/HV copolymer crystals with polyethylene: (a) PHB crystals grown from propylene carbonate solution at 68 °C; (b) PHB/HV crystals grown from octanol solution at 70 °C; (c) PHB/HV crystals grown from triacetin solution at 68 °C; (d) PHB/HV crystals grown from toluene solution at 69 °C.

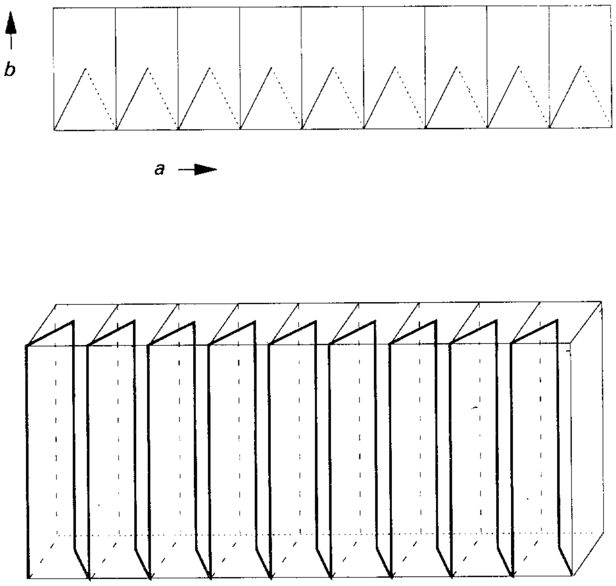


Figure 6 A sketch indicating how the predominant fold direction in PHB crystals could be along the [1 0 0] direction while successive folds are in the [1 1 0] and [1 $\bar{1}$ 0] directions.

decorating crystals, than in the copolymer. This may be due to a less regular fold surface in the copolymer arising from the bulky HV units which are likely to act as defects in the HB lattice. These defects would disrupt the PHB lattice and it would be energetically favourable for the disruptions to be confined mainly to the fold surfaces, which is where the decoration takes place. Thus, we suggest that in the case of PHB crystals, the length of the decorating polyethylene crystals is more likely due to a long-range order in the fold surface, than to a limitation of the nucleation rate at the PHB crystal surface.

Finally we consider the problem of the fold direction. All the experimental evidence points to folding along the [1 0 0] direction, however, this is not possible: when chains fold they must have alternate crystalline stems "up" and "down" in the lattice, whereas all the stems along the [1 0 0] direction must, from crystallographic considerations, be either all "up" or all "down". The only close-packed plane along which regular folding is possible is the (1 1 0) plane. Thus it would appear that there is a serious contradiction.

Before attempting to resolve the problem of the fold direction we should ask how thick the fold surface layer may be in these crystals. We know from a variety of experimental evidence (density [5], X-ray diffraction, and DSC measurements [17]) that the crystallinity of the single crystals is about 80%, or possibly even higher. We also know that the crystallinity is only slightly reduced in the copolymers [17, 18]. Because the crystals are very thin (typically 5 nm) and have high crystallinity, the maximum thickness of the fold surface layer is small, probably not more than 0.5 nm. This leaves little room for many loose folds. We have already noted that we may deduce that the chains are accurately perpendicular to the surface, so that from density considerations [19] it is easy to calculate that at least 70% of the chains must fold back sharply (into an adjacent site). Such tight folding has been shown to be possible in recent work [20] where HB oligomers formed lamellar crystals with thicknesses equal to half their molecular length.

Having deduced that the folding in PHB and PHB/HV crystals should be quite regular, with a high degree of adjacency, we have found experimental evidence, from the polyethylene decoration, which may be taken to support this deduction. The only possible adjacent fold for a chain emerging from a PHB crystal is along one of the $\langle 1\,1\,0 \rangle$ directions. Because the overall direction of folding is along the $[1\,0\,0]$ direction, we are forced to deduce that a chain which folds in the $[1\,1\,0]$ direction at one surface of the crystal folds back at the opposite surface along the $[1\,\bar{1}\,0]$ direction as illustrated in the sketch of Fig. 6. Such a complex and regularly repeating fold structure would imply that there is a special favoured fold conformation, perhaps due to the handedness of the molecule.

4. Conclusion

It has been shown that the morphology of PHB and PHB/HV crystals depends on the solvent from which they were prepared, and that the least aggregation of crystals occurs when they are grown from octanol. It has been possible to identify the screw dislocations which act as branching sites and cause the formation of the crystal aggregates, and it has been demonstrated that the folding in PHB and PHB/HV crystals appears to be quite regular. We have suggested that there may be an underlying crystallographic cause for this regularity.

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References

1. E. A. DAWES, "Microbial Energetics" (Chapman and Hall, New York, 1986).
2. Y. DOI, "Microbial Polyesters" (VCH, New York, 1990).
3. P. A. HOLMES, in "Developments in Crystalline Polymers-2", edited by D. C. Basset (Elsevier, London, 1987) pp. 1–65.
4. P. J. BARHAM, *J. Mater. Sci.* **19** (1984) 3826.
5. P. J. BARHAM, A. KELLER, E. L. OTUN and P. A. HOLMES, *ibid.* **19** (1984) 2781.
6. P. J. BARHAM and A. KELLER, *J. Polym. Sci. B Polym. Phys. Ed.* **24** (1986) 69.
7. E. L. WELLAND, J. STEJNEY, A. HALTER and A. KELLER, *Polym. Commun.* **30** (1989) 302.
8. C. LAUZIER, R. H. MARCHESSAULT, P. SMITH and H. CHANZY, *Polymer* **33** (1992) 823.
9. J. F. REVOL, H. D. CHANZY, Y. DESLANDES and R. H. MARCHESSAULT, *Polymer* **30** (1989) 1973.
10. R. H. MARCHESSAULT, S. COULOMB, H. MORIKAWA, K. OKAMURA and J. F. REVOL, *Can. J. Chem.* **59** (1981) 38.
11. E. L. OTUN, PhD thesis, University of Bristol (1985).
12. H. MITOMO, P. J. BARHAM and A. KELLER, *Polym. J.* **19** (1987) 1241.
13. J. C. WITTMAN and B. LOTZ, *J. Polym. Sci. Polym. Phys. Ed* **23** (1985) 205.
14. *Idem.*, in "Materials Science and Technology", Vol. 12, edited by E. Thomas (VCH, New York, 1993).
15. S. J. ORGAN and A. KELLER, *J. Polym. Sci. Polym. Phys. Ed.* **25** (1978) 2409.
16. D. C. BASSETT, *Polymer* **33** (1992) 2467.
17. P. A. BARKER, PhD thesis, University of Bristol (1992).
18. R. H. MARCHESSAULT, *Macromol.* **19** (1986) 2865.
19. "Faraday Discussions of the Royal Society of Chemistry", Vol. 68 edited by F. C. Frank, J. D. Hoffman, C. M. Guttman and E. A. DiMarzio (Fletcher, Norwich, 1979) pp. 7 and 177.
20. D. SEEBACH, H. M. BÜRGER, H. M. MÜLLER, U. D. LENGWEILER, A. K. BECK, K. E. SYKES, P. A. BARKER and P. J. BARHAM, *Helv. Chim. Acta* **77** (1994) 1099.

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