JOURNAL OF MATERIALS SCIENCE 25 (1990) 1952-1956

Density and crystallinity of poly (3-hydroxybutyrate/3-hydroxyvalerate) copolymers

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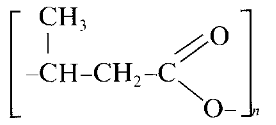
Bacteria can produce a range of optically active copolymers of (R)-3-hydroxybutyrate (HB) and (R)-3-hydroxyvalerate (HV). These copolymers have aroused much scientific interest because of their high crystallinity at all HB : HV compositions which suggests the possibility of inclusion of HB and HV in the same crystal. In this paper we look at the assessment of crystallinity by density measurements taking into account the possible changes in crystal and amorphous densities caused by the rejection or inclusion of HV from the crystallites. The approach developed is applicable to crystallization of copolymers and blends whatever the composition of the crystals and makes a distinction between mass fraction and mole fraction crystallinities for cases where the average molar mass of residues in the crystal and amorphous phases differs. While it is impossible to give values for crystallinity without knowing the degree of inclusion of HV into the crystals it is possible to say that the previous suggestions that the crystallinity remains constant independent of HV content and that there is equal concentration of HV in the crystal and amorphous phases are incompatible with the measured sample densities. We also show that the crystallization temperature is an important factor in the balance between the crystallinity and HV content of crystals.

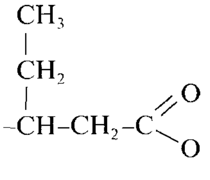
1. Introduction

Poly(3-hydroxybutyrate), PHB, is an optically active biopolymer with the chemical structure:



which is produced by many bacteria as a carbon store if their cell growth and division is inhibited [1, 2]. There is much commercial interest in the possibility of producing such a material in large quantities because it is a thermoplastic that need not rely on oil in its manufacture. There is also a great deal of interest from environmentalists and medical researchers eager for an environmentally benign polymer that will degrade into harmless, naturally occurring small molecules [3]. The exceptional purity and low nucleation density of this homopolymer has been exploited to study the growth and nucleation of spherulites [4, 5].

ICI Biological Products Business, Billingham, are now producing, as well as the homopolymer, a range of copolymers of 3-hydroxybutyrate, HB, with 3-hydroxyvalerate, HV, which has the chemical structure



These HB/HV copolymers have better mechanical properties and degrade less rapidly at processing temperatures [6] than the homopolymer and are and are marketed under the tradename "Biopol". Bluhm and co-workers have shown by NMR analysis that the HB/HV copolymers are random [7].

The definition and measurement of crystallinity in a copolymer system pose many problems. There are two extreme cases: (A) where there is complete exclusion of one monomer from the crystals of the other; and (B) where there is complete inclusion. In either case, measurements of crystallinity are difficult; for example (as we show below), in the case of exclusion the complex relationship between crystallinity and amorphous density makes it difficult to use density as a measure of crystallinity. Similarly, use of heats of fusion would be inappropriate to measure crystallinity in the other extreme because inclusion of defects will significantly lower the heat of fusion of an infinite crystal. These problems have, in the past, been addressed experimentally. For example, branched polyethylene has been studied by Balta Calleja and co-workers [8-11] who deduce, on the basis of thermal and X-ray measurements, that there is partial inclusion of the short branches into the usual, but expanded polyethylene lattice. Theoretical models of co-crystallization have been developed by Sanchez and Eby [12] based on earlier work by Helfand and Lauritzen [13].

In general the expectation is for large comonomer units to be excluded from the crystals (because they cause a high-energy defect). This expectation is in

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contrast to the observations reported by Bluhm *et al.* [7] and Bloembergen *et al.* [14] for the HB/HV copolymer system. They have reported that for crystallization at room temperature, the degree of crystallinity, assessed by FTIR and WAXS, is the range of composition 0 to 27 mol % HV and that the HV co-crystallizes with the PHB. The heat of fusion has also been observed to drop steadily as the HV content increases [6, 7], which would be consistent with the inclusion of the comonomer units.

An attempt to measure crystallinity of HB/HV copolymers has been made by Mitomo *et al.* [15]. In this case the amorphous density was taken to be that of the homopolymer PHB and the crystal density calculated from the crystal densities of the homopolymers PHB and PHV. We shall show this to be quite unsatisfactory.

In the present paper we address the question of the relationship between sample density and crystallinity in a copolymer (especially P(HB/HV)) for general case where there may be any degree of inclusion of comonomer units in the homopolymer crystals. We shall show that while it is not possible to determine the crystallinity of samples from density measurements alone we can demonstrate that there appears to be partial inclusion of HV units in the HB crystals, and that the degree of inclusion is lower at higher crystallization temperatures.

2. Theory

Three degrees of crystallinity can be defined:

1. the volume fraction of crystal,

$$X^{\mathrm{v}} = \frac{\text{volume of crystals}}{\text{volume of sample}} = \frac{V_{\mathrm{c}}}{V_{\mathrm{s}}}$$

2. the mass fraction of crystal,

$$X^{\mathrm{m}} = \frac{\text{mass of crystals}}{\text{mass of sample}} = \frac{M_{\mathrm{c}}}{M_{\mathrm{s}}}$$

3. the molar fraction of crystal,

$$X = \frac{\text{number of residues in crystals}}{\text{number of residues in sample}}$$

It is easily shown that

$$X^{\mathrm{m}} = \frac{\varrho_{\mathrm{c}}}{\varrho_{\mathrm{s}}} X^{\mathrm{v}} \qquad (1)$$

and

$$X = \frac{\bar{\mu}_{\mathrm{s}}}{\bar{\mu}_{\mathrm{c}}} X^{\mathrm{m}} \qquad (2)$$

where ϱ_{c} is the density of the crystal phase, ϱ_{s} is the macroscopic density and $\bar{\mu}_{\mathrm{c}}$ and $\bar{\mu}_{\mathrm{s}}$ are the average molar masses of residues in the crystals and whole sample, respectively. For homopolymers, and for copolymers in which the comonomers are evenly distributed between the crystalline and amorphous phases, $X^{\mathrm{m}} = X$ and so the distinction is not often made. However, for copolymers where the two comonomers are not uniformly distributed between the crystalline and amorphous phases it is important to be clear which degree of crystallinity a particular method gives:

(i) density will measure X^{v} or X^{m};

(ii) infra-red spectroscopy will measure X for the monomer species giving rise to the bands observed;

(iii) WAXS will measure something close to X^{m}. The electron density is nearly proportional to the mass but comonomers in the crystal may not give purely coherent diffraction.

The general equation to give the mole fraction of crystal from density measurements is

$$X_{\mathrm{d}} = \frac{\bar{\mu}_{\mathrm{s}}\varrho_{\mathrm{c}}}{\bar{\mu}_{\mathrm{c}}\varrho_{\mathrm{s}}}\left(\frac{\varrho_{\mathrm{s}} - \varrho_{\mathrm{a}}}{\varrho_{\mathrm{c}} - \varrho_{\mathrm{a}}}\right) \qquad (3)$$

where ϱ_{a} is the density of the amorphous phase. We shall write v_{s}, v_{c} and v_{a} for the mole-fraction of HV in the whole sample, the crystals and the amorphous phase, respectively. Thus we have

$$\bar{\mu}_{\mathrm{s}} = \mu_{\mathrm{HB}} + v_{\mathrm{s}}\mu_{\mathrm{CH_2}} \qquad (4)$$

and

$$\bar{\mu}_{\mathrm{c}} = \mu_{\mathrm{HB}} + v_{\mathrm{c}}\mu_{\mathrm{CH_2}} \qquad (5)$$

where μ_{HB} is the molar mass of HB and $\mu_{\mathrm{CH_2}}$ is the molar mass of CH_2. If V_0 is the unit cell volume, a function of v_{c} which may depend on sample composition and crystallization conditions, then

$$\varrho_{\mathrm{c}} = \frac{4\bar{\mu}_{\mathrm{c}}}{V_0 N_{\mathrm{a}}} = \frac{4(\mu_{\mathrm{HB}} + v_{\mathrm{c}}\mu_{\mathrm{CH_2}})}{V_0 N_{\mathrm{a}}} \qquad (6)$$

The density of a wholly amorphous sample can be measured for various HV contents and, as we shall show, is linear in v_{c} to a good approximation. We therefore write

$$\varrho_{\mathrm{a}} = \varrho_0 + m v_{\mathrm{a}} \qquad (7)$$

where ϱ_0 and m are established exerimentally.

If there is any tendency for HV to be excluded from the crystals then the amorphous phase will be enriched in HV. In fact it is simple to show that

$$v_{\mathrm{a}} = \frac{v_{\mathrm{s}} - v_{\mathrm{c}}X}{1 - X} \qquad (8)$$

Assuming the degree of crystallinity assessed by density measurements, X_{d}, to be a good measure of the molar crystallinity, X, in the last equation, and substituting Equations 7 and 8 into Equation 3 it is a matter of algebra to show that

$$X_{\mathrm{d}}^2 + X_{\mathrm{d}}\left(\frac{D_{\mathrm{c}} - FRD_{\mathrm{s}} - m(v_{\mathrm{s}} + FRv_{\mathrm{c}})}{mv - D}\right) + FR\left(\frac{mv_{\mathrm{s}} - D_{\mathrm{s}}}{mv_{\mathrm{c}} - D_{\mathrm{c}}}\right) = 0 \qquad (9)$$

where $D_{\mathrm{c}} = \varrho_{\mathrm{c}} - \varrho_0$, $D_{\mathrm{s}} = \varrho_{\mathrm{s}} - \varrho_0$, $F = \bar{\mu}_{\mathrm{s}}/\bar{\mu}_{\mathrm{c}}$ and $R = \varrho_{\mathrm{c}}/\varrho_{\mathrm{s}}$. This can be used to illustrate the effect on X_{d} of different assumptions concerning the HV content of the crystal phase.

3. Experimental procedure

3.1. Materials

A variety of samples covering a composition range of 0 to 27 mol % HV were supplied by ICI Biological Products Business, Billingham, from their "Biopol" range. These were produced by *Alcaligenes eutrophus* with propionic acid introduced into the feedstock to induce HV copolymerization [16, 17]. An enzymatic extraction process removes most of the unwanted cell debris leaving ~2% impurity. Compositions had been determined by ^{1}H solution nuclear magnetic resonance [14].

Samples were supplied as off-white powders, these were purified by washing in boiling methanol to remove low molecular weight degradation products, drying, then dissolving in chloroform and filtering out any remaining debris. The samples were then dried in a vacuum oven at ~60° C until they had constant mass, leaving a film of polymer about 1 mm thick.

3.2. Methods

All densities were measured at 20° C by flotation in aqueous salt (NaCl or KBr) solutions. The solutions were prepared to cover the range of densities required in 0.001 g cm^{-3} steps. This was preferred to a density gradient column because the solutions have a longer life than a column, do not become cluttered with old samples, are less sensitive to temperature fluctuations and can give a value more quickly.

The method of melting the samples was chosen to minimize thermal degradation, which is particularly severe if samples are kept above 200° C in well-ventilated ovens [18, 19]. Samples were melted by hot pressing at 200° C for 2 min. Amorphous samples were readily obtained by quenching from the melt into liquid nitrogen. Once such a sample warms to room temperature the density began to increase after about 1 min, presumably due to the onset of crystallization. Accordingly, all measurements of amorphous density were made within 30 sec. Crystalline samples were prepared by quenching from the melt into silicone oil at the required crystallization temperature. Care was taken to allow the samples sufficient time to reach their maximum possible crystallinity, which can take several weeks [14]. The samples were kept in the oil bath until their densities were constant.

Unit cell parameters of similar samples were measured by Martinez-Salazar *et al.* [20].

4. Results and discussion

Fig. 1 gives the data on the variation of amorphous density with HV content. It can be seen that the amorphous density of the samples decreases as the HV content increases, probably because the longer side group disrupts the packing of the chains. The data in Fig. 1 suggest a linear relationship between amorphous density at 20° C and HV content. Linear regression analysis gives a best fit to the equation

$$\varrho_a = 1.1784 - 0.067 v_s \text{ g cm}^{-3} \qquad (10)$$

The density of crystalline copolymer samples also decreases as the HV content increases; this is illustrated in Fig. 2 for samples crystallized at 52° C. However, this reduction in sample density need not indicate a drastic drop in the crystallinity because we do not know how much of the HV is incorporated in the crystals and how much is rejected into the amorphous phase. We can only calculate the crystallinity of the homopolymer; in fact the homopolymer when crystallized at 52° C gives a molar crystallinity of 68% + 2%. Further, we can calculate the range of possible values of molar crystallinity from density (X_d) for the copolymers using Equation 9. An example of such a calculation is shown in Fig. 3 where we present the possible values of the molar crystallinity for the sample with 27 mol % HV crystallized at 52° C. It can be seen that the sample density may be consistent with the near constant crystallinity reported by Marchessault and co-workers [7, 14] only if the HV content of the crystals is 5% to 10%. Accordingly, either the HV units are partially excluded from the crystals or crystallinity decreases as the HV content of the copolymer increases.

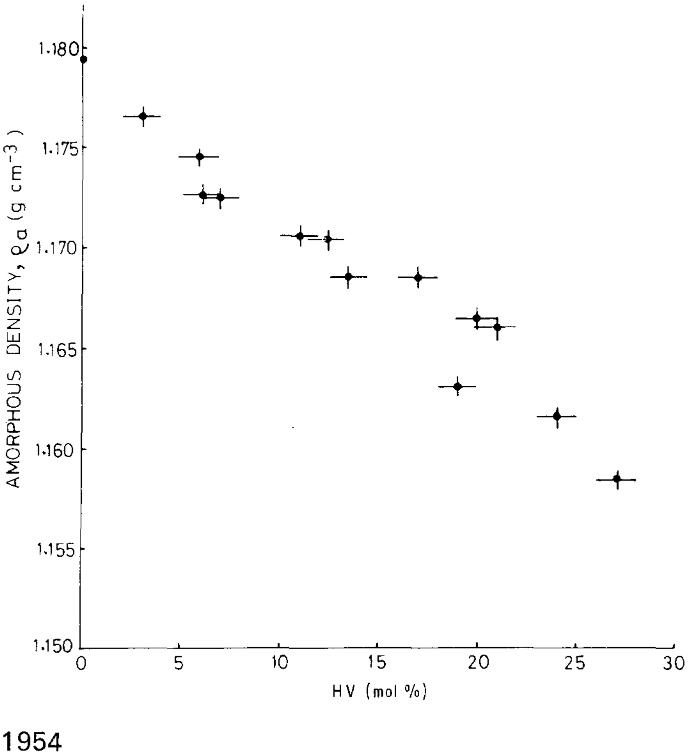

Figure 1 A graph showing the densities of wholly amorphous samples containing different amounts of HV.

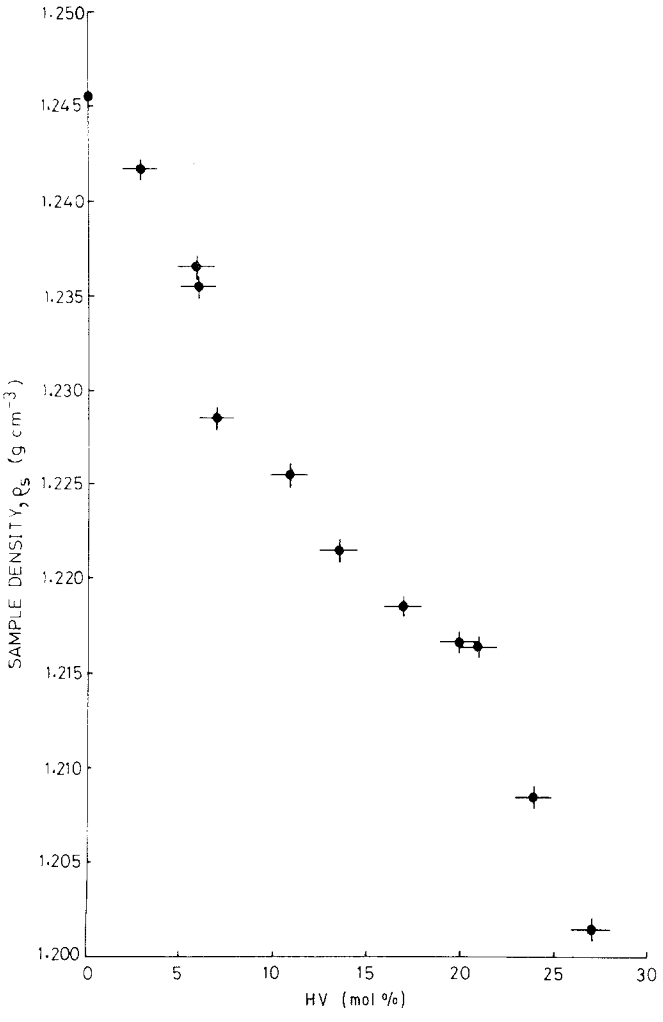

Figure 2 A graph showing the density of samples containing different amounts of HV that have reached their maximum crystallinity at 52° C.

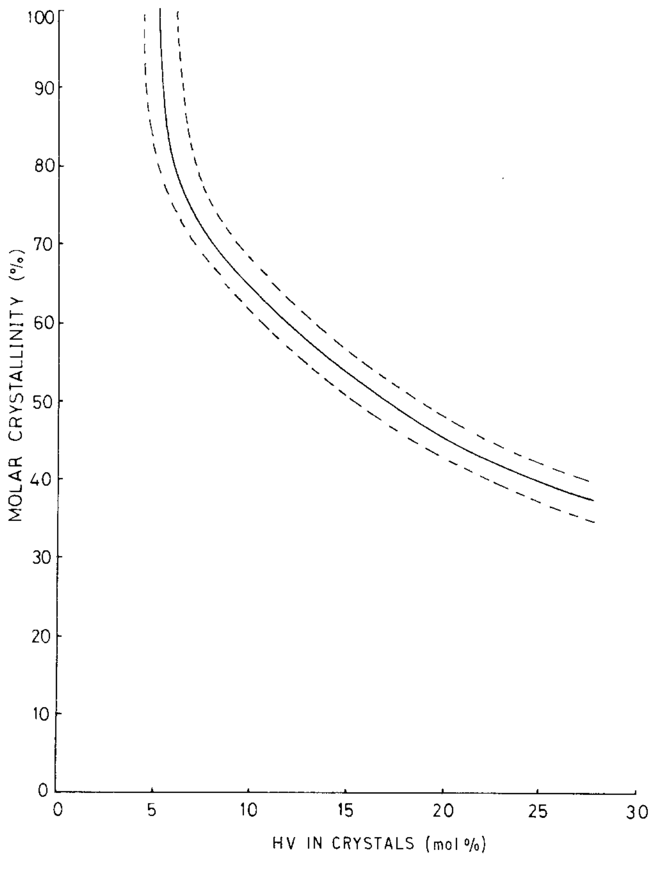

Figure 3 A graph of the possible values of crystallinity of a sample containing 27 mol % HV crystallized at 52° C, showing how the calculated crystallinity according to Equation 2.5 depends on what the HV content of the crystals is taken to be. (——) Mean value, (— —) error limits.

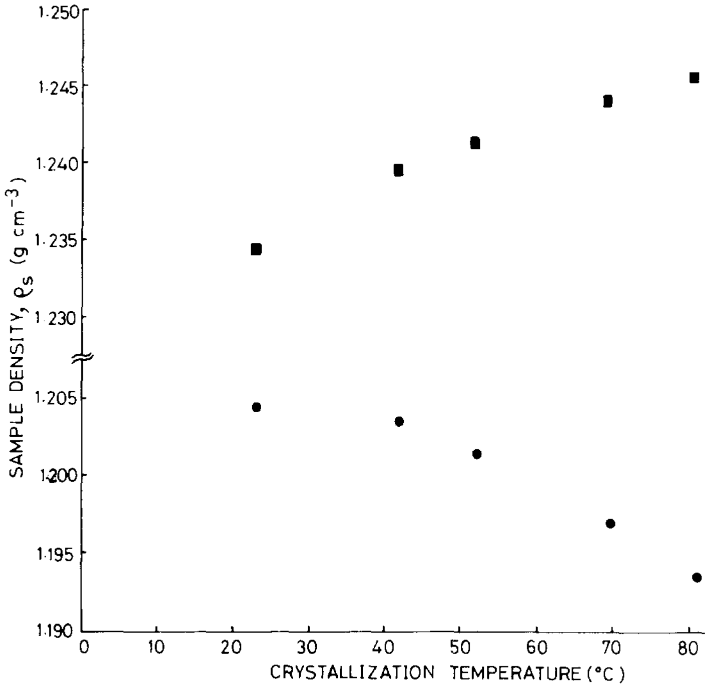

Figure 4 A graph showing the variation of maximum sample density with crystallization temperature for two samples of different HV contents (density measured at room temperature). (■) 3 mol % HV, (●) 27 mol % HV.

The variation of sample density with crystallization temperature is illustrated for two copolymers in Fig. 4, one with a high and the other with a low HV content. The low HV content sample behaves as one would expect: at a higher crystallization temperature the samples have higher density which we interpret as being due to higher crystallinity. However, the density of the sample with high HV content decreases as the crystallization temperaure increases. This is most easily explained as being caused by increasing exclusion of HV units from the crystals as the crystallization temperature is raised. This causes the density of the amorphous phase, and hence the whole sample, to decrease. In such a case it becomes impossible to determine the changes in crystallinity with crystallization temperature; however, by considering the totality of our data we would expect the samples crystallized at higher temperatures to have more perfect crystals (i.e. less inclusion of HV) but lower overall crystallinity. Greater exclusion of HV from the crystals at lower supercoolings is consistent with the fact that there is an energy penalty for the inclusion of HV, because the higher chain mobility will allow the minimization of these energy defects.

5. Conclusions

We have shown that the measurement of crystallinity of copolymers by density is less straightforward than it is for homopolymers. In this context we note that Mitomo *et al.* [15] did not consider all the complications in their analysis, which should accordingly be treated with great caution. It is, in fact, impossible to find the crystallinity of P(HB/HV) from density measurements unless the HV content of the crystals is known, a topic which we are persuing and intend to publish on in the near future. However, the sample densities are not necessarily inconsistent with crystallinities that are nearly independent of sample HV content as suggested by Bloembergen *et al.* [14] but this would necessitate exclusion of HV from the PHB crystals, contrary to their suggestions. Further, we have shown that the crystallization conditions are as important as the HV content of the sample. These affect both the crystallinity, which appears to increase with increasing crystallization temperature, and HV content of the crystals, which appears to decrease with increasing crystallization temperature.

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Received 27 February
and accepted 30 August 1989