On the equilibrium melting temperature of polyhydroxybutyrate

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The equilibrium melting temperature, as determined from Hoffman–Weeks plots, has been measured as a function of the molecular weight for several batches of polyhydroxybutyrate. In all the batches there is a significant increase in the equilibrium melting temperature with the molecular weight. However, different polymer batches give non-superimposable Hoffman–Weeks plots and equilibrium melting temperatures. In particular, at the highest molecular weights studied, we can find anomalously high equilibrium melting temperatures. The equilibrium melting temperatures vary from ca. 179°C for polymers with molecular weights as low as 20 000 to typically 186°C for polymers with molecular weights ca. 100 000; however, for a polymer with a molecular weight of ca. 300 000, we have recorded an equilibrium melting temperature as high as 198°C.

(Keywords: polyhydroxybutyrate; equilibrium melting temperature; molecular weight)

INTRODUCTION

The equilibrium melting temperature of polymer crystals is an important thermodynamic parameter, which is needed as input for theories of polymer crystallization. In particular, the supercooling of the system can only be determined once the equilibrium melting temperature is known. The predictions of crystal thicknesses and, more importantly, the growth rates are strongly dependent on the supercooling.

Several techniques have been used to measure equilibrium melting temperatures of polymer crystals. These include: the Hoffman–Weeks plot[1]; extrapolation of the melting temperature to infinite crystal thickness using the Gibbs–Thompson equation[2]; and the very slow heating of specially prepared and well annealed samples[3]. For most polymers all three techniques give good agreement, and the equilibrium melting temperatures are found to have only a small dependence on the molecular weight. It has been shown that we should expect the melting temperature to increase as the inverse molecular weight decreases[4]. Such behaviour has been observed in practice, in for example polyethylene[5].

In the case of polyhydroxybutyrate (PHB), we have reported values for the equilibrium melting temperature[6,7]. In our original study of the physical properties of PHB[6], we used a plot of the inverse lamellar thickness against the measured melting temperature to obtain an equilibrium melting temperature of 196°C. However, in a subsequent study[7], where we used a polymer from a different batch, we found a lower equilibrium melting temperature (188°C) using the Hoffman–Weeks method.

In this paper we attempt to address the issue of the apparent variability of the equilibrium melting temperature of PHB between various batches. We begin by comparing the results obtained from Hoffman–Weeks and Gibbs–Thompson plots for one polymer and show that the two methods do indeed give closely similar results. We thereafter use the Hoffman–Weeks approach for several series of specially prepared PHB samples of differing molecular weights. Finally, we shall discuss critically the results and the possibilities that the assumptions underlying the extrapolation techniques are not valid in some cases.

EXPERIMENTAL

Preparation of samples

We obtained five different PHB samples from different fermentation batches from ICI BioProducts and Fine Chemicals. These polymers were then deliberately degraded by heating to temperatures in the range 180–200°C for various times. We then had a total of 20 polymers, which were used in the experiments. The molecular weights of all the samples were measured using gel permeation chromatography (g.p.c.) after they had been crystallized for use in the Hoffman–Weeks experiments. We found that there was no further significant degradation of the samples provided the crystallization temperature was below 150°C. We show in *Table 1* the weight-average molecular weights and the polydispersity of these samples.

Crystallization of samples

The samples were crystallized isothermally at temperatures between 80 and 150°C in a Linkam microscope hot stage; the temperature was controlled to better than ±0.5°C. The times of crystallization were sufficiently long that all the material that would crystallize at the

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Table 1 Molecular weights of the polymers

Polymer batch	Polydispersity (M_w/M_n)	Weight-average molecular weight
GSS1		164 000
		138 000
	2.79	121 000
		48 000
'Fluff'	2.63	283 000
	2.22	207 000
		141 000
		119 000
	3.11	111 000
		61 000
G04	2.09	130 000
		120 000
	2.28	110 000
		94 000
		50 000
		51 000
T20	4.86	19 000
		21 000
'Original'	2.69	358 000
	2.17	198 000

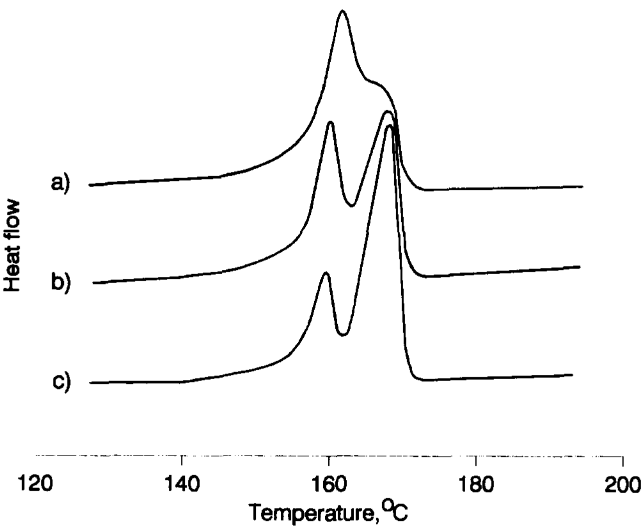

Figure 1 D.s.c. melting peaks as a function of heating rate for PHB crystallized at 80°C. Heating rate: (a) 20°C min^{-1}, (b) 10°C min^{-1}, (c) 5°C min^{-1}

temperature did so. Typically we used a crystallization time of four days for the highest crystallization temperatures, using self-seeding to initiate crystallization where appropriate.

Measurements of melting temperatures

The melting temperatures were measured by differential scanning calorimetry (d.s.c.), using a Perkin–Elmer DSC7, at a heating rate of 20°C min^{-1}. The peak positions were determined and corrected using an indium standard. Where there were two d.s.c. endotherms, we took the lower endotherm as being indicative of the crystals formed at the crystallization temperature, and the higher one as being due to material that had annealed or thickened during the heating in the d.s.c. Note that we did not use any samples with crystallization temperatures less than 70°C; in such samples the annealing effects were so pronounced that we were unable to determine the position of the first melting peak.

The effect of the annealing process is illustrated in *Figure 1* where we show a series of d.s.c. traces of a sample crystallized at 80°C heated at different rates. In all these thermograms two melting endotherms can be seen. At the lowest heating rate, most of the polymer melts at the higher temperature; as the heating rate is increased, so the proportion of the polymer melting at the lower temperature is increased. Similar changes in the relative areas of two melting peaks have been seen in many other systems. It is normally argued that the changes are due to a solid-state process (annealing or crystal thickening) occurring during heating. We take the same view in this case, and hence we argue that it is the lower peak which truly represents the polymer crystallized at the crystallization temperature.

RESULTS

Comparison of Gibbs–Thompson and Hoffman–Weeks plots

We show in *Figures 2* and *3* Gibbs–Thompson and Hoffman–Weeks plots for one of our samples, the material used in our original study[6]. The extrapolated equilibrium melting temperature from the Hoffman–Weeks plot is 195 ± 2°C and that from the Gibbs–Thompson plot is 198 ± 3°C. We regard these results as indicating that the agreement between the two methods is sufficiently

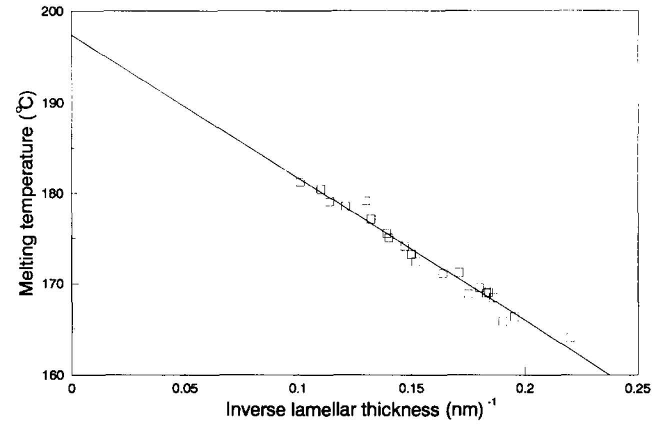

Figure 2 A graph showing the variation in melting temperature with inverse lamellar thickness (the Gibbs–Thompson plot) for PHB with M_w 358 000

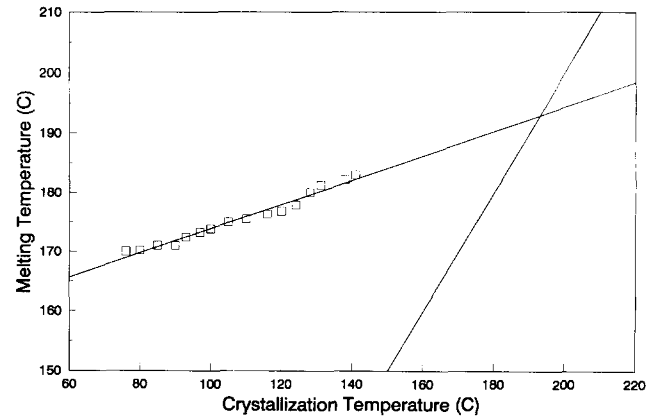

Figure 3 A graph showing melting temperature as a function of crystallization temperature (the Hoffman–Weeks plot) for PHB with M_w 358 000

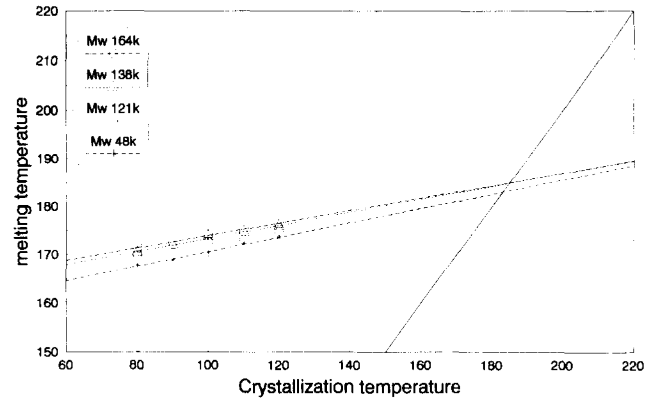

Figure 4 Hoffman–Weeks plots for PHB of various molecular weights derived from batch GSS1

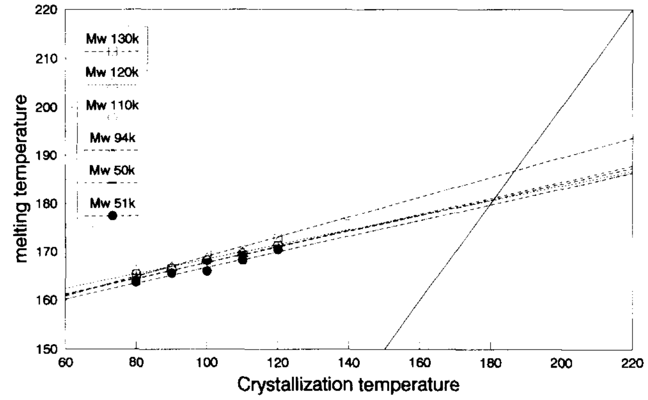

Figure 5 Hoffman–Weeks plots for PHB of various molecular weights derived from batch G04

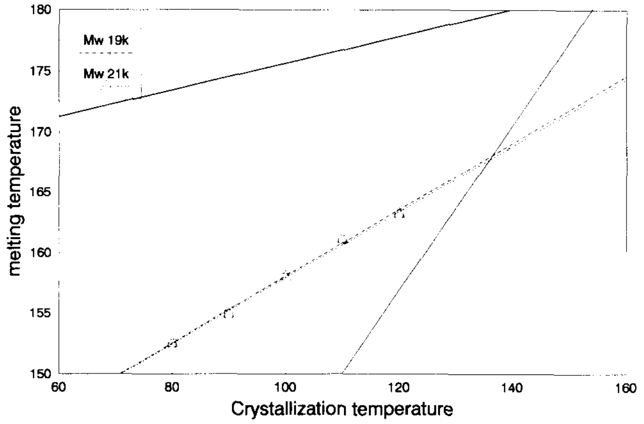

Figure 6 Hoffman–Weeks plots for PHB of various molecular weights derived from batch T20

good that we may use only the Hoffman–Weeks method in our more detailed study.

Hoffman–Weeks plots

We show in *Figures 4–7* the Hoffman–Weeks plots for each of the polymer batches; straight lines have been fitted to the data for each molecular weight. *Figures 4*, *5* and *6* include data for polymers with molecular weights up to 200 000. In each batch a clear increase of extrapolated equilibrium melting temperature is seen as the molecular weight is increased. The equilibrium melting temperatures are quite similar for polymers of similar molecular weights; this can be seen in *Figure 8*, which is a plot of extrapolated equilibrium melting temperature as a function of molecular weight for all the data in *Figures 4*, *5* and *6*. However, while the equilibrium melting temperatures are in good agreement between the different batches, the actual recorded melting temperatures are quite different. We illustrate this by replotting the data for two polymers from different batches, but which have closely similar molecular weights, in *Figure 9*. Now it is clear that these two polymers give the same extrapolated equilibrium melting temperature but different slopes on the Hoffman–Weeks plots.

The Hoffman–Weeks plots in *Figure 7* include one polymer with a molecular weight approaching 300 000. This polymer behaves quite differently from the other, degraded polymers from the same batch; it gives a much higher extrapolated equilibrium melting temperature and the 'Hoffman–Weeks' slope is higher than for the other polymers. We should note that this polymer behaves in a quite similar manner to the polymer used in our original studies, whose molecular weight was ca. 350 000 and whose Hoffman–Weeks plot is shown in *Figure 3*.

If we include all the measured equilibrium melting temperatures in a plot of melting temperature as a function of molecular weight, we obtain the graph shown in *Figure 10*. This shows a markedly strong dependence of melting point on molecular weight, which is most unusual.

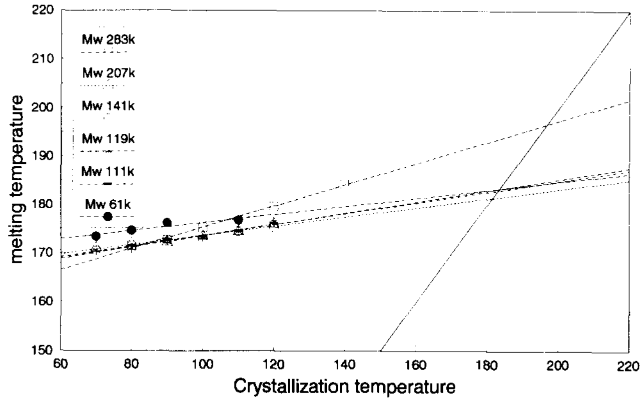

Figure 7 Hoffman–Weeks plots for PHB of various molecular weights derived from 'fluff' and 'original' batches

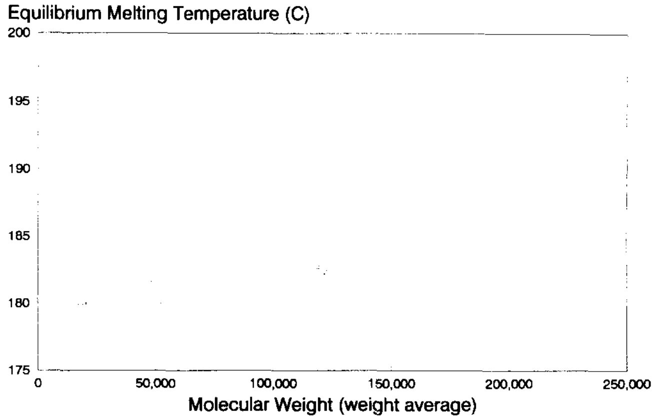

Figure 8 A graph showing extrapolated equilibrium melting temperature as a function of molecular weight for the data represented in *Figures 4* to *6*, where all molecular weights lie below 200 000

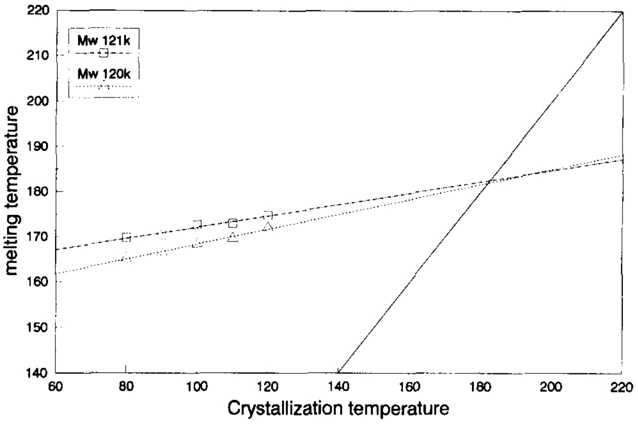

Figure 9 A comparison between the variation in melting point with crystallization temperature for PHB of molecular weight 121 000 derived from batch GSS1 and PHB of molecular weight 120 000 derived from batch G04

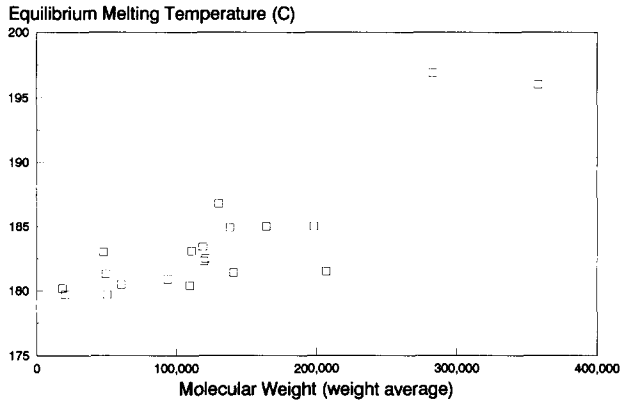

Figure 10 A graph showing extrapolated equilibrium melting temperature as a function of molecular weight for all the PHB samples examined

Highest observed melting temperatures

To construct the Hoffman–Weeks plots we have taken the melting temperature of the samples as the position of the peak of the lower-temperature endotherm. We justify the use of the peak position by arguing that this represents the melting temperature of the majority of the material crystallized at the crystallization temperature. However, the d.s.c. traces contain more information, which should be considered when estimating the equilibrium melting temperature. Clearly, no crystal can exist at a temperature above the equilibrium melting temperature. It has been argued that one way to determine the equilibrium melting temperature is to heat a sample as slowly as possible (so that it perfects itself) and record the temperature at which the last trace of crystallinity disappears. In practice we find that the heating rate has little effect on the final melting temperature as seen in the d.s.c. after appropriate corrections have been made; this can be seen from the traces in *Figure 1* and the data in *Table 2*. As an aside, the data in *Table 2* show a remarkable and most unusual effect: the final melting temperature of the samples crystallized at the lower temperature, 80°C, are higher than those for the sample crystallized at the higher temperature, 120°C. We believe that this behaviour (which we only saw in a few cases) is due to some annealing effect in the samples crystallized at the lower temperature.

We have collected together in *Table 3* the highest, final measured melting temperatures (all corrected for heating-rate effects using indium standards) that we have recorded in each of our samples; these data do show a similar trend to the equilibrium melting temperatures derived by the Hoffman–Weeks method. The highest molecular weight samples have actual recorded highest melting points greater than the equilibrium melting temperature for the lower molecular weight materials.

DISCUSSION

Several distinct anomalies in the melting behaviour of PHB samples have been revealed by the experiments reported above. These may be summarized in three categories. First, there is the variation in the slopes of the 'Hoffman–Weeks' plots for polymers from different batches, which nevertheless still give the same equilibrium melting temperatures. Secondly, there is the unusually strong molecular weight dependence of melting temperatures shown in *Figure 8*. Thirdly, there is the apparent sudden increase of ca. 10°C in equilibrium melting temperature for polymers of molecular weights above ca. 300 000. We shall discuss each of these issues later, but first it is instructive to examine carefully the basis of the Hoffman–Weeks method to see how reliable it really should be.

The Hoffman–Weeks method to estimate equilibrium melting temperature

The Hoffman–Weeks method is to plot melting temperatures as a function of crystallization temperatures. A crystal grown at the equilibrium melting temperature

Table 2 Heating-rate effects on the final melting temperatures

Crystallization temperature (°C)	Heating rate (°C min^{-1}) 5	10	20
80	176.3	176.2	176.0
120	175.1	173.8	175.7

Table 3 Highest recorded final melting temperatures

Weight-average molecular weight	Maximum recorded melting temperature (°C)
164 000	183.3
138 000	182.5
121 000	181.2
48 000	179.9
283 000	190.5
207 000	182.7
141 000	182.7
119 000	182.7
61 000	180.8
130 000	185.6
120 000	177.8
110 000	176.8
94 000	176.4
50 000	176.6
51 000	176.0
19 000	172.7
21 000	176.1
198 000	185.1

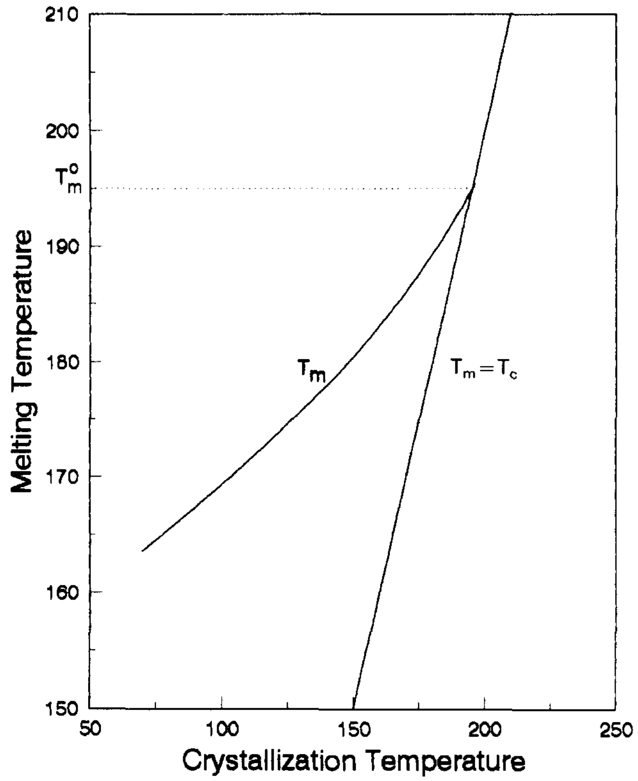

Figure 11 A graph showing the theoretical Hoffman–Weeks plot for PHB

would, of course, melt at its crystallization temperature; therefore by extrapolating the measured melting point data to the temperature where the melting point equals the crystallization temperature, we can find the equilibrium melting temperature. In practice, the extrapolation always used is a linear one; this is justified by the fact that in almost all cases the experimental data lie on a good straight line. Indeed, examination of any of the Hoffman–Weeks plots in this paper shows that the data do indeed show a linear relation between melting point and crystallization temperature.

The reduction in the melting temperature from the equilibrium value is usually accounted for by the limited crystal size and may be expressed theoretically by the Gibbs–Thompson equation:

$$T^\circ_m - T_m = 2\sigma_e T^\circ_m/(\Delta H l) \qquad (1)$$

where T°_m is the equilibrium melting temperature, T_m is the observed melting temperature, σ_e is the end-surface free energy of the crystal, ΔH is the enthalpy of fusion and l is the crystal thickness. The crystal thickness is itself a function of the crystallization temperature and is most commonly given by the nucleation-based theories of Hoffman *et al.* (e.g. ref. 8) by:

$$l = 2\sigma_e T^\circ_m/(\Delta H \Delta T) + \delta l \qquad (2)$$

where ΔT is the supercooling and δl is a small term whose exact dependence on the crystallization temperature is not important here. These two equations may be combined to produce theoretical 'Hoffman–Weeks' plots. This we have done, using values for the parameters appropriate to PHB (from ref. 6), in *Figure 11*. Clearly, this simple theoretical modelling of the Hoffman–Weeks plot is not strictly adequate, since it does not account for the temperature dependence of the heat of fusion. However, the general trend, that we should expect slight curvature to the Hoffman–Weeks plots, remains valid. The curvature in Hoffman–Weeks plots will only become a significant problem when the extrapolation is over a large temperature range. When the highest crystallization temperatures are close to the equilibrium melting temperature, as is usually the case, any error incurred by making a linear extrapolation will be small.

In our work with PHB the highest crystallization temperatures are typically 40°C lower than the extrapolated equilibrium melting temperatures. Accordingly we may expect the problem of curvature to affect our results. We may therefore tend to underestimate the equilibrium melting temperature. However, the problem of extrapolation is the same for all our samples, so it is unlikely that this can provide an explanation for the apparent increase in equilibrium melting temperature for molecular weights ca. 300 000.

Annealing effects

We have already noted that the polymer crystals are able to perfect themselves during heating in the d.s.c. We believe that this perfectioning involves the crystals increasing their thickness. It is well known that the rate at which such crystal thickening occurs increases as the temperature increases. Thus it could be argued that those crystals with higher melting temperatures (grown at higher temperatures) will see higher temperatures in the d.s.c. heating experiments and hence will be subject to faster thickening. It is then possible that all the polymer thickens during the heating so only one d.s.c. peak may be seen and that this peak corresponds to thickened material. In that case we would overestimate the melting temperature of crystals grown at high temperatures. We might argue that any observed melting temperatures above some particular high temperature where the thickening process becomes very rapid should be ignored.

In a separate study, using synchrotron radiation to follow the thickening of PHB during heating (to be reported elsewhere[9]), it has been found that the thickening process is a combination of a solid-state thickening and a melting and recrystallization process. The rate of increase of crystal thickness becomes very rapid at temperatures above 175°C. We may therefore argue that when we observe a melting peak at a temperature above 175°C it has probably been affected by some annealing and hence we should ignore it as it is not representative of the material crystallized at the crystallization temperature.

If we replot our data using the criterion that we ignore any measured melting points above 175°C, we do indeed see a reduction in the equilibrium melting temperatures for the higher-molecular-weight samples. The equilibrium melting temperature of the sample with molecular weight 283 000 is reduced to 188°C, and that of the sample with molecular weight 358 000 is reduced to 189.5°C. These temperatures, while significantly reduced, still show a marked increase over those for slightly lower molecular weights. We note that the data in *Table 3* actually show higher recorded melting temperatures than these extrapolated values; hence we believe that they are too low.

Probably the main reason for the reduction in the melting temperatures when ignoring the higher observed melting data comes from the curvature in the Hoffman–Weeks plots as described in the preceding section. If this is the case, we are able to place some

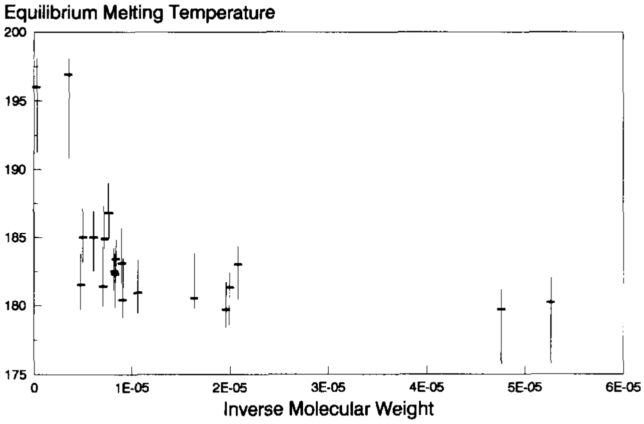

Figure 12 A graph showing equilibrium melting temperature as a function of inverse molecular weight for all the samples used, including error bars showing the reasonable limits of accuracy

estimate of the degree to which the problem of curvature may cause us to underestimate the equilibrium melting temperatures. We have seen that by removing the higher-temperature data the extrapolated melting temperature falls by ca. 8°C; hence it would be reasonable to assume that this is the sort of error we may expect in all our measurements.

Variation in the slope of the Hoffman–Weeks plots

We were most surprised to find the wide and quite consistent differences in the actual melting temperatures of seemingly identical polymers that came from different fermentation batches. We were even more surprised when the Hoffman–Weeks plots extrapolated to the same equilibrium melting temperatures. If we assume that there are some impurities in the samples, which act as solvents and hence tend to lower the measured melting temperatures, the quality of these 'solvents' must be very poor so that the equilibrium dissolution temperature is only slightly lower than the equilibrium melting temperature. It seems unlikely that such a poor solvent could so significantly reduce the melting temperatures. An alternative is to assume that there is a non-solvent impurity present which affects the surface energies of the crystals. If the surface energy is reduced, a crystal would melt at a higher temperature. We favour this latter argument to explain the differences in the slopes of the Hoffman–Weeks plots.

Best estimates of the equilibrium melting temperatures

We find ourselves in great difficulty in trying to decide on the best estimates for the equilibrium melting temperature of PHB crystals. We can clearly see that there is a larger dependence of equilibrium melting temperature on molecular weight than is usual in polymers. The increase of equilibrium melting temperature by ca. 5°C as the molecular weight increases from 20000 to 200000 is quite astonishing, but well documented and quite reproducible. The implication is that the end-groups on the chains play a very significant role in the crystallization process.

As the molecular weight is increased above 200000 we find a further large increase in equilibrium melting temperature. We can offer no rational explanation for such strange behaviour. The only possible explanation we could suggest is that there is a different, high-melting crystal structure in these materials, which perhaps occurs only at the high temperatures. However, X-ray diffraction offers no evidence for such behaviour[10].

We have made some arguments above that may permit us to explain away some of the anomalous behaviour: ignoring experiments where we observed high melting temperatures; the possible curvature of the Hoffman–Weeks plots; and the thickening of crystals during heating. However, none of these provide sufficient error to remove the strong molecular-weight dependence of the equilibrium melting temperature. Accordingly we believe that the data produced by extrapolation from the Hoffman–Weeks plots provide the best available estimate for the equilibrium melting temperature.

We have made a plot in *Figure 12* of these 'best estimates' with error bars representing the range of errors we believe are reasonable in each measurement taking into account all the arguments made above. In this figure we have plotted the equilibrium melting temperature as a function of the inverse molecular weight. The available theories for the temperature dependence of equilibrium melting temperature suggest that such plots should be linear[4]; clearly our results are non-linear. We offer no explanation of this behaviour; we merely report it and hope others can make sense from it.

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