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Nucleation behaviour of poly-3-hydroxy-butyrate

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Poly-3-hydroxy-butyrate (PHB) is a thermoplastic polyester produced by bacterial fermentation. Because of this bacterial origin PHB is a very pure polymer. This high purity in turn leads to very few (if any) heterogeneous nuclei, which gives a much wider scope for a systematic study of nucleation behaviour and the effect of nucleating agents than was possible before. It is shown that in pure PHB nucleation is sporadic. The nucleation rate may be measured over a temperature range of some 100° C. The nucleation rate data are recorded in the range where homogeneous nucleation is possible and are at least consistent with it. When foreign particles are added the nucleation rate is modified. Two distinct types of behaviour are observed. One may be interpreted quantitatively as the local raising of the crystal melting point due to the constraints of the actual presence of a surface; and the other as being due to epitaxial growth on the foreign surface. Detailed kinetic data are presented to support these conclusions.

1. Introduction

Poly-3-hydroxy butyrate (PHB) is a thermoplastic polyester produced by bacterial fermentation. It was first isolated by Lemoigne [1] about 50 years ago and subsequent work has shown that the polymer serves as an energy and carbon storage product [2]. PHB is relatively abundant in the environment, being found in a wide variety of bacteria [3–6]; the proportion of PHB in these cells is normally low – between 1 and 30%. However, under controlled fermentation conditions of carbon excess and nitrogen limitation overproduction of PHB can be encouraged leading to yields of *c.* 70% of dry cell weight [7]. Recently ICI Agricultural Division have developed a pilot scale facility for the production of PHB [8]. Unlike conventional thermoplastics, there are no catalyst residues in the polymer. The major impurities are inorganic nitrogen, phosphorous and sulphur-containing compounds which are present at concentrations less than 200 ppm.

The crystal structure of PHB has been reported on by various authors [9–11], as has some work on single crystals of low molecular weight material [12]. With the new availability of PHB in larger quantities we have already published some details of crystal morphology, growth rate, and thermodynamic constants for PHB [13].

In this paper we present details of our study of nucleation in which we have taken advantage of the high purity, and hence very low degree of heterogeneous nucleation of PHB, which make it an ideal model system for nucleation studies. We have studied the nucleation behaviour of pure PHB and then added various impurities, observing their effect on nucleation behaviour and nucleation rates as a function of temperature. We have observed a wide variety of behaviour which we shall describe. However, we shall concentrate here on three kinds of nucleation; nucleation of pure PHB and nucleation of PHB onto talc and onto saccharin surfaces.

Once we have described these various types of nucleation behaviour we shall move on to consider their theoretical interpretation, and we shall show that with minor modifications to existing nucleation theories we can explain completely all the various types of behaviour. While the theories we shall use are not of themselves original, we believe that this is the first time that they have been brought together in this way and shown actually to predict nucleation behaviour.

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2. Types of nucleation observed in PHB

Five distinct types of nucleation in PHB have been observed. These are described separately below as Types A to E. All the nucleation experiments were carried out using the Mettler hot stage on the optical microscope. The standard experimental procedure was to melt the sample and then cool it at ~ 20° C min^{-1} (i.e., as fast as the stage will allow) to a crystallization temperature, usually between 110 and 120° C, and then observe nucleation and growth. In Type A, nucleation was never observed within 35 min at 110° C. In Types B and C nucleation usually occurred within a few minutes of reaching 120° C and more or less instantaneously at 110° C. In Types D and E nucleation occurred during cooling at a temperature between 120 and 130° C.

2.1. Type A: nucleation from the pure melt

This is the usual very slow nucleation occurring when PHB is cooled from a melt at or above ~ 210° C. Nucleation does not occur at predetermined sites.

2.2. Type B: self-seeding

When PHB is heated to not more than ~ 15° C above its melting point (the temperature at which birefringence disappears), on cooling and recrystallizing at the same temperature as the initial sample there is usually an increased nucleation density. The nucleation density is greater for lower superheatings. There is no increased nucleation density if the melt temperature exceeds 205° C or if the superheating exceeds 20° C. The effect is also reduced if the melt is held at high temperature for more than a few minutes. For example if a sample, crystallized at 120° C with a spherulite size of ~ 2 mm and melting point 182° C, is held at 188° C for 1 min before being cooled to 120° C and crystallized, the resulting spherulite size is ~ 50 μm, while if it is held at 188° C for 10 min the resulting spherulites crystallized at 120° C are ~ 1.5 mm in diameter.

2.3. Type C: enhanced self-nucleation

This is really an extension of self-seeding. If the sample is heated to no more than 1° C above its melting point (temperature at which birefringence disappears), then on cooling to a crystallization temperature the spherulites simply reappear, just as the reverse of melting, and not by the usual radial growth process. This is illustrated by Figs. 1, 2 and 3 of [14] which show the initial growth, melting and recrystallization of some spherulites of polethylene. We discovered the effect in polyethylene but just the same sort of behaviour is observed in PHB.

Typically a sample heated to 182° C after melting at 181.5° C can be recrystallized in a few minutes at 130° C. This effect is only observed if the melt temperature is less than 188° C, i.e., if a sample melts at 189° C, then no enhanced nucleation can be obtained.

2.4. Type D: nucleation by impurities

This type of nucleation in fact seems to work with any impurity but we shall use talc as the example here; other impurities have different temperatures and rates associated with them. Essentially the impurity seems to act so as to stabilize the self-seeding type of nucleation. Thus if the impurity is added directly to the melt it will have no nucleating effect – only after the PHB has crystallized around the talc will it act as a nucleating agent on subsequent melting and recrystallization. The nucleating effect can be seen from Figs. 1 to 3. In Fig. 1 we show the growth of a PHB spherulite at 120° C in a melt to which talc has been added. In Fig. 2 we show the same area recrystallizing at 120° C after melting at 185° C for 1 min, the large number of small spherulites each having a talc particle at its centre. In Fig. 3 we show the same area crystallizing again after melting at 205° C for 1 min, and again we see only one large spherulite, the talc having lost its effect at the high melting temperature. In practice the talc particles will act as nuclei provided the melt temperature never exceeds 205° C. Once again, just as for self-seeding, there is a time dependence, and to some extent a dependence on the actual melting point of the spherulites. We indicate in Fig. 4 the temperature–time regions in which talc nucleation always, never and sometimes, works. The talc particles used in this work had a diameter of *c.* 5 μm.

2.5. Type E: nucleation by saccharin

This type of nucleation has been observed only with saccharin; it is the "classical" nucleation behaviour where the foreign material will always act as a nucleus, whether it is added directly to the melt or whether the polymer has first been allowed to crystallize around it. Saccharin has a compara-

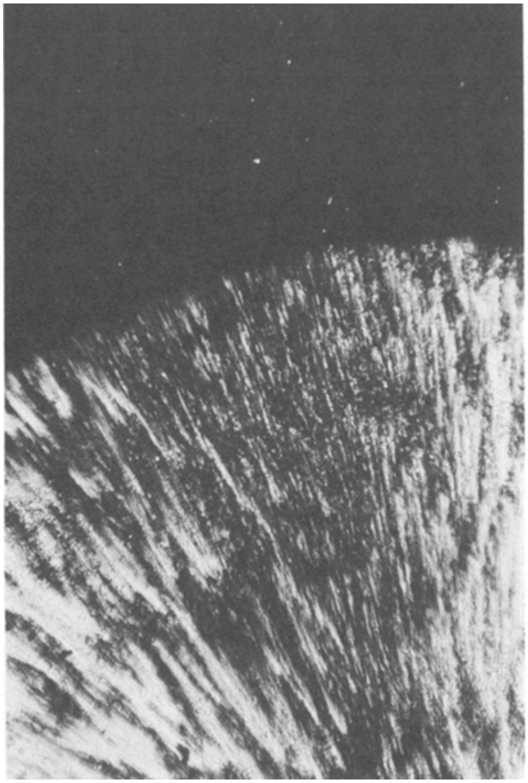

Figure 1 The growth of a PHB spherulite at 120° C from a melt to which talcum powder has been added.

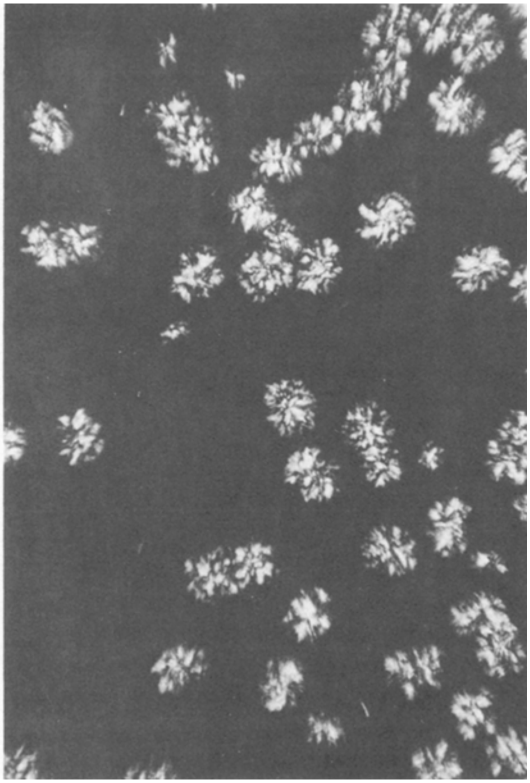

Figure 2 The same region as Fig. 1 crystallizing at 120° C after melting the fully crystallized sample at 185° C for 1 min.

tively low melting point, 226° C. Crystals nevertheless are stable in a PHB melt at temperatures up to 225° C and will act as nuclei on subsequent cooling, independent of the melt temperature. The nucleation on saccharin is shown in Fig. 5. This is directly comparable to Figs. 1 to 3 for nucleation in the presence of talc. It can be seen that saccharin crystals are just as efficient as nucleating agents as talc particles. It can also be seen that nucleation occurs preferentially on certain crystal faces; although it will occur eventually on any face, nucleation always appears first on the (1 1 1) crystal faces. The oxygen atoms on the 1 1 1 crystal face of saccharin project above the surface and are spaced ~ 1.185 nm apart. PHB has an axial repeat of ~ 0.596 nm and will fit with little distortion onto the saccharin (1 1 1) crystal face. The saccharin crystals used in this work had a longest dimension of *c.* 50 μm.

3. Nucleation kinetics in PHB

The nucleation rate, and its variation with temperature has been studied for three types of nucleation, A, D, and E above. The same basic experimental techniques were used in each case. Before measuring the nucleation rate it was first determined that nucleation was sporadic in these cases. This was done by checking that at a constant temperature (100° C for pure PHB, 120° C for PHB with 1% talc added and 140° C for PHB with 1% saccharin crystals added), the number of new nuclei per unit volume of uncrystallized polymer appearing in an interval of 2 min was randomly distributed about a mean value. One example, for the pure PHB melt, is given in Fig. 6. This histogram is compiled from very many sets of experiments. In all cases we stopped counting new nuclei after approximately 15% of the polymer had crystallized. In all cases the data formed a Gaussian distribution, indicating that the nucleation was indeed at random.

For the detailed studies of nucleation rate as a function of temperature we prepared several slides of each type of sample, all of equal weight (250 mg). These were melted at 200° C for 2 min in the case of pure PHB and PHB with 1% saccharin

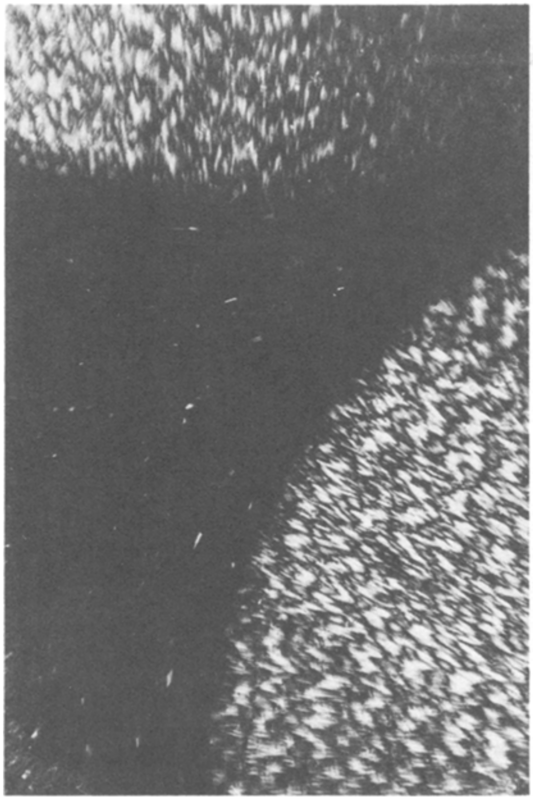

Figure 3 The same region as Figs. 1 and 2 recrystallizing at 120° C after melting the fully crystallized sample at 205° C for 1 min.

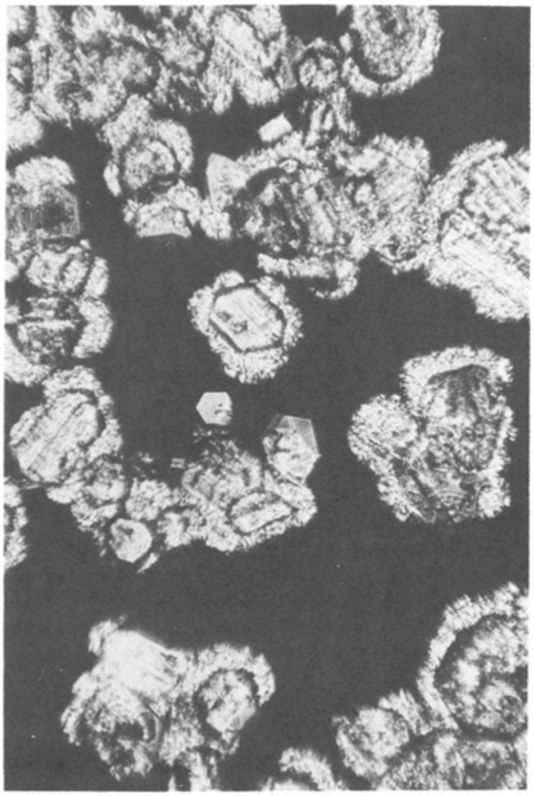

Figure 5 The growth of PHB spherulites form a melt to which saccharin crystals have been added.

crystals added, and at 195° C for 5 min for the PHB with 1% talc added. The slides were then cooled to the required temperature (a fresh slide being used for each experiment to avoid possible problems of degradation) and photographs taken, from which the number of nuclei initially present was determined. Further micrographs were taken at later times and from these the nucleation rate was determined.

The results of these studies are shown in Figs. 7 to 9 where the nucleation rate is plotted as a function of temperature for the three types of sample.

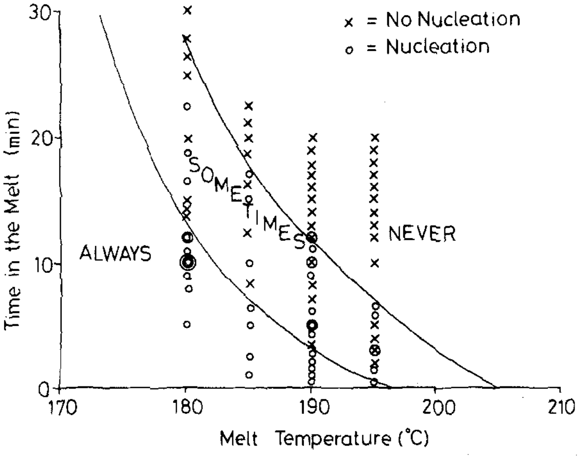

Figure 4 A diagram showing the regions of melt temperature and time for which nucleation of PHB by talcum powder always, sometimes and never occurs.

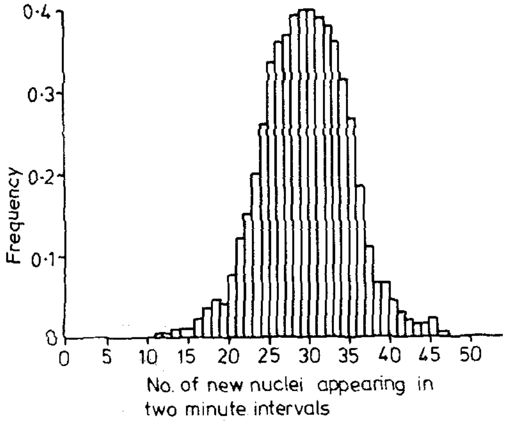

Figure 6 A Histogram showing the frequency of nucleation events in a pure PHB melt at 100° C.

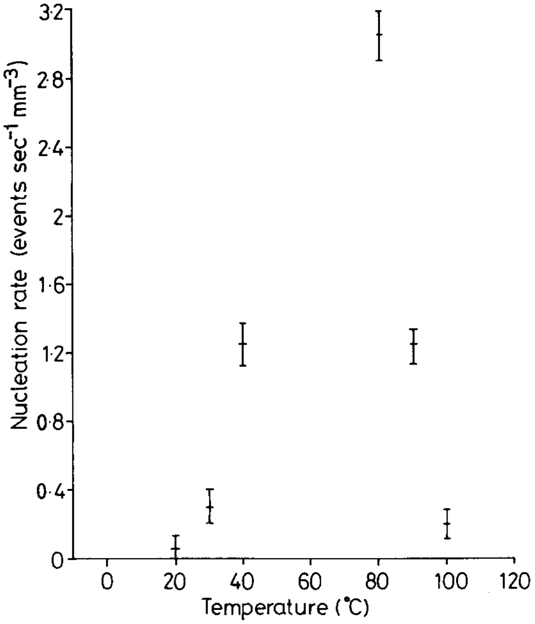

Figure 7 A graph showing the variation in nucleation rate with crystallization temperature for pure PHB crystallized from the melt.

The data in Figs. 7 and 8 at temperatures between 20 and 40° C were obtained from samples quenched from the melt into liquid nitrogen and then heated to the required crystallization temperature. This technique could not be applied to the saccharin-filled material since this crystallized even during such severe quenching. The error bars on the data points in Figs. 7 to 9 indicate the standard deviation observed from a number (always at least 6) of independent measurements of the nucleation rate. The standard deviations on these data are less than that of the data in Fig. 6 since the measurement periods were chosen so that many more nuclei formed during each period.

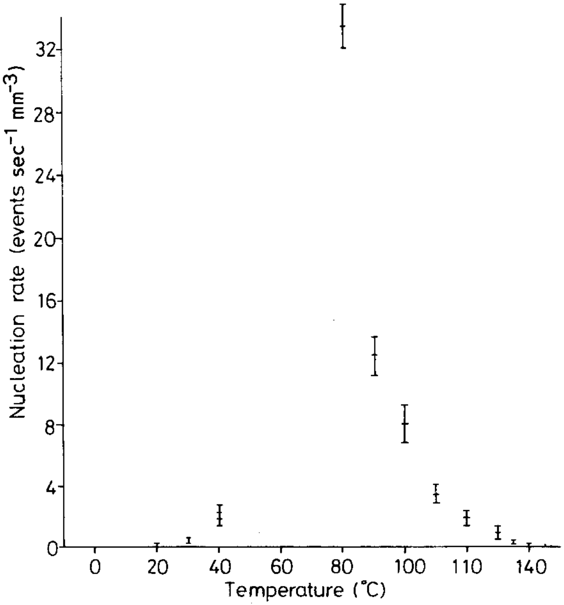

Figure 8 A graph showing the nucleation rate from a PHB melt doped with 1% talc after remelting at 195° C for 5 min as a function of the crystallization temperature.

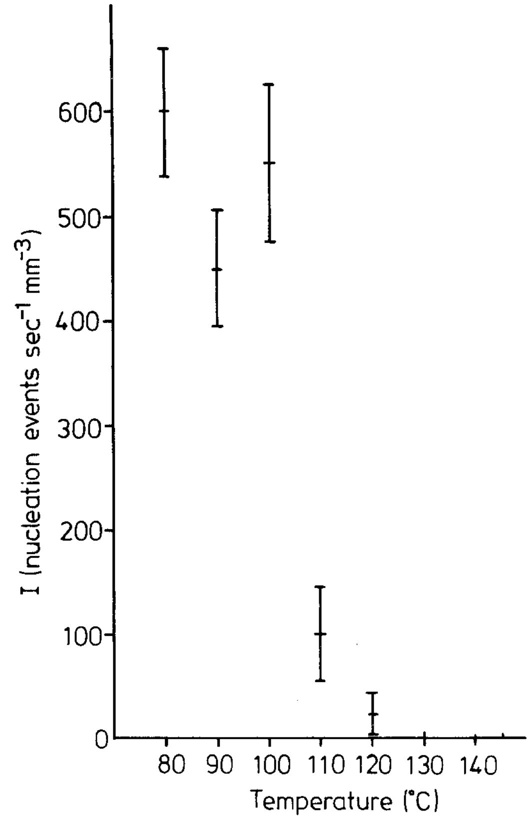

Figure 9 A graph showing the nucleation rate from a PHB melt with 1% saccharin added as a function of crystallization temperature.

4. Theoretical interpretation of nucleation rate data

The usual way to consider nucleation is to calculate the work required to form a nucleus, in terms of surface free energies and the bulk free energy of fusion, as a function of crystal dimensions. This leads to a surface which in general will have a saddle point corresponding to a critical nucleus. The work to form this critical nucleus, $\Delta\phi^*$, may be calculated and the rate of nucleation is then proportional to $\exp(-\Delta\phi^*/kT)$. Various cases have been considered in the literature; the review by Price [15] details the derivations of all those of

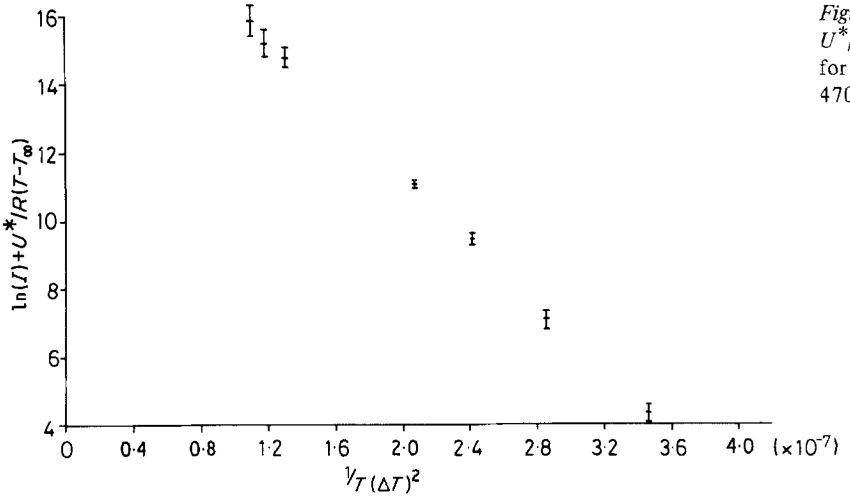

Figure 10 A graph of $\ln(I) + U^*/R(T-T_\infty)$ against $1/T(\Delta T)^2$ for pure PHB (taking $T_M^0 = 470\,\mathrm{K}$).

interest here, a brief summary being given below. First, in the case of homogeneous nucleation, which is the process of the birth of small regions of the crystalline phase in the pure supercooled melt in the absence of all surfaces, $\Delta\phi^*$ is given by

$$\Delta\phi^* = \frac{32\sigma^2\sigma_e}{(\Delta G_V)^2}$$

where σ is the side surface free energy, σ_e the end, or fold surface free energy and ΔG_V the bulk free energy of fusion. In this treatment only two types of surface are considered; crystal sides and fold surfaces. The shape of the nucleus is assumed to be a rectangular parallelpiped. A more detailed analysis allowing additional faces and free energies leads to a similar result where σ^2 is replaced by the product of two side surface free energies and the numerical factor 32 may be reduced. Secondly, various cases of heterogeneous nucleation may be considered. We shall only be concerned with nucleation at a foreign surface; in this case, if all three dimensions of the nucleus are allowed to change, $\Delta\phi^*$ is given by

$$\Delta\phi^* = \frac{16\sigma\sigma_e\Delta\sigma}{(\Delta G_V)^2}$$

where $\Delta\sigma = \sigma + \sigma_{os} - \sigma_s$, σ_s is the interfacial energy between the foreign surface and the polymer melt, and σ_{os} is the interfacial energy between the polymer crystal and the foreign surface. When the substrate is absent $\sigma_s = 0$ and $\sigma_{os} = \sigma$; hence $\Delta\sigma = 2\sigma$ and we obtain the value of $\Delta\phi^*$ for homogeneous nucleation. The more closely the foreign surface resembles the crystal surface the closer σ_s will be to σ, and the closer σ_{os} will be to zero, and hence $\Delta\sigma$ will become smaller. In the extreme case where the crystal surface is identical to the foreign surface $\Delta\sigma$ becomes zero and $\Delta\phi^*$ is then given by $4b\sigma\sigma_e/\Delta G_V$, where b is the lattice spacing normal to the surface. This is the familiar result of secondary nucleation.

Before we turn to a consideration of the experimental results we must note that ΔG_V is given by

$$\Delta G_V = \Delta H - T\Delta S \qquad (1)$$

and since at $T = T_M^0$ the equilibrium melting point $\Delta G_V = 0$, we may write

$$\Delta G_V = \frac{\Delta H \Delta T}{T_M^0}$$

where ΔH is the enthalpy of fusion and ΔT the supercooling.

Now let us examine the experimental results. First the results for pure PHB; since these data are for pure melts it seems reasonable to examine the possibility of homogeneous nucleation. In such a case we would expect the nucleation rate I to be given by an equation of the form:

$$I = I_0 \exp\left[\frac{-U^*}{R(T-T_\infty)}\right] \times \exp\left[\frac{-32\sigma^2\sigma_e(T_M^0)^2}{(\Delta H)^2(\Delta T)^2 kT}\right] \qquad (2)$$

where the term $\exp[-U^*/R(T-T_\infty)]$ represents the transport of molecules to a growing nucleus. In our previous work [13] we have found from growth rate and other data best fit values for U^*, T_∞, T_M^0, ΔH, σ and σ_e as $U^* = 10.25\,\mathrm{kJ\,mol^{-1}}$, $T_\infty = -48°\,\mathrm{C}$, $T_M^0 = 470\,\mathrm{K}$, $\Delta H = 146\,\mathrm{J\,mol^{-1}}$, $\sigma_e = 38 \pm 6 \times 10^{-7}\,\mathrm{J\,cm^{-2}}$ and $\sigma\sigma_e = 11.60 \times 10^{-12}\,\mathrm{J\,cm^{-4}}$.

In Fig. 10 we have plotted $\ln(I) + U^*/R(T-T_\infty)$

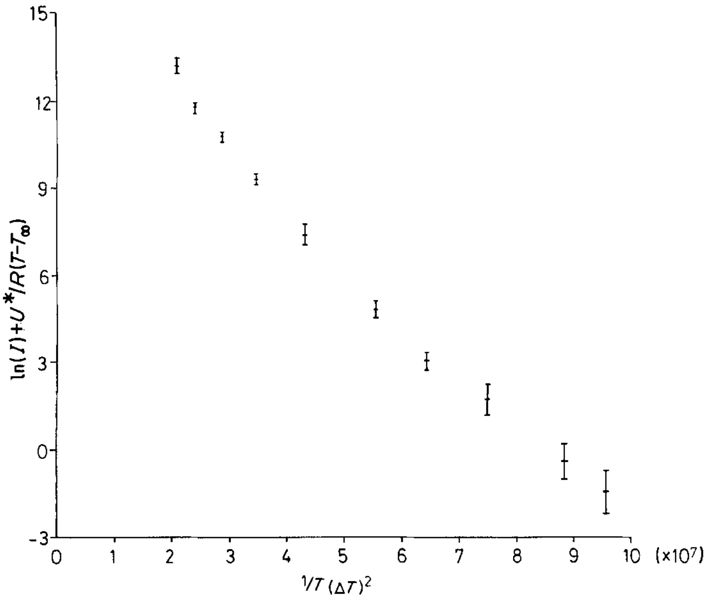

Figure 11 A graph of $\ln(I) + U^*/R(T - T_\infty)$ against $1/T(\Delta T)^2$ for PHB with 1% talc added (taking $T_M^0 = 470\,\text{K}$).

against $1/T(\Delta T)^2$, taking the above values for T_∞, U^* and T_M^0. The data fall very well on a straight line of slope $4.83 \times 10^7\,\text{K}^3$ which, assuming homogeneous nucleation, suggests $\sigma^2\sigma_e \simeq 3.100 \times 10^{-18}\,\text{J cm}^{-6}$. This value is a little lower than may be expected from our previous estimates of σ and σ_e. Thus we might expect nucleation to be occurring heterogeneously at a surface. The only surfaces present are the glass slide and cover slip. In order to test for nucleation at these surfaces we prepared thinner samples using much smaller amounts of material (20 mg per sample). If the glass surface affects the nucleation we should expect a higher nucleation rate in these samples; this was not observed†.

Now let us examine the data from the talc-doped PHB melts. In Fig. 11 we have plotted the data in the same way as for the pure melt, which shows clearly that the results do not lie on a straight line. Thus the effect of the talc is not simply to cause heterogeneous nucleation at a foreign surface – something else must be involved.

When talc is added to the PHB melt it does not act as a nucleating agent – it only has a nucleating effect on remelting after PHB has first been crystallized around it. Furthermore it loses its nucleating effect once the melt is heated above ~ 210° C. We may deduce from this that nucleation does not occur directly at the PHB/talc interface, but is influenced by some association which occurs between crystalline PHB and talc but which may be destroyed by high temperatures. There are two possibilities; either the PHB molecules which formed a crystalline layer around the talc particles are prevented from melting – thus leaving a polymer crystal substrate for subsequent nucleation – or the PHB molecules are partially absorbed onto the talc particles so as to reduce their entropy in the molten state. In the first case we should expect the nucleation rate to be given by

$$I = I_0 \exp\left[\frac{-U^*}{R(T - T_\infty)}\right] \exp\left[\frac{-4b\sigma\sigma_e T_M^0}{kT\Delta H\Delta T}\right]$$

and in the second by

$$I = I_0 \exp\left[\frac{-U^*}{R(T - T_\infty)}\right] \exp\left[\frac{-32\sigma^2\sigma_e T_M^{0\,2}}{kT(\Delta H)^2(\Delta T)^2}\right]$$

with T_M^0 now greater than in the case of homogeneous nucleation since T_M^0 is defined by $T_M^0 = \Delta H/\Delta S$ and ΔS is reduced by the constraint of partial adsorption. In practice it is not possible to fit the data to a relationship of the first kind but it does fit one of the second kind if T_M^0 is taken as 495 K. This is illustrated in Fig. 12 where $\ln(I) + U^*/R(T - T_\infty)$ is plotted against $1/T(\Delta T)^2$ (with $\Delta T = 495 - T$), and the slope of the resulting straight line is 5.20×10^7. We should expect the ratio of the slope of this line to the line for nucleation from the pure melt to be the ratio of

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† The pre-exponential term I_0 in Equation 2 may be calculated approximately. Typically it has a value of ~ 10^{34} events $\text{cm}^{-3}\,\text{sec}^{-1}$. In our experiment we find a value of approximately 10^{12} events $\text{cm}^{-3}\,\text{sec}^{-1}$. We make no further comment on this wide discrepancy.

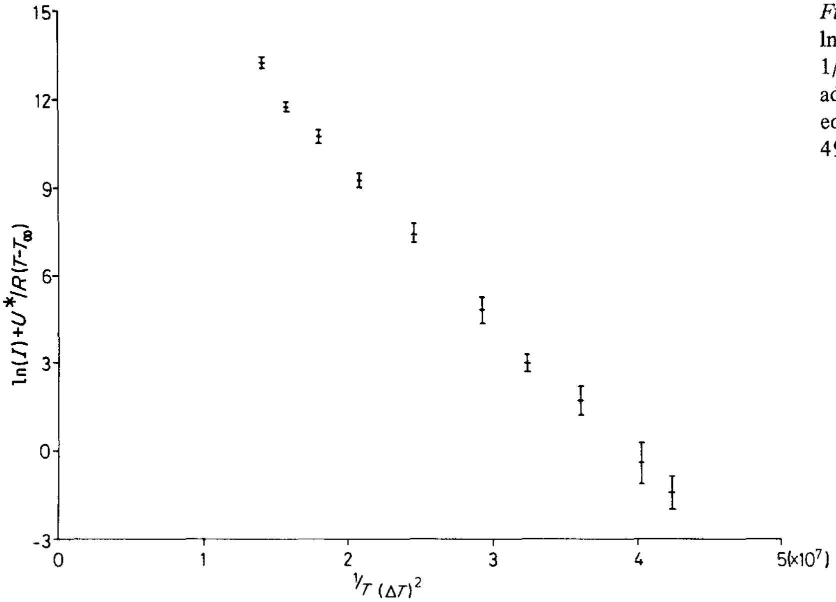

Figure 12 As Fig. 11, a graph of $\ln(I) + U^*/R(T - T_\infty)$ against $1/T(\Delta T)^2$ for PHB with 1% talc added, but this time taking the equilibrium melting point $T_M^0 = 495$ K.

the squares of the equilibrium melting points; the ratio of the slopes is 1.08 and of the squares of the melting points 1.11. This again shows a very good agreement between the theoretical prediction and experiment.

The nucleation behaviour in the presence of saccharin is different in that it acts as a nucleating agent even when added to a supercooled melt, nucleation occurring preferentially on certain crystal faces. We can therefore deduce that nucleation occurs at the saccharin/polymer interface and accordingly we should expect the nucleation rate to be given by

$$I = I_0 \exp\left[\frac{-U^*}{R(T-T_\infty)}\right] \exp\left[\frac{-16\sigma\sigma_e\Delta\sigma T_M^{0\,2}}{kT(\Delta T)^2(\Delta H)^2}\right]$$

If we assume, as seems reasonable from the observations reported above, that in the melt PHB molecules are adsorbed onto the saccharin surface in approximately their own crystallographic form (i.e., the growth is epitaxial), then the term σ_{os} should be approximately equal to σ_s. In Fig. 13 $\ln(I) + U^*/R(T - T_\infty)$ is plotted against $1/T(\Delta T)^2$ for PHB nucleated by saccharin. I is in arbitrary

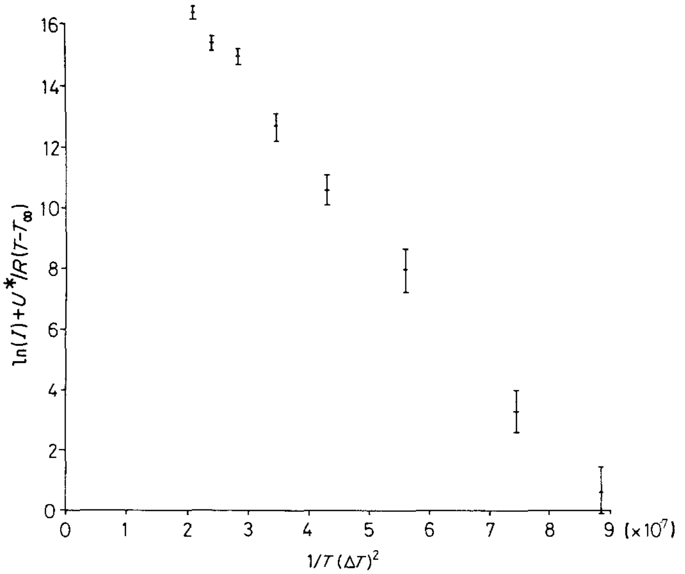

Figure 13 A graph of $\ln(I) + U^*/R(T - T_\infty)$ against $1/T(\Delta T)^2$ for PHB with 1% saccharin added.

units and T_M^0 is taken at 470 K, as for the pure PHB melts. The slope of the straight line is $2.33 \times 10^7\,K^3$, which compares with the expected value taking $\sigma_{os} = \sigma_s$ of $2.42 \times 10^7\,K^3$ (i.e. one half the value of the slope obtained from the pure PHB melts).

We may therefore draw the conclusion that pure PHB melts may undergo homogeneous nucleation, that the nucleating effect of talc is due to the reduction in entropy of partially adsorbed molecules, and that the nucleating effect of saccharin is due to adsorption of molecules onto the surface in what is close to their crystallographic form.

5. Conclusions

The work reported here has shown that there are several nucleation mechanisms which may occur in crystallizing from the melt. It remains to be seen to what extent these mechanisms are general and to what extent they are limited to PHB. PHB has proved, by the nature of its high purity, to be an ideal material for nucleation studies.

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