Hydrolytic degradation of isolated poly(β-hydroxybutyrate) granules

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The kinetics of chain degradation of purified granules of poly(β-hydroxybutyrate) in 3.0 N HCl at the boil were followed using gel permeation chromatography to detect changes in molecular weight of the residue. The results showed that degradation corresponded to a model of random scission of the molecules of both the crystalline shell and the non-crystalline core indiscriminately. Differential scanning calorimetry and wide-angle X-ray diffraction results demonstrated that the molecules recrystallize due to chain scission and annealing at the temperature at which degradation is performed (104.5°C). Transmission electron microscopy of enzyme-isolated, spray-dried granules revealed a retention of the surface shell throughout the reaction with conversion of the core to a porous lamellar texture.

(Keywords: poly(β-hydroxybutyrate); hydrolytic degradation; kinetics)

INTRODUCTION

Poly(β-hydroxybutyrate) (PHB) is a chiral biopolyester produced by many bacterial strains in the form of submicrometre granules in the cytoplasmic fluid. They constitute a carbon reserve in a wide variety of bacteria. Their structure was examined by transmission and scanning electron microscopy and has been shown to consist of two different components: a solid shell composed of overlapping lamellar crystals and a soft non-crystalline core[1].

In order to examine the crystalline shell of PHB granules using electron diffraction, a treatment allowing the removal of the non-crystalline matter from the core using concentrated HCl is necessary[1]. X-ray diffraction results showed that granules purified using sodium hypochlorite were crystalline (~40%) after drying. Although it was known that the non-crystalline core of such granules was preferentially hydrolysed under strongly acidic conditions, the effects of a milder hydrolysis treatment on the granule texture are unknown[2]. Since the shells retained their integrity on hydrolysis, it might be expected that the hydrolysis rate of the crystalline phase is different from that of the non-crystalline phase.

The homogeneous hydrolytic degradation of polyesters occurs through random scission of the ester linkages[3]. Heterogeneous hydrolysis (buffered aqueous solutions at pH 7.4) of PHB also proceeds via random scission of the polymer chains throughout the whole polymer matrix for solution-cast as well as melt-crystallized film and fibre samples[4,5]. Under these conditions, both the non-crystalline and crystalline domains are hydrolysed indiscriminately[2-6]. In a study aimed at understanding the selective degradation of PHB single crystals upon exposure to gaseous methylamine, Welland *et al.*[7] showed, by molecular-weight determination and small-angle X-ray diffraction results, that folds of PHB single crystals (and possibly other disordered regions of the crystals) were hydrolysed preferentially to yield a molecular-weight limit corresponding to the crystalline stems within the crystals.

Thus, in a solid substrate composed of folded-chain lamellar crystals, the fold surfaces are more likely to be the reactive site. Depending on the permeability of the solid substrate and on the solubility of the degraded products, the overall hydrolysis should decrease after the disordered fold surface is removed. An evaluation of the hydrolytic process kinetics of the PHB granules by conductometric titration of their carboxyls should provide information as to where the carboxylic termini are located with respect to the granule morphology.

Also of interest in this study is the resulting short-chain product, which could be used in further chemical syntheses. Because PHB is a biocompatible polyester, oligomeric chains of this polymer could be useful for the synthesis of graft or block polymeric structures with amphiphilic or biodegradable characteristics. The synthesis of poly(β-hydroxyalkanoate) (PHA) conjugates with carbohydrates and other synthetic polymers has been reported[8]. The chemical reaction to form the conjugate by reaction with the terminus (hydroxyl or carboxyl) of a PHB chain was carried out in solution. If the synthesis of drug–polymer conjugates was carried out on solid submicrometre-sized PHB granules rather than on solubilized oligomeric chains, it could lead to the production of a new generation of biocompatible drug delivery systems.

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PHB has been proposed for the production of sutures, orthopaedic devices and drug delivery tablets and for subcutaneous implantation[9,10]. *In vitro* and *in vivo* degradation studies have been reported in the literature[4–6,11–14]. The results showed that at physiological pH values (7.0 to 7.4) high-molecular-weight PHB and poly(β-hydroxybutyrate/valerate) (PHB/V) degrade relatively slowly, and that, to trigger the *in vivo* biodegradation, a pretreatment with 10 Mrad γ irradiation was required[11]. Korsatko *et al.*[13] reported the use of PHB as a drug delivery system and concluded that PHB of molecular weight higher than 10^5 was not desirable for long-term medication dosage.

The results presented in this study are aimed at describing the morphological changes occurring within PHB granules upon acid hydrolysis at 104.5°C and observing the kinetics of such a process. Weight loss, g.p.c., conductometry, d.s.c., WAXD and TEM were used to monitor the changes throughout the hydrolytic process.

EXPERIMENTAL

Samples

Three samples were used to perform the hydrolytic degradation experiments. A spray-dried sample (BXG08) of enzymatically purified PHB homopolymer granules provided by Marlborough Biopolymers (ICI), UK, and a freeze-dried sample (IH11/1HYP) of sodium hypochlorite-purified granules[15,16] provided by Dr B. Ramsay of Ecole Polytechnique de Montreal. A third sample was prepared by annealing a portion of the BXG08 sample (ANBXG08) for 48 h at 120°C.

Hydrolytic degradation

First, 0.5 g of each sample was placed in 100 ml round-bottomed flasks with 50 ml of 3.0 N HCl and refluxed at a temperature of 104.5°C for time periods varying from 1 to 18 h. The hydrolysed samples were then rapidly cooled to room temperature by immersing the flasks in ice–water. The acid suspensions were then successively washed and centrifuged (5000*g*) three times in distilled water and twice in ethanol. The resulting pellets were then dried at 60°C overnight, weighed and kept for later examination.

Molecular-weight determination

All molecular-weight data were obtained using a Spectra Physics g.p.c. system equipped with an SP 4290 Hewlett–Packard integrator and a SP 8430 refractive-index detector with two PlGel 30 cm linear columns and a 500 Å Ultrastyragel 30 cm column obtained respectively from Polymer Laboratories Inc., Amherst, MA, and Millipore (Waters), Toronto, Ont. Chloroform was used as a solvent and eluant at a flow rate of 0.7 ml min^{-1}. The sample concentration was 10 mg cm^{-3}. Monodisperse (narrow-cut) polystyrene (PS) samples of weight-average molecular weights ($\bar{M}_w$) ranging from 1.8×10^6 to 3.25×10^3 purchased from Polymer Laboratories Inc., Amherst, MA, were used as standards. The Mark–Houwink parameters used in the universal calibration method[17] were for PS[18] $a = 0.794$ and $K = 4.9 \times 10^5$ and for PHB[19] $a = 0.78$ and $K = 1.18 \times 10^4$.

X-ray powder diffractograms

A Philips PW 1730 X-ray generator with a Ni filter to provide a Cu K_α radiation ($\lambda = 0.1542$ nm) and equipped with an automatic wide-angle X-ray powder diffractometer was used to obtain diffractograms of all samples. Every scan was recorded in the range of $2\theta = 8$ to 36° at a scan speed of 2° min^{-1}. Determination of the crystallinity level of the samples was done by evaluating the ratio of the peak surface area to the total surface area under the diffractogram, and the scattering profile of the amorphous material was taken into account.

Transmission electron microscopy

All samples were deposited on carbon-coated 200 mesh copper grids and were examined in a Philips EM400T electron microscope equipped with a low-dose unit. The micrographs were recorded using diffraction contrast in the bright-field mode at an accelerating voltage of 120 kV and with an objective aperture of 20 μm.

Thermal analysis

A Perkin–Elmer DSC-7 instrument equipped with an intracooler unit was used to obtain the melting temperature and enthalpy of fusion of the samples before and after hydrolysis. The instrument was calibrated using indium ($T_m = 156.4$°C and $\Delta h_f = 12.43$ J g^{-1}). Samples weighing between 5 and 10 mg were used and each scan was performed in the range 30–190°C at a heating rate of 20°C min^{-1}.

Conductometric titration

Conductometric titration was performed for carboxylic group quantification using a procedure developed by Scallan *et al.*[20]. A 200 mg sample of hydrolysed PHB was dispersed in 100 ml of 1 mN NaCl solution until the obtention of a homogeneous suspension. Before the conductivity of the suspension is measured, 0.5 ml of 0.1 N HCl was added to the suspension. The titration involved an initial addition of 0.5 ml aliquots of 0.01 N NaOH for the neutralization of the HCl initially added. Aliquots of 0.25 ml of 0.01 N NaOH were added during the titration of the carboxylic groups of the sample. After complete neutralization of the latter, aliquots of 0.5 ml of 0.01 N NaOH were added to obtain an alkali excess. A delay of 3 min was allowed between each addition. The titrations were performed under a nitrogen atmosphere with a low stirring rate. An automated system was used to collect the data. It consisted of a conductivity meter (Metrohm 660 sleeveless immersion cell with a built-in temperature probe) and a titration burette (Metrohm 665 Dosimat), all interfaced to an AT-286 personal computer.

RESULTS AND DISCUSSION

The number-average molecular weight ($\bar{M}_n$) and crystallinity level of the PHB homopolymer samples used in this study are reported in *Table 1*. It can be observed

Table 1 Number-average molecular weight, polydispersity index and crystallinity level of bacterial PHB homopolymer granule samples determined by g.p.c.

Sample	$\bar{M}_n$[a]	$\bar{M}_w/\bar{M}_n$[b]	χ[c] (%)
BXG08	174 100	2.5	70
ANBXG08	138 100	2.3	80
IH11/1HYP	268 100	2.0	30

[a] Number-average molecular weight
[b] Polydispersity index
[c] Crystallinity determined by X-ray diffraction

that the sodium hypochlorite-purified sample has a molecular weight higher than that of the other two samples. These results indicate that isolation by sodium hypochlorite digestion under controlled conditions[15,16] is a satisfactory isolation process in comparison with the enzymatic one[21]. However, for environmental reasons, the use of chlorine compounds is not recommended. The granules isolated using sodium hypochlorite show a lower level of crystallinity. *Table 1* also reveals that the annealing treatment performed on sample ANBXG08 resulted in a decrease in $\bar{M}_n$ probably due to thermal degradation[22-24]. It was assumed that predegradation of the sample did not affect the subsequent hydrolytic degradation process.

Hydrolytic degradation

The theory for random degradation[3] of polymers states that the degree of degradation, α_d, of a polymer sample, at a certain stage of the degradation process, is expressed by:

$$\alpha_d = s/(L_0 + 1) \quad (1)$$

where s is the number of broken links per chain and L_0 is the number-average chain length of the polyester before degradation. When L_0 is large, equation (1) reduces to:

$$\alpha_d = s/L_0 \quad (2)$$

The number-average chain length, L_t, at any time t during the degradation process, can be expressed by:

$$L_t = L_0/(s + 1) \quad (3)$$

When the rate of breaking links is independent of both the position of the link in the chain and the chain length, as is the case for hydrolytic degradation (random scission of the polymer chains), the process is a linear function of time that can be expressed by:

$$\alpha_d = k_d t \quad (4)$$

or

$$s = L_0 k_d t \quad (5)$$

where k_d is the hydrolytic degradation constant (h^{-1}) and t is the time of hydrolysis (h). The combination of equations (3) and (5) leads to:

$$1/L_t - 1/L_0 = k_d t \quad (6)$$

which shows that, early in the degradation process, the number-average chain length should decrease linearly as a function of time.

These equations are normally used for samples that are at an early stage of the degradation process and they take into account all the fractions produced during the reaction (from the monomer to the undegraded polymer). In the hydrolytic degradation process described above, the ethanol-soluble oligomers and water-soluble monomer were discarded and were not considered when evaluating the number-average molecular weights from which the kinetics of the reaction were derived. Thus the results described below are not absolute in terms of the random scission model.

Figure 1 shows a linear relationship between $1/L_t - 1/L_0$ and time up to 18 h for sample BXG08. Samples ANBXG08 and IH11/1HYP deviate from linearity after 14 h of hydrolysis. These results suggest that for all samples the degradation proceeds through random scission of the polymer chains up to 14 h of hydrolysis. Since a weight loss was recorded the values of L reported here do not account for the fraction that has been solubilized during the degradation process. The annealing treatment performed on sample ANBXG08 and the dense shell of sample IH11/1HYP due to isolation with sodium hypochlorite may be responsible for the overall slower rate of chain scission. A 'level-off' degree of polymerization seems to have been reached after 14 h for the two latter samples.

Table 2 shows the $\bar{M}_n$ of the samples at various times. A rapid decrease in $\bar{M}_n$ is observed within the first hour of hydrolysis for all samples as well as a decrease in polydispersity for both BXG08 and ANBXG08. Sample IH11/1HYP showed little variation in its polydispersity.

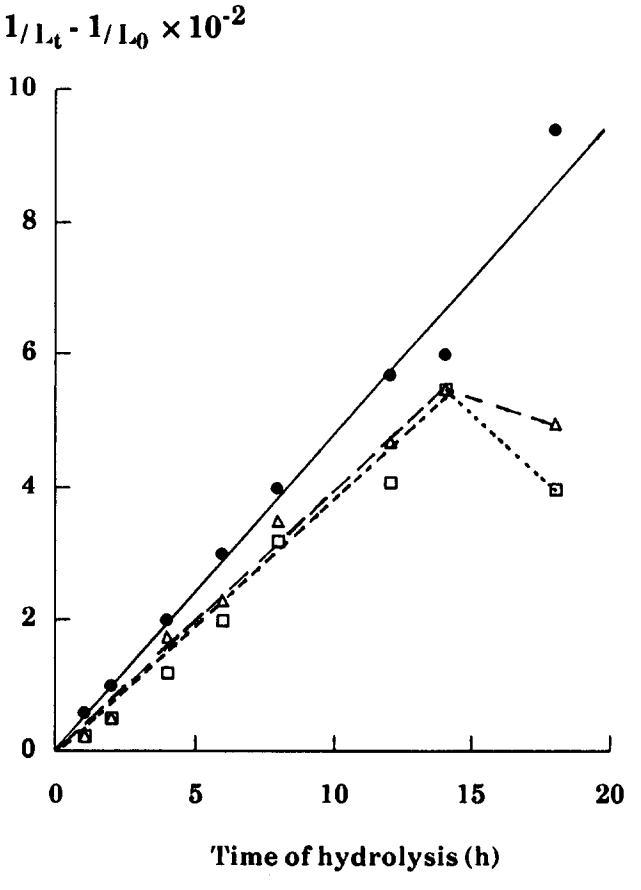

Figure 1 Relationship between $1/L_t - 1/L_0$ and time of hydrolysis: (●) BXG08; (△) ANBXG08; (□) IH11/1HYP. Sample BXG08 shows linearity up to 18 h of hydrolysis while linearity stops after 14 h for samples ANBXG08 and IH11/1HYP

Table 2 Molecular weights of bacterial homopolymer granule samples after hydrolytic degradation in 3.0 N HCl at 104.5°C determined by g.p.c.

Time (h)	BXG08		ANBXG08		IH11/1HYP	
	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
0	174 100	2.5	138 100	2.3	268 100	2.0
1	13 800	2.3	19 800	2.0	24 800	2.0
2	8 500	2.2	11 300	1.8	12 400	2.4
4	4 200	2.2	4 600	2.3	6 600	2.3
6	2 800	2.1	3 400	2.0	4 100	2.0
8	2 200	2.1	2 400	1.8	2 600	2.4
12	1 500	1.4	1 800	1.6	2 100	2.3
14	1 400	1.4	1 600	1.4	1 600	2.8
18	900	2.0	1 700	1.5	2 100	2.1

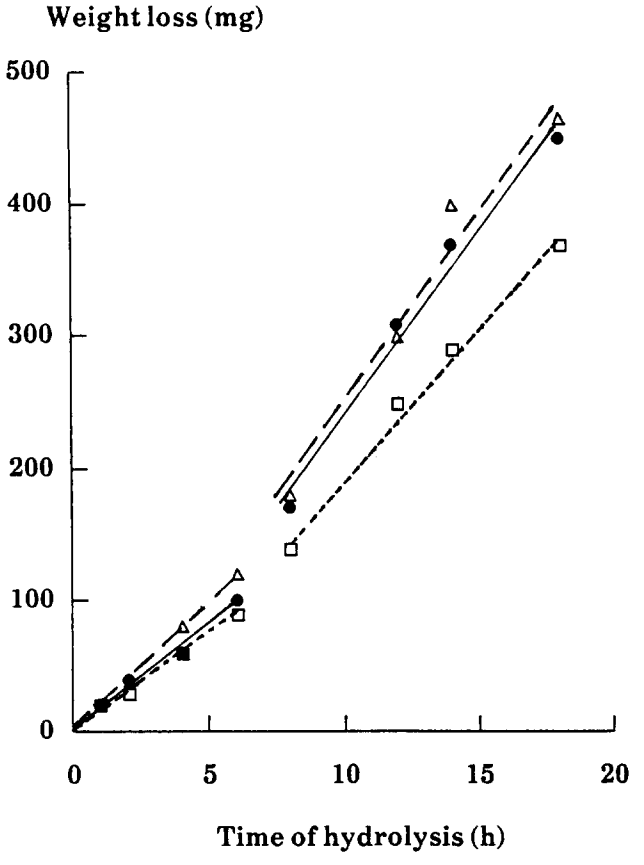

Figure 2 Relationship between weight loss and time of hydrolysis: (●) BXG08; (△) ANBXG08; (□) IH11/1HYP. All samples, initially 500 mg, showed an initial period lasting 6 h after which a second degradation stage took place with an increased degradation rate constant

Table 3 Degradation and weight-loss rate constants for hydrolytic degradation of bacterial PHB homopolymer granule samples in 3.0 N HCl at 104.5°C; the initial weight of all samples was 500 mg

Sample	k_d[a] ($\times 10^{-3}$ h^{-1})	k_I[b] (mg h^{-1})	k_{II}[c] (mg h^{-1})
BXG08	4.86	15.87	28.24
ANBXG08	3.98	19.77	29.55
IH11/1HYP	3.80	15.76	22.73

[a] Degradation rate constant
[b] Weight-loss rate constant induction period
[c] Weight-loss rate constant of the second stage

The decrease in $\bar{M}_n$ is accompanied by a weight loss, as is demonstrated in *Figure 2*, which also shows that all samples went through a two-stage degradation process. Initially, a slow rate of weight loss was observed, which lasted until 20% of the starting material was degraded and solubilized; afterwards, the second stage of the reaction (onset after 6 h of hydrolysis) was triggered. During the second stage of the hydrolytic process the weight-loss rate constants of all three samples increased to nearly double the value derived from the initial period. The $\bar{M}_n$ of all samples after 6 h of degradation ranged from 2800 to 4100, which corresponds to chain segments bearing on average 32 to 48 monomer units. The two different weight-loss rate constants are presented along with the degradation rate constants in *Table 3*.

Morphology of hydrolysed PHB granules

Figures 3a and *3b* show the X-ray diffractograms of the IH11/1HYP sample taken before and after 8 h of degradation. All samples showed a similar increase in crystallinity during the hydrolysis. It suggests that the non-crystalline high-molecular-weight molecules of the core, which were cut into smaller segments during the first 8 h of hydrolysis, rearranged to form crystals growing by intussusception, i.e. from the shell–core interface inwards. The temperature at which the hydrolytic degradation process was performed (104.5°C), nearly 100°C above the T_g of PHB, accelerated the recrystallization process.

These results are in line with the data of the thermal analyses presented in *Table 4*. During the initial period, the melting temperature (T_m) of all samples decreased by 4 to 8°C, which, based on the assumption that a depression in T_m is solely due to the finite crystal thickness, indicates a decrease in the thickness of the crystals formed and which is associated with the decrease

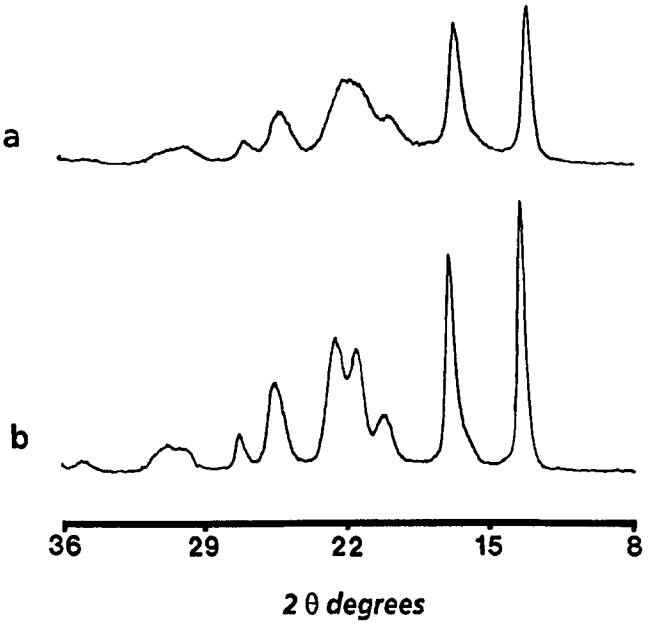

Figure 3 Wide-angle X-ray powder diffractograms of (a) sample IH11/1HYP before hydrolysis and (b) sample IH11/1HYP after 8 h of hydrolysis

Table 4 Melting point[a] and enthalpy of fusion variations[a] of bacterial PHB homopolymer granule samples after hydrolytic degradation in 3.0 N HCl at 104.5°C

Time (h)	BXG08 T_m[b] (°C)	BXG08 Δh_f[c] (J g^{-1})	ANBXG08 T_m[b] (°C)	ANBXG08 Δh_f[c] (J g^{-1})	IH11/1HYP T_m[b] (°C)	IH11/1HYP Δh_f[c] (J g^{-1})
1	167.9	112.4	171.8	126.8	168.8	111.6
2	165.8	120.1	171.8	131.3	165.9	116.0
4	163.5	124.9	169.3	139.9	162.6	120.6
6	162.0	126.6	167.2	133.5	160.5	124.4
8	157.4	125.9	165.0	131.1	159.2	125.2
12	137.6	122.2	159.0	116.4	155.4	123.4
14	130.4	120.1	154.0	124.6	157.2	123.4
18	124.6	109.0	154.3	104.7	156.3	113.1

[a] Derived from d.s.c. thermograms (heating rate of 20°C min^{-1})
[b] Peak melting temperature
[c] Enthalpy of fusion

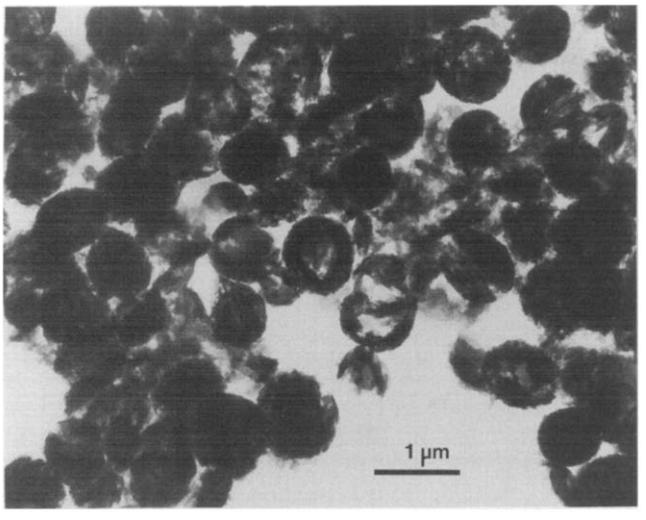

Figure 4 Micrograph of sample BXG08 after 14 h of hydrolysis

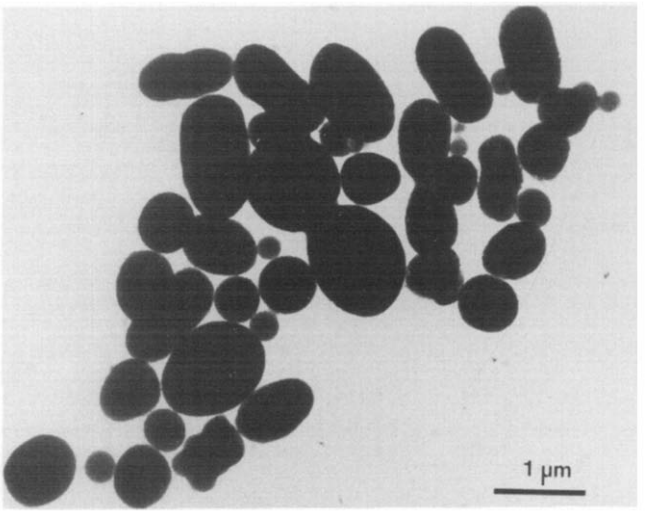

Figure 5 Micrograph of sample IH11/1HYP after 14 h of hydrolysis. As opposed to *Figure 4*, the lamellae on the shells of sample IH11/1HYP cannot be distinguished from one another and seem to be welded together

in $\bar{M}_n$ (ref. 25). Attempts to perform small-angle X-ray diffraction (SAXD) on both ANBXG08 and IH11/1HYP granule samples were unsuccessful due mainly to the high level of scattering caused by the voids left in the granule cores after hydrolysis. Thus the decrease in thickness of the lamellar crystals of the shells could not be confirmed by X-ray analysis.

Associated with the decrease in T_m was an increase in the enthalpy of fusion (Δh_f), which reached a maximum (125–127 J g^{-1}) between 6 and 8 h of hydrolysis for BXG08 and IH11/1HYP samples. Owing to the annealing treatment of sample ANBXG08, Δh_f maximum was reached after only 4 h of hydrolysis and the value of 139 J g^{-1} obtained approached that derived by Barham *et al.*[26] for PHB crystal (146 J g^{-1}) grown from the melt and annealed until no further increase in thickness was detected. The increase in Δh_f implied a higher level of crystallinity and degree of perfection of the crystals formed, which confirms the X-ray data presented in *Figure 3*. For the second stage of the hydrolysis process, both T_m and Δh_f decreased continuously.

Figure 4 is a micrograph of BXG08 taken after 14 h of hydrolysis. Electron diffractograms recorded from fragments of shell residues are typical of PHB single crystals, and the spacings correspond to those of lamellar single crystals grown from dilute solutions[1]. As opposed to the hydrolysed sodium hypochlorite-purified granules shown in *Figure 5*, the overlapping lamellar crystals of the shell residues can easily be distinguished from one another (see *Figure 4*). The lamellae seem to be much wider than the needle-shaped PHB single crystals that are grown from dilute solutions[1]. Identical results were obtained from the ANBXG08 sample. The electron diffraction patterns obtained from sample IH11/1HYP hydrolysed shells are similar to those obtained in the case of BXG08 sample, although they are less well defined owing to overlapping of the lamellae into thicker material.

GENERAL CONSIDERATIONS AND CONCLUSIONS

As reported by Doi *et al.*[4–6], heterogeneous degradation of PHB has two stages. In the first stage, random scission of the ester links reduces the molecular weight and allows a reorganization to a lamellar morphology; weight loss is negligible. In the second stage of the degradation process, an appreciable weight loss of the sample is observed. When strong acid is used to catalyse the reaction, as in this study, considerable reduction in $\bar{M}_n$ is observed during the first stage of the reaction. As a result, a small weight loss, which increases slowly up to 6 h of hydrolysis, is immediately monitored. The rapid decrease in $\bar{M}_n$ is a sign of the instant permeation of the small protonated water molecules through the granule shell. The small weight-loss rate observed during the induction period is thus not due to a permeation problem, as was suggested for the buffered hydrolysis of film samples[4–6], but rather to the competition between the solubilization and recrystallization of the hydrolysed fragments.

By the end of the initial period ~20–25% of the polymer molecules were lost and the crystallinity of the granules increased considerably, reaching a maximum after 6 h of degradation. Preliminary results of hydrolytic degradation of the same three samples at room temperature, using identical acid concentration (HCl, 3.0 N), revealed that the decrease in $\bar{M}_n$ was far less than observed at a temperature of 104.5°C and no significant increase in crystallinity could be detected even after 7 days of hydrolysis. These results indicated that, even under strong acid conditions, high-molecular-weight PHB granules are fairly stable at room temperature. This emphasizes the importance of understanding the intra- and extracellular enzymatic degradation processes that, in a matter of days or even hours (depending on whether the granules have been chemically, thermally or physically modified), can degrade granules of high-molecular-weight PHB molecules down to the monomer units.

The higher weight-loss rate constant (*Table 3*) of sample ANBXG08 during the initial period of the hydrolytic degradation process can be explained in terms of contact surface between the acidic solution and the PHB material. It is known that, upon annealing, a contraction of the core molecules towards the crystalline shell gives rise to the formation of microvoids inside the

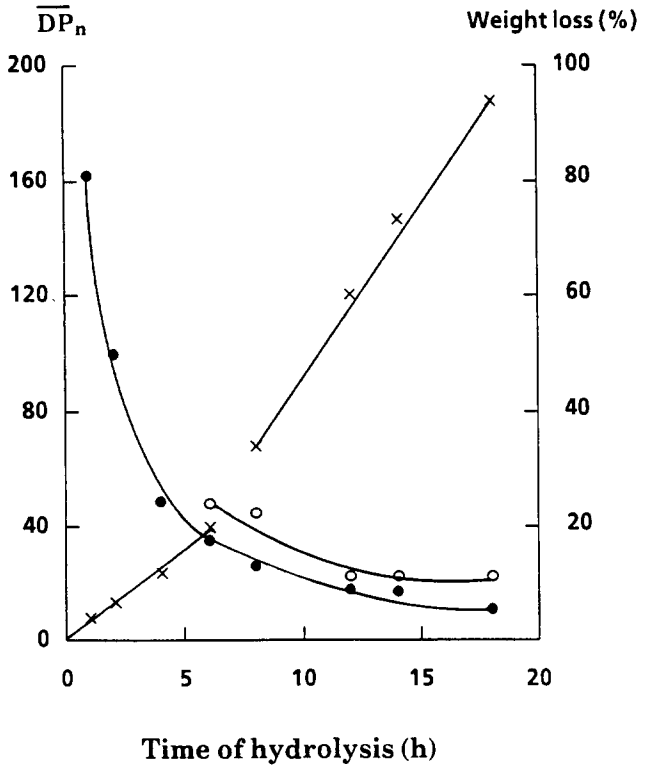

Figure 6 Number-average degree of polymerization of BXG08 PHB samples as determined by g.p.c. (●) and conductometry (○) and percentage of weight loss (×) as a function of hydrolysis time

granules[2]. Such a change in the morphology of the granules allows more of the acidic solution, once it has penetrated the shell, to come into contact with the core molecules, resulting in a higher weight loss.

The reason for the difference in morphology between the crystals present at the PHB granule surface and single crystals grown from dilute solutions is not clear at the moment. One hypothesis could be that, during their growth at the surface of the PHB granules, single crystals seeded by distinct heterogeneous nuclei impinge on each other, which hinders their lateral growth, leading to more or less square-shaped folded-chain lamellae.

The difference observed in the morphology of the hydrolysed shells of sample IH11/1HYP and those of samples BXG08 and ANBXG08 is not well understood. The shells of sample IH11/1HYP seem to be made of strongly adhering lamellae, which form a thick block (see *Figure 5*). The dense shell is probably responsible for the slower weight-loss rate constant during the second stage of the hydrolytic process as was observed in our experiments (see *Table 3*).

The linear relationship between weight loss and time, for both stages of the reaction, suggests that the reaction is of zero order at least up until 80% weight loss. Thus, the crystalline as well as the non-crystalline domains are degraded indiscriminately, as was previously reported[4–6]. This implies that the carboxylic termini are probably distributed randomly at the surface as well as within the lamellar crystals of the granule's shell. Although some of these groups may be inaccessible for chemical reaction, it is expected that many of them are located at the crystal surfaces. Conductometric titrations of the carboxylic termini was used to establish the number of groups accessible to alkaline titration. The results are shown in *Figure 6*, where the average degrees of polymerization ($\overline{DP}$) found by the conductometric titration method and by g.p.c. are plotted against the time of hydrolysis for sample BXG08. The weight loss of BXG08 is plotted for reference. The difference between the two $\overline{DP}$ curves allows the conclusion that approximately 70–75% of the carboxyl termini are accessible. Since the recrystallization effect due to annealing was completed in the early stages of hydrolysis, it is proposed that carboxyls may have been rendered inaccessible during the annealing process, where a possible interdigitation of the chains of adjacent lamellae may have taken place, entrapping them inside the crystalline phase. The conclusion is dependent on the precision and comparability of the two different methods for measuring $\overline{DP}$. The Appendix shows the equations used to evaluate the $\overline{DP}$ from the conductometric titration.

Comparison of the thermal analysis results presented in *Table 4* with those reported by Marchessault *et al.*[25] for oligomers of PHB show that the same trend is followed (decrease in T_m with decrease in $\overline{DP}$), although the T_m they measured were better defined due to the fractionation they performed on their samples to obtain narrow-cut oligomer aliquots.

In conclusion, it was established that the kinetics based on the residue of acid-catalysed hydrolysis of high-molecular-weight PHB molecules contained in granules follow a random scission degradation model (see *Figure 2*). The degradation process is a two-stage reaction. In the first stage (initial period) the weight loss for all three samples was moderate (rate constant $k_I \approx 16$–$20\,mg\,h^{-1}$) while the crystallinity, as shown by the WAXD and d.s.c. data, increased, reaching a maximum after 6 h of hydrolysis. In the second stage of the hydrolytic process, the weight-loss rate constant increased to nearly twice the value for the initial period ($k_{II} \approx 22$–$30\,mg\,h^{-1}$). The combined effects of the hydrolytic degradation, which randomly reduces the PHB residue, and the unique morphology of the isolated PHA granules could in the future lead to practical application where PHB–drug conjugate particles would be used in delivery systems. This oligomer, produced with about 5% weight loss, is pertinent to the production of amphiphilic molecules as described by Yalpani *et al.*[8], since the molecules have hydroxyl and carboxyl termini.

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APPENDIX

The $\overline{DP}$ of the hydrolysed BXG08 samples was determined using the equation:

$$\overline{DP} = n/C \tag{A1}$$

Here n is the number of moles of repeat units present in the sample and C is the number of carboxylic groups present in the sample (number of polymer chains), which in turn is determined through:

$$C = [\text{COOH}] \times w/1000 \tag{A2}$$

where w is the weight of the sample in grams. [COOH] is the concentration of carboxylic groups obtained by conductometry (expressed in meq/kg) as expressed by:

$$[\text{COOH}] = 1000 \times N_{\text{NaOH}}(x-y)/w \tag{A3}$$

where N_{NaOH} is the normality of the titrant, x is the volume of 0.01 N NaOH used to neutralize the acid (ml) and y is the volume of base to neutralize the initial 0.1 N HCl added.

Finally, the proportion of accessible carboxylic groups of the substrate (r) is determined using:

$$r = 100 \times \overline{DP}_{\text{gpc}}/\overline{DP}_{\text{cond}} \tag{A4}$$

where $\overline{DP}_{\text{gpc}}$ is the number-average degree of polymerization of the sample as determined by gel permeation chromatography and $\overline{DP}_{\text{cond}}$ is the number-average degree of polymerization of the sample as determined by conductometry.