ELSEVIER

PII: S0032-3861(97)10172-0

Polymer Vol. 39 No. 15, pp. 3387–3392, 1998
© 1998 Published by Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0032-3861/98/$19.00+0.00

Hydrolytic degradability of poly(3-hydroxyoctanoate) and of a poly(3-hydroxyoctanoate)/poly(*R,S*-lactic acid) blend

D. Mallardé[a], M. Valière[a], C. David[a], M. Menet[a] and Ph. Guérin[b,*]
[a]*ESCOM, 13 Boulevard de l'Hautil, 95092 Cergy Pontoise, France*
[b]*Laboratoire de Physico-chimie des Biopolymères, UMR CNRS 27, Université Paris XII, 2 rue Henry Dunant, 94320 Thiais, France*
(Revised 29 September 1997)

Films of bacterial poly(3-hydroxyoctanoate) (PHO) ($M_n = 85\,000$) pure or blended with racemic poly[(*R,S*)-lactic acid] (PLA50) of low molecular weight ($M_n = 1100$) were prepared by solvent casting techniques and incubated in buffer solutions at 37°C and at different pH. Changes in weight, molecular weight and morphology were recorded during hydrolysis experiments. The PHO hydrolytic degradation process was very slow: after 5 months of degradation, only 0.1% of ester functions were hydrolyzed. In the case of a PHO/PLA50 blend (80/20, w/w), after 1 month, no more PLA50 was present in the film and PHO molecular weight decrease was significantly more important than in the case of pure PHO degradation; the hydrolytic scission of PHO chains was enhanced by the presence of PLA50. Differential scanning calorimetry achieved on PHO/PLA50-blended samples displayed a lack of complete miscibility between both components. In the early stage of ageing, an increase of crystallinity for the different materials was observed in all cases. © 1998 Published by Elsevier Science Ltd. All rights reserved.

(Keywords: Poly(3-hydroxyoctanoate); Poly[(*R,S*)-lactic acid]; Hydrolytic degradation)

INTRODUCTION

The bacterial production of many types of poly(3-hydroxyalkanoates) (PHAs), as intracellular inclusion bodies in a wide variety of microorganisms, and poly[(*R*)-3-hydroxybutyric acid] (PHB), a crystalline optically active polyester[1–3], are under study. The major interest of the microbial polyesters family lies in the use of these materials for environmental and biomedical applications, such as biocompatible and (bio)degradable thermoplastics or elastomeric polymers. Two degradation processes can be involved according to the environment and the polymer: simple hydrolysis (non-enzymatic) or/and enzymatic action of extracellular depolymerases (biodegradation)[4]. The degradability of bacterial polyesters depends on the chemical structure of the PHA and consequently on its morphology, and on the living systems type used in the case of biodegradation [5,6].

A strict control of the degradation kinetics is the driving force for the increasing use of biodegradable polymers in medicine and in the environment. Extensive efforts have been devoted to the study of the effect of copolymerization and polymer blending on the physical and chemical properties of the final products. The emergent class of multicomponent systems has been explored in the field of biodegradable polymers to tailor-made materials with desired features[7–9]. Blending of PHB or PHBV (random copolymers containing 3-hydroxybutyrate and 3-hydroxyvalerate as repeating units) with other polymers has been the subject of several studies to provide potential methods for improving the processing characteristics, for the goal of enlarging the range of applicability of these biopolymers[10–12]. Blends can produce either homogeneous or phase-separated morphologies[13]. Properties such as miscibility, crystallization, melting and biodegradability of PHB blended with optically active or racemic poly[(*S*) or (*R,S*)-lactic acid] (P[(*S*) or (*R,S*)-LA]) have been investigated[13]. Concerning biodegradation, it has been shown that the rate of enzymatic surface erosion decreased with increasing P[(*R,S*)-LA] content in the blend film, and the hydrolytic scission of PHB polymer chains was accelerated by blending. However, the rate of enzymatic hydrolysis was much faster than the rate of non-enzymatic hydrolysis[14]. Binary blends of PHB ($M_n >$ 200 000)/P[(*S*) or (*R,S*)-LA] were miscible over the whole composition range when polylactic acid had a low molecular weight ($M_n \sim 2000$), while blends with high molecular weight P[(*S*)-LA] ($M_n = 160\,000$) displayed biphasic separation[15].

Poly(3-hydroxyoctanoate) (PHO), produced from bacteria *Pseudomonas oleovorans* by fermentation with sodium octanoate as the carbon source, is a thermoplastic elastomer of approximately 30% crystallinity by weight[16,17]. This polymer exhibits elastomer behaviour at room temperature, with the crystalline regions acting as physical crosslinks, and displays elastic properties, while PHB is a rigid brittle plastic. The biodegradation of PHO is not evident compared to PHB or PHBV and only a few specific extracellular depolymerase systems are active. They are extracted from *Pseudomonas fluorescens*[18] and *Pseudomonas maculicola*[19]. It is therefore necessary to consider a simple hydrolysis instead of biodegradation for environmental applications and in the case of biomedical or pharmaceutical uses.

In this paper we report the hydrolytic degradation of PHO, pure or blended with poly[(*R,S*)-lactic acid]

* To whom correspondence should be addressed

oligomers, related to temporary applications using this fascinating polymer.

EXPERIMENTAL

Materials

Poly(3-hydroxyoctanoate) used in this study was obtained by fermentation of *Pseudomonas oleovorans* as follows.

Pseudomonas oleovorans was obtained from the American Type Culture Collection (ATCC 29347) and was maintained at − 80°C in a nutritional medium. Before fermentation experiments, the strain was grown on nutrient agar plates using medium E with 10 mM octanoate sodium salt as carbon source. Medium E is a solution of distilled water containing 1.1 g l^{-1} of $(NH_4)_2PO_4$, 5.8 g l^{-1} of K_2HPO_4, 3.7 g l^{-1} of KH_2PO_4, 10 ml l^{-1} of 0.1 M $MgSO_4$ solution and 1 ml l^{-1} of a microelement solution (a 1 N HCl solution containing 2.78 g l^{-1} of $FeSO_4{\cdot}7H_2O$, 1.98 g l^{-1} of $MnCl_2{\cdot}4H_2O$, 2.8 g l^{-1} of $CoSO_4{\cdot}7H_2O$, 1.67 g l^{-1} of $CaCl_2{\cdot}2H_2O$, 0.17 g l^{-1} of $CuCl_2{\cdot}2H_2O$ and 0.29 g l^{-1} of $ZnSO_4{\cdot}7H_2O$). For the preculture, 200 ml of medium E and 0.332 g (10 mM) of sodium octanoate as the carbon source were prepared. The pH was adjusted to 7.1 and the flask was autoclaved at 121°C for 20 min. After the solution was cooled down to 31°C, bacteria were inoculated from the plate by a sterile procedure and the flask was continuously shaken under aerobic conditions at 200 rpm.

When the optical density of the preculture at 660 nm reached a value of 2, 12 l of medium E from a 15-l fermentor, prepared under the same conditions as the preculture, were inoculated with 200 ml of the preculture. Samples were periodically withdrawn from the fermentor and the optical density was recorded. During growth, additional sodium octanoate solution (12.9 g in 100 ml distilled water) was added to the fermentor until the optical density reached $2n$ (n = 1, 2,...) and then early stationary phase.

After reaching the early stationary growth phase, the cells were harvested by centrifugation, and the biomass was frozen and lyophilized (117 g). Intracellular PHO was extracted from the lyophilized cells by refluxing in 1.5 l chloroform. After a reflux period of 3 days, the solution was filtered and the solvent was removed by evaporation until a remaining residue of concentrated polymer solution of 80–100 ml was obtained. The polymer (30.5 g) was precipitated into 800 ml of vigorously stirred methanol, collected by filtration and dried under vacuum for 48 h at 40°C.

The PLA50 was supplied by Phusis Company (France). Molecular weights were measured by size exclusion chromatography (s.e.c.).

Preparation of samples

PHO and PHO/PLA50 samples were dissolved in chloroform. The polymer films were prepared by conventional solvent-casting techniques from the chloroform solutions of polymers (100 mg ml^{-1}) using glass plates as casting surfaces. The films (initial weights, 85 mg; initial film dimensions, 2.2 × 7.2 mm and 0.07–0.09 mm thick) were aged for 3 weeks at ambient temperature, in order to let them reach a stabilized crystallinity before the experiments.

Blend films were composed of 20% PLA50 and 80% PHO (w/w). When more than 20% PLA was added, the films were no longer visually homogeneous.

Hydrolysis experiments

Samples were placed in bottles containing aqueous buffer solutions at 37°C. Three pH buffer solutions were used: pH 4, pH 7 and pH 10. The pH 4 buffer solution contained 0.056 M citric acid, 0.068 M sodium hydroxide and 0.044 M sodium chloride. The chemical composition of the pH 7 buffer solution was 0.029 M sodium hydroxide and 0.050 M potassium dihydrogen phosphate. The pH 10 buffer solution contained 0.013 M sodium tetraborate and 0.018 M sodium hydroxide. All three buffer solutions were supplied by Fluka.

Pure PHO sample degradation was carried out at pH 10. Blended samples of PHO/PLA50 were degraded at pH 4, pH 7 and pH 10.

Quantitative analysis of polymers

Methanolysis of polymers. For determination of the composition of PHO and PHO/PLA50-blended films, approximately 4 mg of sample were mixed with 1 ml chloroform in a small screw-capped test tube. The solution was shaken vigorously and put under ultrasound for 10 min. Then, 0.85 ml of 2% (v/v) sulfuric acid (a very low concentration of acid to avoid methoxylation of β-hydroxy acid methyl esters) in methanol was added, and the solution was heated for 6 h at 98°C. After cooling the samples, 1 ml of demineralized water was added and tubes were shaken vigorously for 20 s. The chloroform layer was collected and analyzed by GC. Tetradecane was used as internal standard.

Synthesis of standards. 3-Hydroxyhexanoic acid methyl ester, 3-hydroxyoctanoic acid methyl ester, 3-hydroxydecanoic acid methyl ester and 3-hydroxydodecanoic acid methyl ester were synthesized through Reformatsky reaction[20–22] from 2-bromoethanoic acid methyl ester and appropriate aldehyde. A mixture of toluene/ether (30/10, v/v) was used as solvent.

3-Hydroxyhexanoic acid methyl ester: n.m.r. (270 MHz, $CDCl_3$, TMS), ^1H: δ = 0.96 ppm (t; J = 6.5 Hz; 3H, H-6), 1.38–1.50 (m; 2H, H-5), 1.56–1.63 (m; 2H, H-4), 2.43–2.65 (m; 2H, H-2), 3.75 (s; 3H, CO_2CH_3), 4.07–4.16 (m; 1H, H-3), 4.36 (s; broad; 1H, OH), ^{13}C: δ = 13.12 ppm (C-6), 17.99 (C-5), 37.79 (C-4), 40.45 (C-2), 51.38 (CO_2CH_3), 68.14 (C-3), 172.92 (C-1); GC (t_R = 9.5 min).

3-Hydroxyoctanoic acid methyl ester: n.m.r. (270 MHz, $CDCl_3$, TMS), ^1H: δ = 0.89 ppm (t; J = 6.5 Hz; 3H, H-8), 1.22–1.38 (m; 6H, H-5 to H-7), 1.54–1.60 (m; 2H, H-4), 2.44–2.64 (m; 2H, H-2), 3.74 (s; 3H, CO_2CH_3), 3.97 (s; broad; 1H, OH), 4.04–4.13 (m; 1H, H-3), ^{13}C: δ = 13.41 ppm (C-8), 22.01 (C-7), 24.44 (C-6), 30.94 (C-5), 36.07 (C-4), 41.04 (C-2), 51.05 (CO_2CH_3), 68.09 (C-3), 172.52 (C-1); GC (t_R = 14.0 min).

3-Hydroxydecanoic acid methyl ester: n.m.r. (270 MHz, $CDCl_3$, TMS), ^1H: δ = 0.88 ppm (t; J = 6.5 Hz; 3H, H-10), 1.15–1.39 (m; 10H, H-5 to H-9), 1.49–1.55 (m; 2H, H-4), 2.37–2.57 (m; 2H, H-2), 3.07 (s; broad; 1H, OH), 3.72 (s; 3H, CO_2CH_3), 3.87–4.07 (m; 1H, H-3), ^{13}C: δ = 13.41 ppm (C-10), 22.07 (C-9), 24.98 (C-8), 28.74 (C-7), 28.97 (C-6), 31.29 (C-5), 36.16 (C-4), 41.01 (C-2), 50.95 (CO_2CH_3), 67.49 (C-3), 172.51 (C-1); GC (t_R = 17.7 min).

3-Hydroxydodecanoic acid methyl ester: n.m.r. (270 MHz, $CDCl_3$, TMS), ^1H: δ = 0.81 ppm (t; J = 6.5 Hz; 3H, H-12), 1.11–1.32 (m; 14H, H-5 to H-11), 1.49–1.57 (m; 2H, H-4), 2.44–2.67 (m; 2H, H-2), 3.71 (s; 3H, CO_2CH_3), 3.98–4.13 (m; 1H, H-3), 5.00 (s; broad; 1H, OH),

^{13}C: δ = 13.62 ppm (C-12), 22.27 (C-10 to C-11), 25.14 (C-9), 28.95 (C-8), 29.20 (C-6 to C-7)), 31.50 (C-5), 36.30 (C-4), 41.08 (C-2), 51.12 (CO_2CH_3), 67.58 (C-3), 172.78 (C-1); GC (t_R = 21.2 min).

3-Hydroxybutanoic acid methyl ester was supplied by Lancaster and 2-hydroxypropanoic acid methyl ester (corresponding to methanolized PLA) was supplied by Interchim.

Gas chromatography analysis (GC)

The chloroform solutions of methanolyzed polymers were analyzed using a Varian Model 4200 Gas Chromatograph equipped with a 15-m × 0.53-mm Ohio Valley column, film thickness 1 μm, and with a flame ionisation detector. The oven temperature was held for 2 min at 45°C, then increased by 5°C/min to 60°C, held for 1 min at 60°C, increased by 5°C/min to 150°C and held at 150°C for 2 min, and eventually increased by 30°C/min to 230°C and held at 230°C for 3 min. The carrier gas was nitrogen, the injector temperature was 250°C. A 1-μl portion of the chloroform was injected. Under these conditions, the retention times of the different β-hydroxyalkanoic acid methyl esters were as follows (min): C-4, 5.6; C-6, 9.5; C-8, 14.0; C-10, 17.7; C-12, 21.2. The retention time of 2-hydroxypropanoic acid methyl ester was 2.6 min.

Gravimetric study

Mass loss was evaluated by weighing the specimens. It was deduced from the following relationship:

$$\text{mass loss } (\%) = (W_0 - W_t)/W_0 \quad (1)$$

where W_0 and W_t were the initial weight and the weight at time t, respectively. W_t was obtained after drying the samples at 60°C under vacuum for 1 week.

Molecular weight measurements

Molecular weights were determined by s.e.c. with a Waters apparatus (510 pump), using a set of five columns (Waters: 10^4 Å, 10^3 Å, HR2, HR1, HR0.5). Sample concentration was 6 mg/ml. The mobile phase was tetrahydrofuran, with an eluent flow rate of 1.1 ml/min. A refractive index detector (Waters 410) was used for detection. For calibration, polystyrene standard solutions were injected.

Thermal analysis

Thermal analysis was carried out using a Perkin-Elmer differential scanning calorimeter (DSC 7). DSC investigations were achieved in two steps. For the first run, the samples were scanned at a rate of 10°C/min; the second run was performed at the same rate just after a quenching of the samples. The values reported concerning the change of the crystallinity of the samples were calculated from the following relationship:

$$\frac{\Delta H(t) - \Delta H(0)}{\Delta H(0)} = \frac{X(t) - X(0)}{X(0)} \quad (2)$$

where $\Delta H(0)$ and $\Delta H(t)$ correspond, respectively, to the melting enthalpy values at time 0 and t, and $X(0)$ and $X(t)$ are the crystallinity degrees at time 0 and t, respectively.

N.m.r. spectra

^1H and ^{13}C n.m.r. spectra were obtained on chloroform-d solutions at 20°C on a Jeol JNM EX-270 n.m.r. spectrometer at 270 MHz. The ^1H n.m.r. spectra were referenced to TMS and the ^{13}C n.m.r. spectra to chloroform.

Fourier transform infrared spectroscopy

Infrared spectra were detected using a Nicolet Fourier transform infrared spectrophotometer (Impact 400), collecting 42 scans for each sample. The resolution was 4 cm^{-1}.

Analysis of buffer extraction products

After degradation, buffer solutions were extracted with ethyl acetate. The extracts were evaporated under vacuum, and then analyzed by s.e.c. and infrared spectroscopy.

RESULTS AND DISCUSSION

Materials

The determination of the composition of PHA repeating units is very important in regard to degradation studies, considering that PHA composition closely depends on the growth substrate used and on the bacterial fermentation conditions. Lenz and co-workers have already characterized, using GC analysis of methanolyzed PHO samples, two different types of PHO whether an additional carbon source was added or not into the fermentor in the course of the fermentation[23]. In order to identify and to quantify the composition of the different repeating units of PHO samples, standards have been synthesized according to Reformatky's reaction and used in the analysis of methanolyzed PHO. *Table 1* displays the composition of the PHO sample: 3-hydroxyoctanoate units represent the main proportion (82%) of the five different side chains; 3-hydroxyhexanoate units (14.6%) are also present in the macromolecular chain, whereas only a small proportion of 3-hydroxydodecanoate (2.0%), 3-hydroxydecanoate (1.2%) and 3-hydroxybutanoate sequences (0.2%) are present. This result confirms that *P. oleovorans* produces polyesters with variable compositions according to the experimental conditions. It will be possible to modulate PHO properties by changing the polymer structure.

PHO and PHO/PLA50 blend properties are presented in *Table 2*. Results from s.e.c. and d.s.c. measurements

Table 1 Composition of PHO units (mol%)

Material	HB	HH	HO	HD	HDD
PHO	0.2	14.6	82.0	1.2	2.0

HB, 3-hydroxybutyrate; HH, 3-hydroxyhexanoate; HO, 3-hydroxyoctanoate; HD, 3-hydroxydecanoate; HDD, 3-hydroxydodecanoate

Table 2 Properties of the materials under study

Material	M_n	I_p	T_g (°C)	T_m (°C)	ΔH_m (J/g)
PHO	85000	1.5	−39	52	22
PLA50	1100	1.3	−12		
Blend PHO + 20%PLA50 (w/w)	80000/1000		−39/−10	49	16

M_n, number average molecular weight determined by s.e.c. (THF, PS standards); I_p, polydispersity index determined by s.e.c.; T_g, glass temperature determined by d.s.c.; T_m, melting temperature determined by d.s.c.; ΔH_m, melting enthalpy determined by d.s.c.

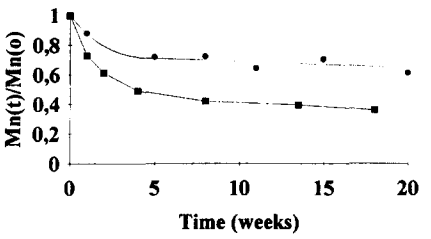

Figure 1 Evolution of the ratio of number average molecular weight $M_n(t)$ to initial $M_n(0)$, $(M_n(t)/M_n(0))$, in the course of hydrolytic degradation of PHO films at pH 10 (●) and PHO/PLA50 films at pH 10 (■)

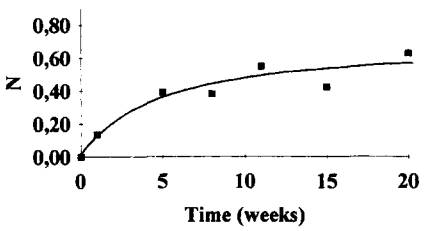

Figure 2 Evolution of the average number of ester bond cleavages per original molecule of PHO with time degradation for PHO films at pH 10 (■)

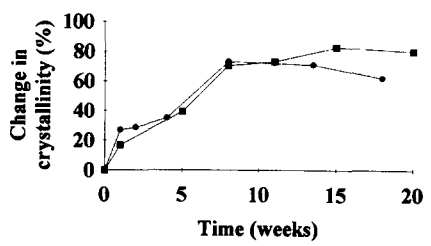

Figure 3 Evolution of crystallinity rate change with degradation at pH 10 for pure PHO (●) and PHO/PLA50 (■) samples

showing the initial PHO/PLA50 blend samples revealed two separated peaks (M_n = 80 000 and M_n = 1000), two glass transition temperatures (−39 and −10°C) corresponding, respectively, to PHO and PLA, and a melting transition temperature at 49°C relative to PHO. These data show that blend components are not miscible.

Degradation of pure PHO at pH 10

s.e.c. analyses were performed both on phosphate buffer extraction products and on pure PHO films, before and during degradation. PHO chromatograms remained monomodal along all the degradation. *Figure 1* displays the curve $M_n(t)/M_n(0)$ as a function of time; this curve presents two different stages of evolution: in the first period, an important M_n decrease occurred, corresponding to ester bonds cleavages in the material.

After 8 weeks, the rate of decrease of M_n also decreased, as shown by the slight change in molecular weight values. After 20 weeks of hydrolysis, only 0.8 ester scissions occurred per mole of PHO, which was representative of a very low degradation. The average number (N) of ester bond cleavages per original macromolecule of PHO was calculated using Eq. (3)[24]:

$$N = \frac{\overline{M}_n(0)}{\overline{M}_n(t)} - 1. \quad (3)$$

Figure 2 represents the evolution of N values as a function of time. According to the literature[25], if the chain scission is a random phenomenon, the value of N is anticipated to be a linear function of time. In the case of PHO, N was not a linear function of time, which means that ester cleavages occurred at preferential domains in the macromolecular chains. The presence of pendant alkyl groups with different sizes, and therefore variable hydrophobic effects, must conduct to some water-accessible ester groups with different hydrolysis reactivities due to their chemical environment. Kumagai and Doi[26], in the case of PHB, have shown that the degradation rate depends not only on the chemical structure of the monomer units, but also on the sequence distribution in the polymer chain, and consequently on the polymer morphology.

S.e.c. analysis of phosphate buffer extraction products showed the release of hydrosoluble oligomers in water after 8 weeks of degradation. Infrared spectra of these oligomers were obtained, and they were similar to initial PHO spectra but displayed significant acid absorption bands at 1600 and 3300 cm^{-1}. This result is consistent with the hydrolysis process of ester functions in PHO in the course of degradation. Gravimetric experiments showed that the mass loss of PHO samples induced by degradation was negligible (mass loss < 1% after 8 weeks).

D.s.c. investigations were achieved on PHO samples before and during degradation. A broadening of the melting transition area was observed. In the first stage of hydrolysis (from initial time to 8 weeks), the crystallinity rate increased, whereas during the second step of degradation it became stabilized (*Figure 3*).

All these phenomena can be explained by a new organization of the PHO molecular segment chains induced by a preferential degradation in the amorphous phase. This phenomenon occurring in the amorphous phase during non-enzymatic degradation was described for lactic acid and hydroxyalkanoate polymers[27,28].

Degradation of PHO/PLA50 blended samples: comparison with the degradation of pure PHO

D.s.c. experiments showed dramatic changes in the morphology of blended samples in the course of hydrolysis. It is important to note that no exothermal crystallisation peak was detected during the first heating, and that the glass transition temperature and melting temperature remained constant. *Figure 3* displays the curve representing the change of crystallinity of blended samples at pH 10. As in the case of pure PHO specimens, d.s.c. data showed that the PHO crystallinity rate in PHO/PLA50 samples increased by around 70% in the first stage of degradation (from initial time to 8 weeks). Then, the crystallinity rate became stabilized. In this case, also, the significant change in the morphology can be due to perturbations of the chain scission rates and to a new organisation of PHO macromolecules.

Figure 1 shows plots representing $M_n(0)/M_n(t)$ *versus* degradation time obtained in the case of pure PHO and the PHO component in the PHO/PLA50 blend after immersion in a buffer solution at pH 10 and 37°C. It is obvious that in the first 5 weeks of degradation, PHO M_n decrease was much more important in blended samples than in unblended samples. On the other hand, along all of the second stage of degradation (from 5 to 18 weeks), the PHO M_n decrease rate was identical in blended specimens and in unblended specimens: the rate constants corresponding to the first and the second stages of degradation were calculated from the slopes of the two parts of the curves $M_n(t)/M_n(0) = f(t)$, their

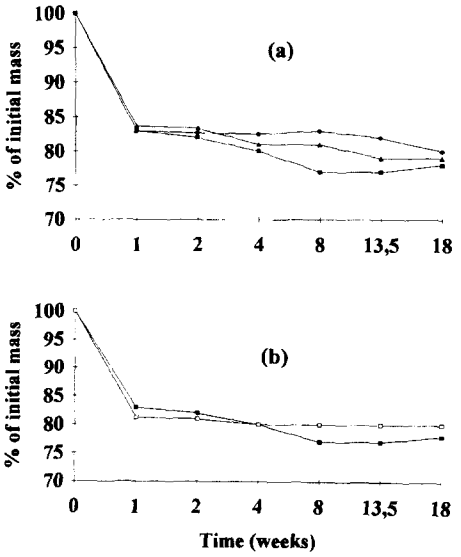

Figure 4 (a) Evolution of mass loss as a percentage of initial mass of the blended films, for samples immersed in buffer solutions at pH 10 (■), pH 7 (▲), pH 4 (●) and at 37°C. (b) Comparison at pH 10 of mass loss as a percentage of initial mass of the film from gravimetric data (■), and of PLA from GC measurements (□)

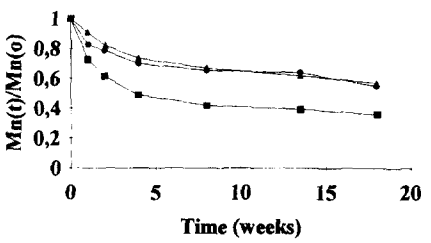

Figure 5 Evolution of the ratio of number average molecular weight $M_n(t)$ to initial $M_n(0)$, $(M_n(t)/M_n(0))$, in the course of hydrolytic degradation of PHO/PLA50 films at pH 10 (■), pH 7 (▲) and pH 4 (●)

values were (week^{-1}): −0.1 and −6 for pure PHO, respectively, in the first and second stages of degradation, and −0.3 and −6 for blended samples. These data suggest that PLA50 speeded up PHO hydrolysis only during the first stage of degradation.

Figure 4a presents the evolution of mass loss as a percentage of initial mass of the film in the course of degradation, for samples immersed in buffer solutions at pH 4, pH 7 and pH 10. The comparison between the three gravimetric curves is shown; the mass loss was more important when samples were immersed in a buffer solution at pH 10.

Figure 5 represents the curves $M_n(t)/M_n(0)$ in the course of hydrolytic degradation of blended samples at the three different pH values. The decrease of molecular weight was much more important at pH 10 than at other pH values. The average number, N, of ester bond cleavages per original macromolecule of PHO component in PHO/PLA50 blend were calculated using Eq. (3). After 18 weeks of hydrolysis, N reached 1.8 at pH 10 against 0.8 at pH 4 and pH 7. The speed up of hydrolysis in alkaline media, which is well known for polylactic acid, was already mentioned by Knowles and Hastings in the case of a PHB/PHV copolymer[29].

In the three experiments (blended specimens degradation at pH 4, pH 7 and pH 10), samples lost more than 15% of their initial weight after only 1 week of degradation (*Figure 4a*). Then, the mass loss decreased more slowly, and seemed to become stabilized after 5 weeks around a value of 20%, without any disintegration of samples. The value of 20% mass loss after 5 weeks of degradation was consistent with the initial mass of the PLA50 component in the PHO/PLA50 blend, and suggested that the mass loss in the blend was probably mainly involved in the release of PLA50 degradation products. This phenomenon was described by Kumagai and Doi[26] in the course of the degradation of binary blends of poly(3-hydroxybutyrate) and poly[(*R,S*)-lactic acid]: the mass loss of the blend poly(3-hydroxybutyrate)/poly[(*R,S*)-lactic acid] (75/25, w/w) increased 25% in 11 weeks and the 50/50 (w/w) blend lost 50% of initial mass in the same time. In both cases, mass loss corresponded to the initial proportion of poly[(*R,S*)-lactic acid] in the blend. In order to confirm this hypothesis in the case of the PHO/PLA50 blend, GC analyses were performed on methanolyzed blended samples along all the degradation. The results shown in *Figure 4b* present the comparison of the PHO/PLA mass loss (gravimetric data) at pH 10 and of the PHO/PLA mass loss based on residual PLA in the blend at pH 10, measured by GC. Both curves were equivalent. These results confirmed that the entire mass loss of the film is due to PLA extraction. The extraction of PLA50 oligomers was also confirmed by s.e.c. and d.s.c.: after 1 week of degradation, the PLA50 peak disappeared from the blend chromatogram, and PLA50 glass transition was no longer present on the blend thermogram.

All the experiments previously described (gravimetry, GC, s.e.c., d.s.c.) tend to prove that as long as PLA50 was present in blended samples, it speeded up PHO hydrolysis. This assumption is consistent with the fact that the second stage rate constant of PHO M_n decrease (k_2) was nearly the same in the case of degradation of both blended and unblended samples. As PLA50 dispersed, PHO in the blended samples degraded at the same rate as pure PHO. These experimental results show that, despite the lack of miscibility of the PHO/PLA50 blend, the introduction of PLA50 into PHO speeded up the hydrolysis of the PHO. Local strong interactions between both components could be present at the polymer interface, and they could lead to a specific acid catalysis by lactic acid oligomer carboxyl acid end groups on close PHO ester groups during PLA oligomer hydrolysis. Such a result was shown by Kumagai and Doi[26] in the case of PHB/P[(*R,S*)-LA] nonenzymatic degradation.

CONCLUSIONS

Poly(3-hydroxyoctanoate), due to the presence of highly hydrophobic alkyl pendant groups, is very resistant to hydrolysis. By combination with poly[(*R,S*)-lactic acid] oligomer, which is a very well-known hydrolyzable polymer, it is possible to enhance the degradation of the bacterial polyester during the period when poly(lactic acid) is present in the polymeric material. Further experiments are under way in order to investigate this synergic effect and to test different types of PHO/polyester blends.

ACKNOWLEDGEMENTS

We thank Phusis Company for providing PLA50 oligomers.

REFERENCES

1. Lemoigne, M., *Ann. Inst. Pasteur (Paris)*, 1927, **41**, 148.
2. Braunegg, G. and Lefebvre, G., *Kem. Ind.*, 1993, **42**, 313.
3. Steinbuchel, A. and Valentin, H.E., *FEMS Microbiol. Lett.*, 1995, **128**, 219.

4. Göpferich, A., *Biomaterials*, 1996, **17**, 103.
5. Horking, Ph., Marchessault, R., Timmins, M., Lenz, K. W. and Fuller, R. C., *Macromolecules*, 1996, **29**, 2472.
6. Shimamura, E. and Kasuya, K., *Macromolecules*, 1994, **27**, 878.
7. Gajria, A., Duné, V., Gross, R. and McCarthy, S., *Polymer*, 1996, **37**, 437.
8. Dawes, M. C., Shakesheff, K. M., Shard, A. G., Domb, A., Roberts, C. J., Tendler, S. J. B., Roberts, C. J., Tendler, S. J. B. and Williams, P.M., *Macromolecules*, 1996, **29**, 2205.
9. Dubini Plagia, E., Beltrame, P. L., Canetti, M., Senes, A., Marcandalli, B. and Martuscelli, E., *Polymer*, 1993, **34**, 996.
10. Satoh, H., Yoshie, N. and Inoue, Y., *Polymer*, 1994, **35**, 286.
11. Koyama, N. and Doi, Y., *Polymer*, 1997, **38**, 1589.
12. Barhan, P. J. and Organ, S. J., *J. Mater. Res.*, 1994, **29**, 1676.
13. Pearce, R., Jesudason, J., Orts, W. and Marchessault, R.H., *Polymer*, 1992, **33**, 4647.
14. Koyama, N. and Doi, Y., *Can. J. Microbiol.*, 1995, **41**(Suppl. 1), 316.
15. Koyama, N. and Doi, Y., *Polymer*, 1997, **38**, 1589.
16. Gagnon, K. D., Lenz, R. W., Farris, R. J. and Fuller, R. C., *Macromolecules*, 1992, **25**, 3723.
17. Preusting, H., Nijenhuis, A. and Witholt, B., *Macromolecules*, 1990, **23**, 4220.
18. Schirmer, A., Jendrossek, D. and Schlegel, H.G., *Appl. Environ. Microbiol.*, 1993, **4**, 1220.
19. Foster, L. J. R., Zervas, S. J., Lenz, R. W. and Fuller, R. W., *Biodegradation*, 1995, **6**, 67.
20. Cornforth, D. A., Opara, A. E. and Read, G., *J. Chem. Soc. C*, 1969, 2799.
21. Opara, A. E. and Read, G., *J. Chem. Soc. Chem. Commun.*, 1969, 679.
22. Adam, W., Beaza, J. and Liu, J., *J. Am. Chem. Soc.*, 1972, **94**, 2000.
23. Gross, R. A., De Mello, C., Lenz, R. W., Brandl, H. and Fuller, R. C., *Macromolecules*, 1989, **22**, 1106.
24. Doi, Y., Kanesawa, Y., Kuniota, M. and Saito, T., *Macromolecules*, 1990, **23**, 26.
25. Yoon, J. S., Chin, I. J., Kim, M. N. and Kim, C. K., *Macromolecules*, 1996, **29**, 3303.
26. Kumagai, Y. and Doi, Y., *Makromol. Chem. Rapid Commun.*, 1992, **13**, 179.
27. Vert, M., Li, S., Garreau, H., Mauduit, J., Boustta, M., Schwach, G., Engel, R. and Coudane, J., *Angew. Makromol. Chem.*, 1997, **247**, 239.
28. Kanesawa, Y. and Doi, Y., *Makromol. Chem. Rapid. Commun.*, 1990, **11**, 679.
29. Knowles, J. C. and Hastings, G. W., *J. Mater. Sci.*, 1992, **3**, 352.