Structural Changes of Polylactic-Acid (PLA) Microspheres under Hydrolytic Degradation

M. F. GONZALEZ, R. A. RUSECKAITE, T. R. CUADRADO

Institute of Materials Science and Technology (INTEMA), National University of Mar del Plata—National Research Council (CONICET), J. B. Justo 4302 (7600) Mar del Plata, Argentina

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ABSTRACT: Low molecular-weight polylactic acid (PLA) was obtained by direct polycondensation of a mixture of 95% L and 5% D-lactic acid isomers, without catalyst, at 195°C. This polymer was used for the synthesis of microspheres by emulsion–solvent evaporation method. Gel Permeation Chromatography (GPC), X-ray Scattering (XRD), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM) techniques were applied to follow morphological and structural changes of particles along *in vitro* degradation at 37°C. The original microspheres were amorphous but could crystallize partially upon heating. Samples stored in a humid environment exhibited an increase in the crystallization capability upon heating. Initial smooth-surface microspheres were transformed to porous particles at the time of degradation at pH = 7 (37°C). The shape of mass loss vs. time curve supports the presence of a heterogeneous bulk degradation process. After hydrolytic degradation the residual particles showed a molecular weight decrease and a crystallinity increase. After 90 days the crystallinity attained a value of 53%. The X-ray diffraction spectrum indicated the formation of a crystalline oligomeric structure. Crystallization of low molecular weight species will not enable the desired PLA absorption in drug delivery systems. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 1223–1230, 1999

Key words: poly (lactic acid); microsphere; degradation; hydrolysis

INTRODUCTION

Bioresorbable aliphatic polyesters derived from lactic acid and glycolic acid are being used clinically as sutures,[1] bone fixation devices,[2] and sustained release drug delivery systems.[3,4] Poly(α-hydroxyacid)s degrade through hydrolysis of the ester groups autocatalyzed by the carboxylic acid end groups, but the mechanism of their degradation in aqueous media is still a matter of discussion in the literature,[5–9]

Although it was demonstrated that esterase-type enzymes accelerate hydrolysis degradation rate,[10] their effect in crystalline poly(lactic acid)s becomes neglected, so, *in vitro* degradation studies without enzymes are significant for practical applications.

It has been demonstrated that the hydrolytic degradation of amorphous poly(D,L lactic acid) devices synthesized from lactide racemic mixture is a heterogeneous process that proceeds at a faster rate at the core than at the surface due to a catalytic effect of degradation products.[11] The diffusion coefficients of the soluble oligomers formed in the degradation process depend on factors like molar mass and degree of matrix swelling.[12]

In controlled release delivery systems based on liberation of drugs from microspheres (i.e., calcitonin for osteoporosis treatment,[13] piroxicam as antiinflammatory drug,[14] growth hormone to improve tissue repair[15]) another factor that influ-

Correspondence to: T. R. Cuadrado.

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ences the degradability of the particle is the surface characteristics. Boury et al.[16] discussed the effect of emulsifiers and fabrication process on microspheres surface properties.

Bergsma et al.[17] reported that small fragments of high crystallinity (M_n = 5500) derived from *in vivo* degradation of high molecular weight poly(L-lactic acid) (bone plates and screws), were found internalized by cells after 3.3 to 5.7 years of implantation. Clinical reports associated patients painless swelling at the site of implantation to this remainder poly(L-lactic acid) material.

When racemic poly(lactic acid) is hydrolyticaly degraded it has been demonstrated that the degradation induces the formation of a crystalline residue associated to oligomeric stereocomplexes.[18–20] This are crystals produced by the selective association between optically active polymers. In the case of poly(lactic acid)s, the stereocomplex crystal is composed of a racemic lattice in which D-polymer and L-polymer are packed side by side in the ratio 1:1.

With the goal of formulation bioabsorbable bone fillers composites loaded with antibiotic we started the study of *in vitro* degradation of PLA particles. Crystallinity, glass transition temperature (T_g), and weight loss pattern are important factors to be considered in the design of macroporous supports.

In this article, we attempt to focus on structural changes produced by degradation of low molecular-weight PLA microspheres synthesized by polycondensation of an initial nonequimolar ratio of lactic acid isomers.

EXPERIMENTAL

Materials

Lactic acid solution (87% w/w), with a 95% of L- and 5% of D-lactic acid isomers, was purchased from Mallinkrodt.

The content of D- and L-isomers was determined by using a polarimeter to measure the optical rotation of the polymer in solution, $[\alpha]_D^{20}$ and using the Fukuzaki[10] calibration curve for similar polymers.

Polylactic acid (PLA) was synthesized by direct polycondensation without a catalyst following the Fukuzaki et al. procedures.[10,21] The lactic acid solution was charged in a glass ampoule, and nitrogen flow was bubbled into the solution from the bottom at a rate of 200 mL/min. Then the ampoule was immersed in an oil bath at a 195°C for 9 h. The polymer thus obtained was used without further purification. Number-average molecular weight (M_n) measured by end-group titration against KOH in benzyl alcohol was 1980. The PLA obtained was amorphous, but it was able to develop partial crystallinity upon heating. The value of crystallization heat measured by DSC was 22.7 J/g.

Microspheres Preparation

Microspheres were prepared by the emulsion–solvent evaporation method.[22] PLA (2.5 g) was dissolved in methylene chloride (50 mL) at room temperature. This polymer solution was then emulsified with 100 mL of PVA aqueous solution (0.2%), and the immiscible phases were mixed at 0°C for 15 min by stirring at 500 rpm. After emulsification, the organic solvent present in droplets was removed by evaporation at room temperature under stirring. The microspheres were washed with distilled water, filtered, and vacuum dried during a week.

In Vitro Degradation

Microspheres (100 mg) were placed in polypropylene test tubes with 10 mL of buffer solution (Titrisol, pH = 7, Merck reagent). Degradation was carried out in an oven at 37°C. After selected degradation times, two specimens were taken out, washed with distilled water, centrifuged, and vacuum dried at room temperature to constant weight.

Analysis Methods

The optical rotation $[\alpha]_D^{20}$ was determined by using a polarimeter Carl Zeiss Jena with D line of sodium (λ = 589.30 μm) at 20°C. The polymer was dissolved in chloroform (1% wt solution).

The percentage of sample weight loss, $W_t\%$, after a degradation time, t, was calculated from:

$$\Delta Wt\% = \frac{100*(W_0 - W_t)}{W_0}$$

where W_0 is the initial sample weight, and W_t is the weight of dried residual microspheres.

Gel permeation chromatograms (GPC) were obtained using a Waters Model 510 apparatus equipped with a differential refraction index detector Waters 410. Samples were dissolved in THF and eluted at 30°C through Ultrastyragel columns of 100 Å, 500 Å, and 10^4 Å.

Thermal analysis (T_g, melting and crystallization heats, relaxation phenomena) was carried out by using a Differential Scanning Calorimeter (DSC) Shimadzu DSC-50 at a heating rate of 10°C/min^{-1} from room temperature to 160°C. The T_g was taken as the onset value of the transition. X-ray diffraction spectra (XRD) were obtained using a Rigaku diffractometer equipped with a CuKα (λ = 1.5405 Å) source and a Ni filter. The degree of crystallinity, X_C %, was evaluated from:

$$X_c\% = \frac{100^*(I_T - I_A)}{I_T}$$

where, I_A is the intensity of the amorphous phase (area under the curve), and I_T is the intensity of the whole sample measured from the X-ray diffraction pattern.

Scanning electron microscopy (SEM) pictures were obtained with a JEOL-LSM-35CF microscope after gold coating.

RESULTS AND DISCUSSION

Analysis of Storage Conditions Effect on the Material

To analyze the influence of aging time and storage sample conditions, several DSC capsules containing ca. 6 mg of as-polymerized PLA material were heated in an oven up to 150°C, kept at this temperature for 20 min, and rapidly quenched to 0°C. Half of these samples were stored in a dissicator, and the others in a 80% relative humidity (RH) environment, both sets, at 5°C. Figures 1 and 2 show DSC thermograms of the samples after different aging times. GPC analysis demonstrated that no changes, either molecular weight or molecular weight distribution, were produced during this process (Fig. 3).

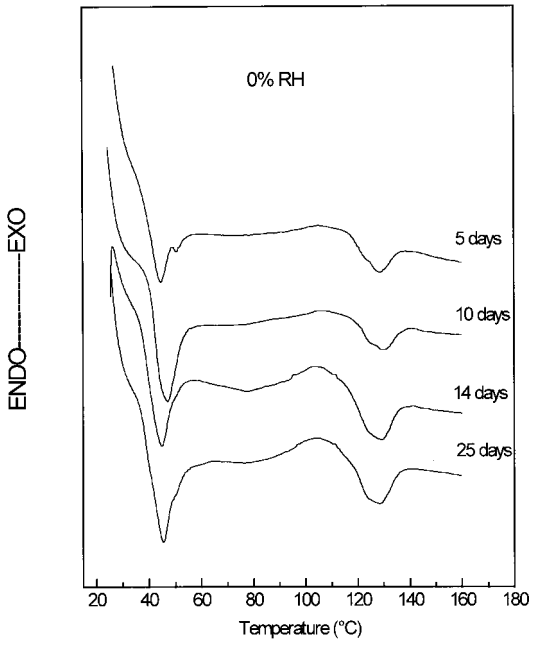

Figure 1. DSC thermograms of as-polymerized PLA at different aging times in dry air conditions (0% relative humidity).

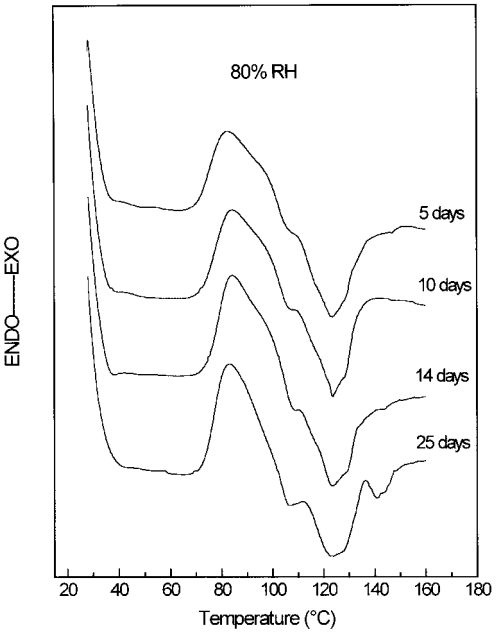

Figure 2. DSC thermograms of as-polymerized PLA at different aging times in 80% relative humidity air conditions.

Samples stored in dry atmosphere exhibited a well-defined glass transition temperature (T_g) value with an associated relaxation enthalpy that increased with aging time. Only a small fraction of the initial amorphous PLA crystallized upon heating (X_c = 3%), showing a lower crystallization temperature (T_c) value while aging time increased.

When the material was kept in humid air it developed more crystallinity upon heating. The fraction of crystalline material increased from 17% (5 days) to 23% (25 days). This is attributed to matrix plastification produced by the presence of water. For these samples T_g could not be detected in the performed DSC thermograms because T_g shifted to the range below room temperature.

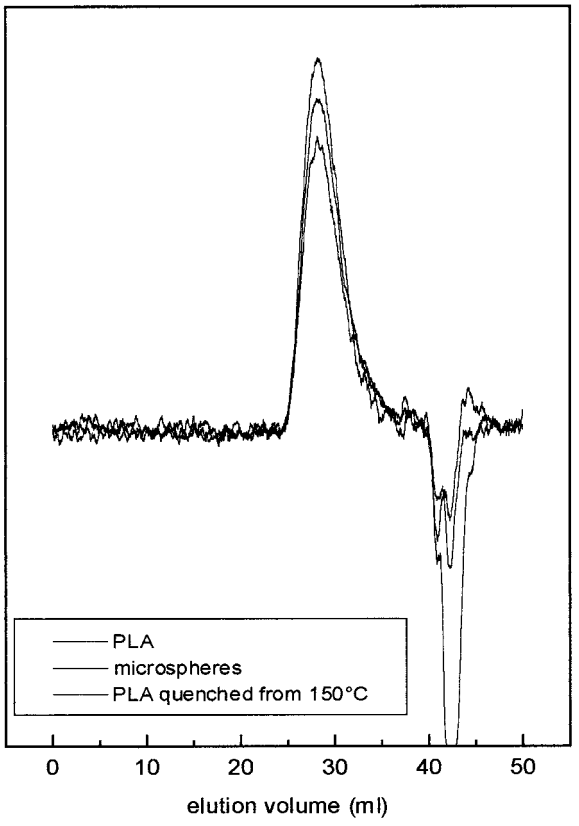

Figure 3. GPC Chromathograms of PLA as polymerized, PLA microspheres, and PLA heated to 150°C and immediately quenched.

Original Microspheres

DSC thermograms of original microspheres are shown in Figure 4. The endothermic peak associated to the T_g is due to the enthalpy relaxation originated from the previous aging.[23] Microspheres crystallized and melted upon heating. The amorphous nature of the original material is revealed by the fact that the crystallization exotherm and the melting endotherm have identical heat contents (same area), as well as by the XRD pattern. By assuming a value of 93 J/g for heat of fusion of 100% crystalline polymer,[24] the crystallinity developed upon heating was about 24%. Along the second DSC scan our polymer showed an increase of T_c and a decrease of crystallization capability (smaller crystallization and melting areas), which could not be associated to any change of molecular weight according to performed GPC measurements. There were not any molecular weight change of the material during our microspheres preparation process (see Fig. 3). T. G. Park[25] reported a molecular weight decrease of the microspheres of PDLA (racemic polymer) for a synthesis method where sonication treatment was applied.

Microspheres Degradation

SEM examination (Fig. 5) revealed that original microspheres exhibited smooth surfaces. After 9 days of *in vitro* degradation the microspheres were still spherical. However, surfaces showed small cracks. After 143 days, large pores were generated inside the microspheres.

Figure 6 shows the pattern of the weight loss evolution. The weight loss curve displayed a fast initial increase, followed by a much slower rise, attaining a 20% level at 90 days. The initial weight loss is attributed to the release of soluble acidic residues from the matrix.

Decrease in weight loss rate can be explain in terms of loss of amorphous phase, which was incorporated along degradation into crystalline domains. This was verified by performing DSC (Fig. 7) and XRD (Fig. 8). Simultaneously, a progressive molecular weight reduction was observed by GPC (Fig. 9).

Along degradation, the crystallization DSC peak decreased while the melting peak increased. This behavior attributing mainly to what could be called "degradation-induced crystallization" was previous reported by Migliaresi et al. [26] DSC thermograms for degraded microspheres are shown in Figure 7. T_g was determined in a second scan

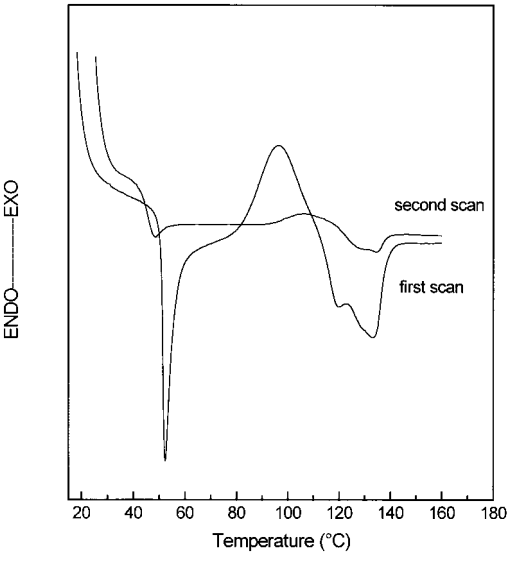

Figure 4. DSC thermograms of original microspheres. A second scan was performed after quenching at the end of the first scan.

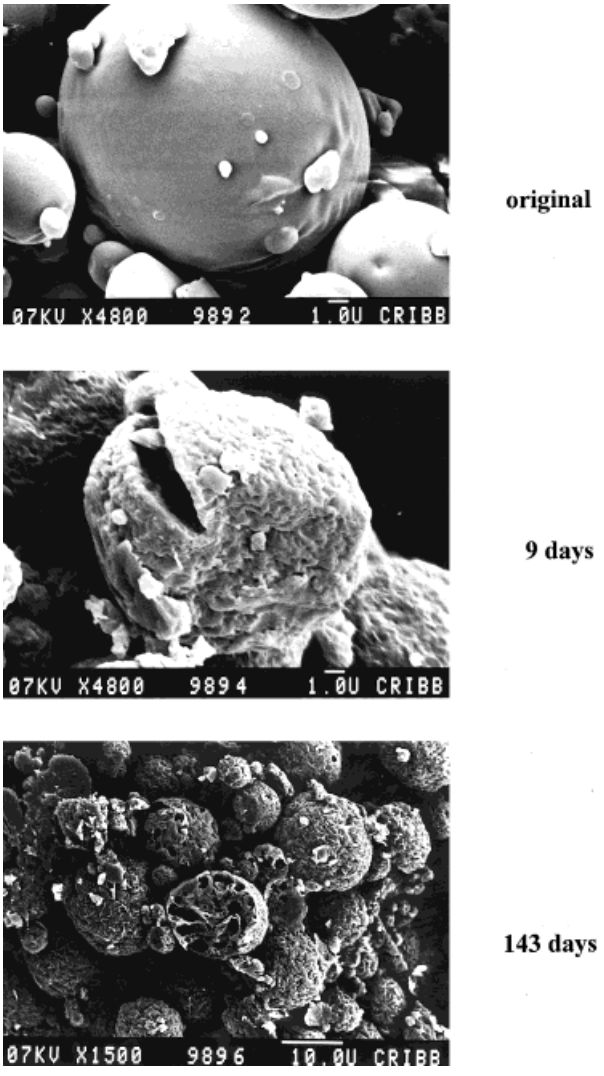

Figure 5. Microspheres SEM images at different degradation times.

after elimination of volatiles. T_g values are represented against degradation times in Figure 10.

We observed that the value of T_g increased during the first degradation period when it was measured in the second DSC scan. The GPC techniques employed to follow the degradation showed evidence of a M_w increase at the analyzed times as results reported by Park[25] for PDLAs (racemic polymer) of various initial molecular weights.

Similar experiences using water as the degradation medium were performed to PLA microspheres. It could be seen that in water there was no increase in T_g values during the DSC second scan as a result of the fast catalytic degradation process (Fig. 11). From this comparative experiment we consider that two phenomena, the lower

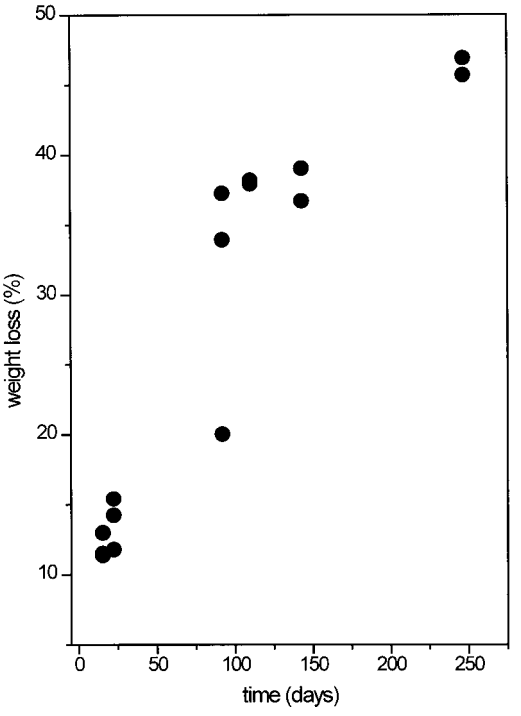

Figure 6. Percentage weight loss vs. degradation times for PLA microspheres kept at pH = 7 and 37°C.

hydrolytic rate plus the lost of soluble very low molecular weight molecules, produced the observed T_g value increase.

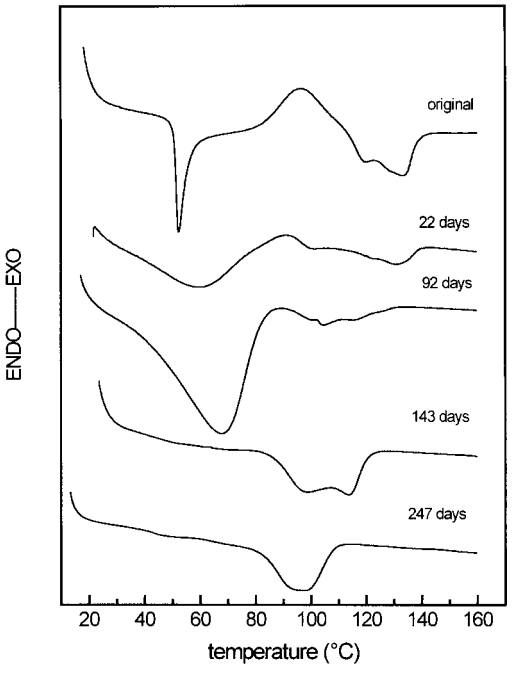

Figure 7. DSC thermograms of PLA microspheres kept at pH = 7 and 37°C for different degradation times.

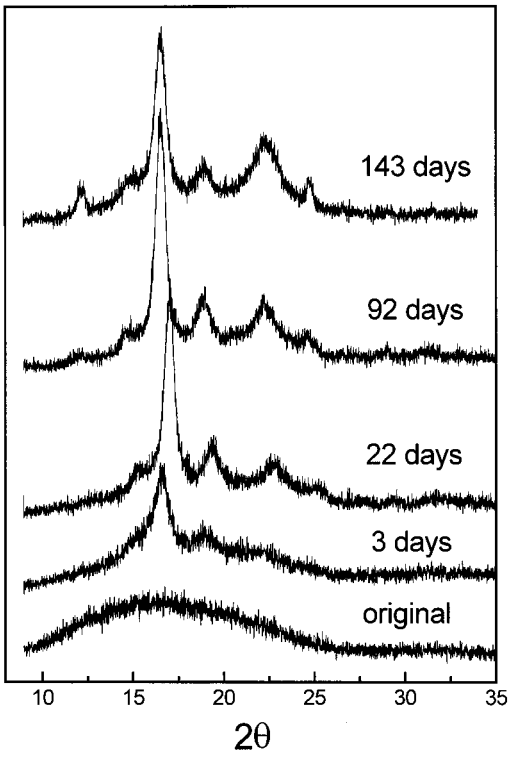

Figure 8. X-ray diffraction patterns of PLA microspheres at different degradation times.

The appearance of crystallinity could be detected by XRD after the first day of aging. The initial X-ray diffraction pattern of a well-defined homocrystal spectrum changed upon degradation, showing the presence of two others peaks that could be attributed to oligomeric stereocomplex crystal formation according to previous published studies.[18–20,27]

After 70 days the percentage of crystallinity (Fig. 12) determined from XRD spectra showed in Figure 8, reached a constant value of 53%, which remained constant along 8 months.

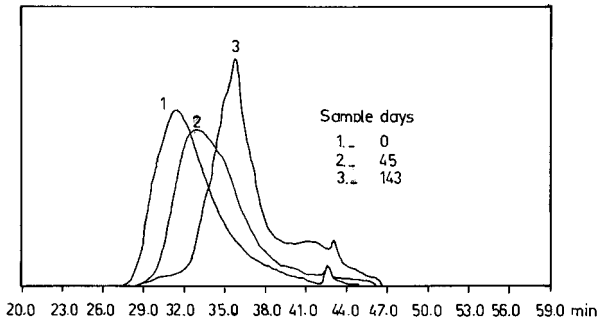

Figure 9. GPC chomathograms of PLA microspheres kept at pH = 7 and 37°C for different degradation times.

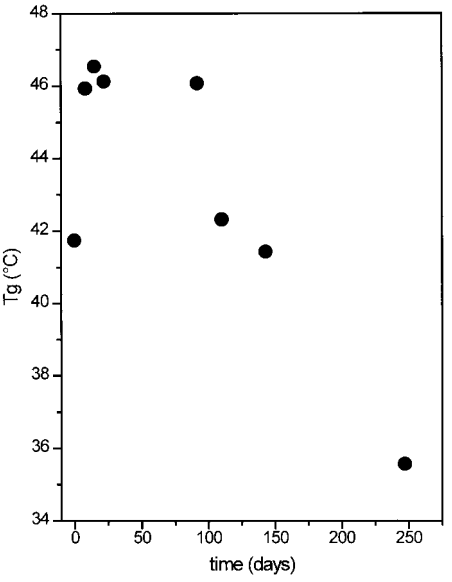

Figure 10. Glass transition temperature of PLA microspheres measured during a second DSC scan after as a function of degradation time.

After 3 months, an increase in the rate of weight loss is observed. At this time the specimens exhibited channels that allowed degradation products to leave the bulk polymer matrix. Migliaresi et al.[26] reported for amorphous P(L)LA (initial M_w = 103,000) degradation, immersed in a Ringer solution, a similar level of degree of crystallinity developed at the time of reaching the molecular weight range of our material.

When the mass loss reached 35%, the rate of degradation slowed down. From this time on the T_g value decreased continuously, and the broad DSC melting peak shifted to lower temperatures, owing to the molecular weight decrease. For

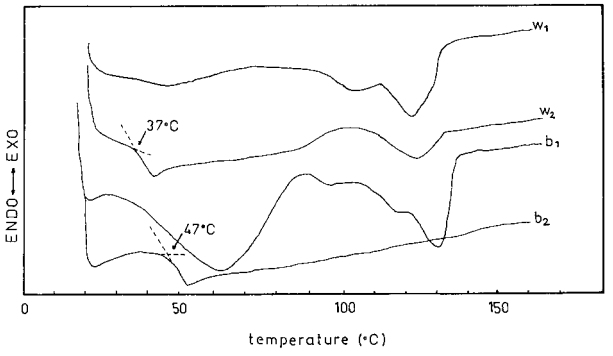

Figure 11. DSC thermograms of microspheres degraded in distilled water (w) and in buffer medium, pH=7 (b) at 37°C. First DSC scan (w_2 and b_2) and second scan after quenching (w_2' and b_2').

PLLA polymers having a M_w lower than 3000, Jamshidi et al.[28] reported broad DSC peaks in the same temperature range. They were attributed to an unstable crystallized region.

The decrease in the rate of mass loss can be explained in terms of the two-phase microstructure of PLA particles. Cleavage of the main chains must start at ester bonds present in the amorphous phase and proceed progressively until the amorphous phase is practically exhausted. After that, the hydrolysis rate may decrease strongly because further degradation can occur only in the crystalline phase.

After 5 months, samples became partially insoluble in THF, the residual particles exhibited bimodal GPC chromatograms, typical of heterogeneous degradation of amorphous devices, and the SEM picture revealed internal microsphere structures with plenty of voids. During this period, the microspheres, however, maintained their spherical shape in the incubation medium. A critical thickness value reported by Grizzi et al.[11] for heterogeneous degradation of amorphous PLA obtained by ring opening polymerization of D,L-lactide was in the range of 200–300 μm. However, Park[29] recently reported heterogeneous bulk degradation for poly(D,L-lactic acid -*co*-glycolic acid) microspheres of 10 μm average diameter.

After 8 months SEM pictures revealed some hollow microspheres fragmentation (Fig. 13).

CONCLUSIONS

The recrystallization heat of PLA original microspheres quenched from the melt is a function of aging time and storage conditions. In a high-humidity environment the aged material showed higher recrystallization capability, progressively increasing with aging time. Samples stored in a dry atmosphere exhibited a constant low recrystallization capability and a growing relaxation enthalpy with time.

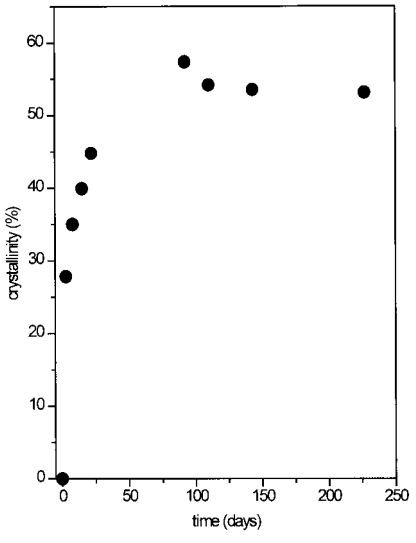

Figure 12. Degree of crystallinity of PLA microspheres versus degradation time.

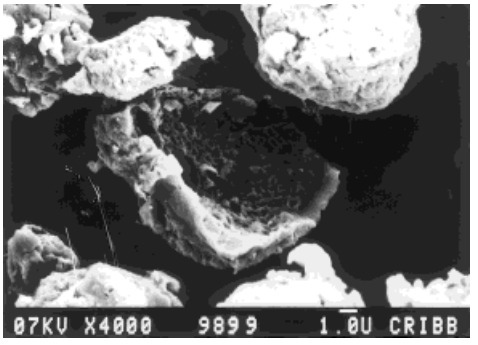

Figure 13. SEM micrograph of a microsphere fragment after 8 months of degradation.

The low molecular-weight amorphous microspheres of 20-μm mean diameter synthesized from a nonequimolar ratio of isomers D- and L-lactic acid by direct polycondensation were able to follow an heterogeneous hydrolytic degradation with the formation of crystalline oligomeric structure.

The medium selected for *in vitro* degradation studies, which influences the evolution of molecules population, is an important factor to take into account at the time of result analyses.

Our results and previous reports already mentioned[11,29] suggested that the critical thickness above which the hydrolytic degradation become heterogeneous should be established in the function of polymer chemistry and morphology, device preparation methods, shape, and dimension.

Degradation patterns of these particles indicate that a drug could be released in a burst after a constant release for 90 days.

The importance of the brittleness and the heterogeneous microspheres structure as well as the delayed bioabsorption of residual particles, which developed higher crystallinity, should be evaluated for any specific biological applications.

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