Hydrolytic Degradation of Glycolide/L-Lactide/ε-Caprolactone Terpolymers Initiated by Zirconium(IV) Acetylacetonate

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ABSTRACT: A series of terpolymers obtained from glycolide/L-lactide/ε-caprolactone were compression molded and allowed to degrade in a pH 7.4 phosphate buffer at 37°C. Analytical techniques such as ^{1}H-NMR, DSC, ESEM and SEC were used to monitor the degradation. Heterogeneous degradation was observed due to internal autocatalysis of carboxylic endgroups formed by ester bond cleavage. Nevertheless, the copolymers present various degradation behaviors depending on the initial composition. The PCL component appears the most resistant to degradation, and the PGA, the most degradable according to compositional changes. Terpolymers could crystallize during degradation even though they were amorphous initially. Degradation-induced increase of crystallinity was also found during hydrolysis. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 2451–2456, 2007

Key words: bioresorbable; biomaterials; crystallization; polyesters

INTRODUCTION

Bioresorbable aliphatic polyesters such as polyglycolide (PGA), polylactide (PLA), and poly(ε-caprolactone) (PCL) are widely investigated for temporary therapeutic applications such as suture materials, bone fracture internal fixation devices, drug delivery systems (DDS), and tissue engineering scaffolds.[1,2] These polymers present outstanding biocompatibility and versatility regarding physical, chemical, and biological properties. Besides, their degradation products can be easily resorbed or excreted by human body.

PGA is the first synthetic polymer used as bioresorbable suture material, although lack of solvent limited its applications in the biomedical field. Homopolymers from L-lactide and ε-caprolactone have been successfully used as osteosynthesis devices and drug delivery substrates, but their degradation proceeds very slowly because of high crystallinity and high hydrophobicity. In contrast, degradation behaviors of copolymers derived from glycolide, lactide, and ε-caprolactone can be tailored by changing the composition, molecular weight, and distribution of monomer units.[3–8] Among them, poly(lactide-*co*-glycolide) (PLG) with various lactide/glycolide ratios has been used as drug delivery carriers and tissue engineering scaffolds.[9–11] These copolymers are in glassy state during degradation because their glass-transition temperature (T_g) is higher than 37°C, which accounts for the low water absorption and slow drug release.

PCL presents great interest in drug delivery due to its high permeability to drugs. This polymer exhibits interesting thermal properties such as low T_g ($-65°C$), low melting temperature ($T_m = 65°C$), and high decomposition temperature ($T_d = 350°C$). Introduction of PCL component into PLGA chains yields terpolymers with lowered T_g, which allows to enlarge the potential applications of bioresorbable polymers. A great deal of work has been devoted to develop various terpolymers with desired degradation profiles,[12–16] thermodynamic properties, and drug release behaviors.[17–19] In fact, poly(glycolide-*co*-lactide-*co*-caprolactone) (PGLC) is most attractive as drug delivery carriers since drug diffusion should be easier from rubbery state polymers with low T_g than from glassy state ones. Investigations on the hydrolytic degradation of PGLC have also been carried out,[12,16] which is of major importance for applications. However, whether these terpolymers exhibit a heterogeneous degradation due to internal autocatalysis remained unknown. On the other hand, most of the research work was based on terpolymers synthesized by using stannous octoate (Sn(Oct)$_2$) as catalyst, which is more or less cytotoxic.[20,21]

In this work, we report on the hydrolytic degradation of PGLC terpolymers initialed by zirconium(IV) acetylacetonate (Zr(Acac)$_4$), a low toxic catalyst.[22] Degradation was carried out on compression molded samples in a pH 7.4 phosphate buffer taken as model of biological fluids. Analytical techniques such as ^{1}H-NMR, DSC, ESEM, and SEC were used to monitor the degradation. The results are reported herein in comparison with literature data.

EXPERIMENTAL

Materials

PGLC terpolymers with different compositions and chain microstructures were synthesized by ring-opening polymerization of glycolide, L-lactide, and ε-caprolactone at 100°C as previously reported,[23] the molar ratio of comonomers/initiator being 1000 : 1.

Degradation

Films of the various terpolymers were prepared by compression molding. Square specimens with dimensions of 10 mm ×10 mm ×1 mm were then cut from the films.

Specimens were placed into vials with 5 mL of 0.13M phosphate buffer solution (pH = 7.4) containing 0.02% of sodium azide to prevent bacterial growth. Degradation was allowed to proceed in an oven thermostated at 37°C.

At each preset degradation time, three samples were withdrawn from the vials and washed with distilled water. After wiping, the specimens were weighed and dried under vacuum at room temperature until constant weight before being subjected to analysis. Water uptake and weight loss values were calculated using the following equations:

$$\text{Water uptake (\%)} = [(W_{\text{wet}} - W_{\text{dry}})/W_{\text{dry}}] \times 100 \quad (1)$$

$$\text{Water loss (\%)} = [(W_0 - W_{\text{dry}})/W_0] \times 100 \quad (2)$$

where W_0 represents the initial weight of the specimens, W_{wet} the wet weight (after degradation and wiping), and W_{dry} the dry weight (after drying under vacuum).

Measurements

The copolymer composition and chain microstructure were examined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy at 300 MHz, using deuterated chloroform (CDCl$_3$) as solvent.

Thermal properties such as glass-transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), crystallization enthalpy (ΔH_c), and melting enthalpy (ΔH_m) were determined by using a TA10 differential scanning calorimeter (DSC) under nitrogen atmosphere at a heating rate of 10°C/min.

Size-exclusion chromatography (SEC) measurements were performed using a setting composed of a Waters 510 HPLC pump, a Waters 410 differential refractometer, and a PLgel 5 m mixed-C 60 cm column, the mobile phase being THF and the flow rate 1 mL min^{-1}. Data were expressed with respect to polystyrene standards from Polysciences.

The cross sections of the films were examined using a Philips XL30 environmental scanning electron microscope (ESEM) under a pressure of 6 Torr at 7°C, and a Wild M7 optical microscope.

RESULTS AND DISCUSSION

The molecular characteristics of the GA/L-LA/CL terpolymers were determined by using ^{1}H-NMR and SEC, as shown in Table I. It appears that the compositions of the copolymers are very close to those of the feeds, indicating a nearly 100% conversion of the monomers. The average lengths of caproyl L_{CL}, lactyl L_{LA}, and glycolyl L_{GA} blocks mainly depended on the composition of the copolymers.[23] The M_n of the copolymers ranges between 16,000 and 40,000, and the polydispersity index between 2.1 and 2.7. Data on Cop4 are not available because of its insolubility in common solvents due to high GA content.

Water uptake and weight loss

Water uptake of a polymer represents the bulk hydrophilicity, which plays an important role in the degra-

TABLE I
Molecular Characteristics of the PGLC Terpolymers

Terpolymer	GA/LA/CL molar ratio in the feed	GA : LA : CL molar ratio in the polymer[a]	M_n[b]	M_w/M_n[b]
Cop1	17.8/54.8/27.4	20.5/55.1/22.4	24500	2.7
Cop2	17.1/68.6/14.3	16.7/69.7/13.6	39600	2.6
Cop3	21.4/35.7/42.9	22.6/36.5/40.9	16000	2.1
Cop4	57.1/28.6/14.3	n.d.[c]	n.d.	n.d.

[a] Determined by ^{1}H-NMR.
[b] Determined by SEC.
[c] n.d., notdetermined due to insolubility.

dation process. As shown in Figure 1, Cop1 exhibited a slow water uptake during the first 3 weeks, followed by a faster water uptake to reach 42% after 5 weeks. Cop2 exhibited a similar and slightly faster water uptake, attaining 45% after 5 weeks, which can be assigned to the higher hydrophilic GA content (20.5% vs. 16.7%). In contrast, Cop4 with nearly 57% GA units appeared the most hydrophilic, 96% of water uptake being detected in the same period. Beyond 5 weeks, Cop1 and Cop4 samples became too fragile or sticky for water uptake measurements, while Cop2 showed a constant water uptake, which attained 103% after 13 weeks. In the case of Cop3, no water uptake data are available, because it became sticky even after 1 week's degradation.

Weight loss of a polymer during degradation reflects the release of soluble oligomers from the bulk sample. Cop4 showed the fastest weight loss as shown in Figure 2. After 1 week's lag time, weight loss of Cop4 increased rapidly and almost linearly to reach 75% after 11 weeks. Weight loss profiles of Cop1, Cop2, and Cop3 appeared very similar. Little weight loss was detected during the first 3 weeks, followed by a slow increase up to 7 weeks. Beyond, faster weight loss was observed, attaining 43, 30, and 40% after 13 weeks' degradation for Cop1, Cop2, and Cop3, respectively.

Microscopic examination

The terpolymers showed different behaviors during degradation in the buffer solution. Cop2 with high LA content and Cop4 with high GA content became more and more fragile. The former remained as a whole, while the latter broke down into pieces. Cop3 with high CL content became softened and viscous. Cop1
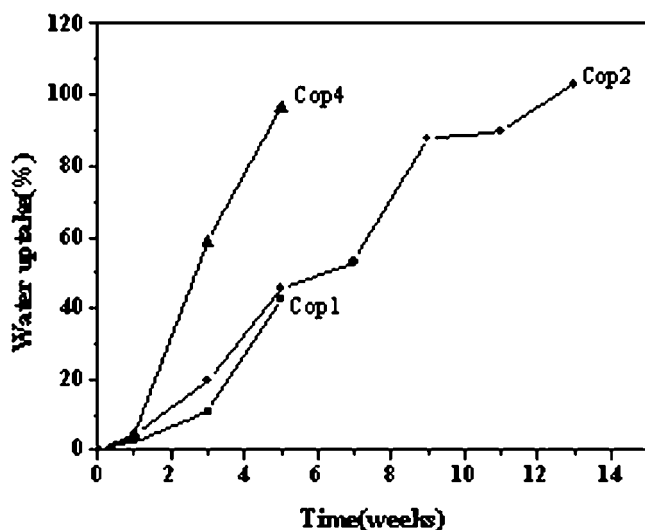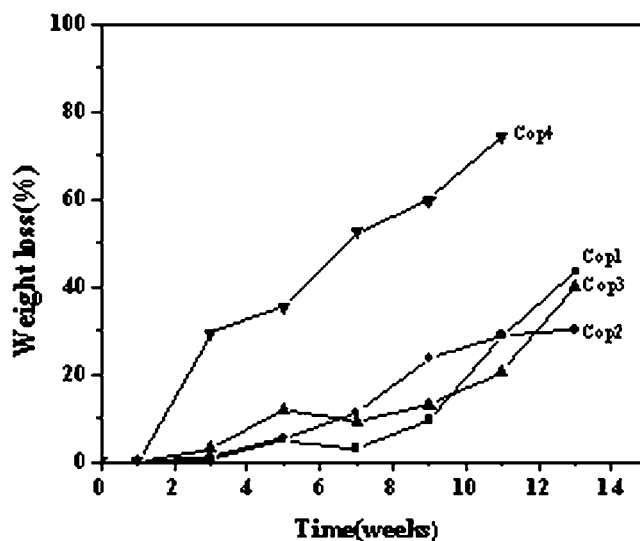

Figure 2 Weight loss changes of the terpolymers during degradation.

with intermediate composition became softened, sticky, and deformed during degradation.

The cross section of the degraded samples was examined by both ESEM and optical microscopy (Fig. 3). ESEM shows that, after 3 weeks' degradation, a surface/interior differentiation appeared. The cross section consists of a surface layer of ca. 50 μm and a porous internal part. The formation of porosity could be assigned to the release of soluble species from the copolymer.[3] Optical microscopy confirmed this observation. Figure 4 shows the cross section of Cop2 after
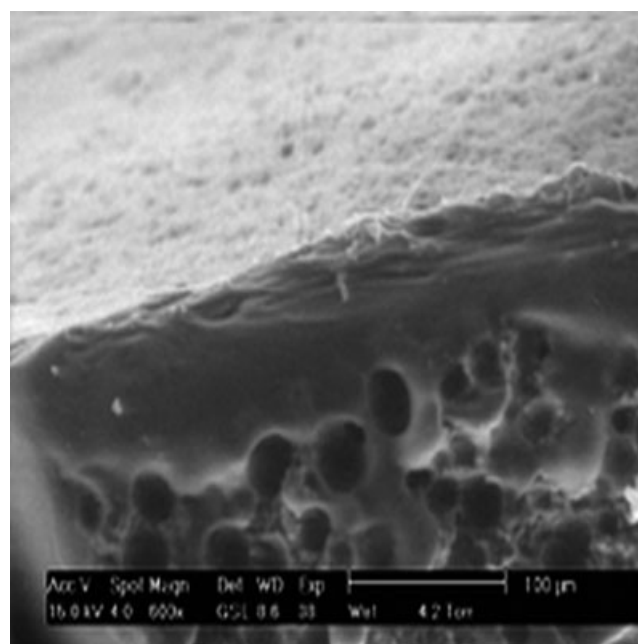

Figure 1 Water uptake changes of the terpolymers during degradation.

Figure 3 Environmental scanning electron micrograph of the cross section of Cop2 after 3 weeks' degradation.
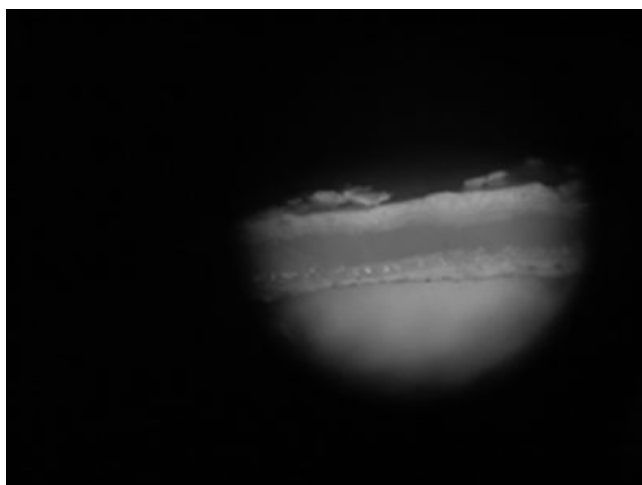
Figure 4 Optical microscope image of the cross section of Cop2 after 11 weeks' degradation.

11 weeks' degradation. A very pronounced surface/interior differentiation was detected: the surface appeared whitish, and the interior translucent.

The surface/interior differentiation has been observed many times during degradation of PLA and PLG copolymers,[3] and assigned to faster internal degradation due to internal autocatalysis. In fact, the PGLC samples were initially homogeneous. Once placed in the degradation medium, water penetrates the sample and hydrolytic cleavage of ester bonds occurs, leading to decrease of molecular weight. Ester bond cleavage produces carboxylic endgroups, which should be able to catalyze the hydrolysis of other ester bonds. However, carboxylic endgroups located near the surface are neutralized by the slightly alkaline buffer solution and lose their catalytic potent, while those located inside accelerate the internal degradation by autocatalysis, thus leading to surface/interior differentiation or faster internal degradation.

Molecular weight changes

Molecular weight is a key parameter that influences polymer degradation. Figure 5 shows the molecular weight changes of Cop2 during degradation as determined by SEC. Initially, Cop2 presents a relatively broad molecular weight distribution with a polydispersity index $\overline{M}_w/\overline{M}_n = 2.6$, $\overline{M}_n$ being equal to 39,600. The SEC curve shifts to longer elution time during degradation, indicating a continuous molecular weight decrease. After 9 weeks, $\overline{M}_n$ decreased to 1900. In the meantime, $\overline{M}_w/\overline{M}_n$ remained almost unchanged.

^{1}H-NMR spectroscopy

Chemical composition changes of the terpolymers were monitored by ^{1}H-NMR as shown in Table II.
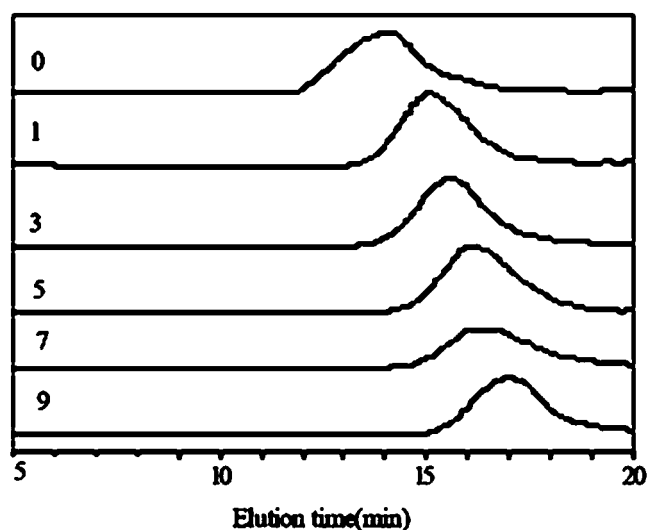
Figure 5 Size exclusion chromatograms of Cop2 after 0, 1, 3, 5, 7, and 9 weeks' degradation.

Cop1 showed a constant decrease of GA and LA contents and an increase of CL content, which seems to indicate that the CL segments are more resistant to hydrolysis than LA and GA ones. In the case of Cop2, however, the LA content increased and both the GA and CL contents decreased. This finding can be attributed to the fact that LA is the major component of Cop2 (ca. 70%), and that LA segments could crystallize during degradation as will be shown in the following. The case of Cop3 resembles to that of Cop1: both GA and LA contents decreased and CL content increased. Insofar as Cop4 is concerned, no NMR data are available because of its insolubility due to high GA content.

Thermal properties

The thermal properties of the various terpolymers were monitored by DSC. Two runs were realized on each sample: after the first heating at 10°C/min, the molten sample was quenched in liquid nitrogen and a second run was immediately performed. The first run allowed evaluating the T_m and ΔH_m of the degraded samples, while the second run was used to visualize the glass transition and cold crystallization.

TABLE II
Chemical Composition Changes of the Terpolymers During Degradation[a]

Time (wk)	Cop1	Cop2	Cop3
0	20.5/55.1/24.4	16.7/69.7/13.6	22.6/36.5/40.9
7	19.0/55.2/25.8	17.5/69.4/13.1	21.8/35.9/42.9
13	17.1/51.8/31.1	14.4/72.8/12.7	21.4/34.3/44.3

All values indicate GA : LA : CL.
[a] Determined by ^{1}H-NMR.

Figure 6 shows the DSC curves of Cop2 after 0, 7, and 13 weeks' degradation. The polymer initially exhibited only a T_g at 24.9°C, indicating that it was intrinsically amorphous. After 7 weeks, however, a melting peak was detected at 82.7°C with a melting enthalpy of 12.6 J g^{-1}. The T_m and ΔH_m slightly increased to 86.1°C and 17.2 J g^{-1} after 13 weeks, respectively. The appearance of crystallinity can be assigned to degradation-induced crystallization of the major component, i.e., L-LA segments, as previously reported in literature.[3,8] T_g decreased from 24.9°C to 19.7°C due to molecular weight decrease.

Table III presents the thermal property changes of the copolymers during degradation. Cop1 is also an intrinsically amorphous polymer with a low T_g at 0.6°C. It remained amorphous during the whole degradation process, with T_g slightly decreasing to −6.1°C. With the highest CL content (40.9%), Cop3 initially exhibited the lowest T_g (−22.6°C) which decreased to −31.9°C after 7 weeks. Cop3 appeared also slightly crystalline with a T_m at 28.9°C and a low ΔH_m due to the PCL-type crystallites. In the case of Cop4 with the highest GA content (57.1%), it initially exhibited a T_g at 30.9°C which decreased to 17.5°C after 13 weeks. A melting peak was detected at 194.0°C with $\Delta H_m = 11.1$ J g^{-1} due to PGA-type crystallites. T_m of Cop4 decreased to 184.7°C, but ΔH_m strongly increased to 46.9 J g^{-1} during degradation, which could be assigned to degradation-induced crystallization of PGA-rich segments and/or preferential degradation of amorphous domains.[3] A second melting peak was also detected after 13 weeks probably due to crystallization of low molecular weight species.

In the literature, attention has rarely been paid to the hydrolytic degradation of PGLC terpolymers. Cai et al. reported the synthesis and degradation of terpolymers synthesized by using stannous octoate as catalyst.[12] Surprisingly, PGLC45/45/10 and PGLC27/63/

TABLE III
Thermal Property Changes of the Copolymers During Degradation

Terpolymer	Time (wk)	T_g (°C)[a]	T_m (°C)[b]	ΔH_m (J/g)[b]
Cop1	0	0.6	–	–
	7	0	–	–
	13	−6.1	–	–
Cop2	0	24.9	–	–
	7	22.5	82.7	12.6
	13	19.7	86.1	17.2
Cop3	0	−22.6	28.9	2.2
	7	−31.6	18.3	2.8
	13	–	–	–
Cop4	0	30.9	194	11.1
	7	12.7	197.3	28.4
	13	17.5	83.7/184.7	23.0/46.9

[a] Determined by DSC (second run after quenching).
[b] Determined by DSC (first run at 10°C/min).

10 (GA/LA/CL) largely swelled up to several times their original volumes and showed rapid water uptake and weight loss. After 10 days' hydrolysis, the cross section of PGLC27/63/10 turned to a porous structure, which was attributed to the swelling and preferential degradation of GA units. However, the composition determined by ^{1}H-NMR showed almost no changes, from 30.8/59.1/11.1 to 29.1/59.5/11.4. No internal autocatalysis was reported.[12] In fact, the porous structure might be caused by the processing of degradation films, which were prepared by solution-casting method. Solvent volatilization inevitably caused pores, which resulted in swelling and high ratio of water uptake. On the other hand, although it was assumed that L-LA segments of PGLC27/63/10 crystallized, no DSC or XRD data were available to confirm this hypothesis. In the present work, the terpolymers initiated by zirconium(IV) acetylacetonate were processed by compression molding. Faster internal degradation was observed due to internal autocatalysis of carboxylic endgroups formed by ester bond cleavage. Degradation-induced compositional and morphological changes were evidenced by NMR and DSC, respectively. Further studies are underway to compare the degradation behaviors of GA/LA/CL terpolymers initiated by zirconium(IV) acetylacetonate and by stannous octoate.

CONCLUSIONS

Poly(glycolide-*co*-L-lactide-*co*-caprolactone) initiated by zirconium(IV) acetylacetonate exhibit low glass-transition temperature, i.e., in the range of 0–31°C depending on the composition. The copolymers present various degradation behaviors. Faster internal degradation was observed due to autocatalysis of carboxylic endgroups. The degradation was also characterized by the appearance of crystallinity due to crys-
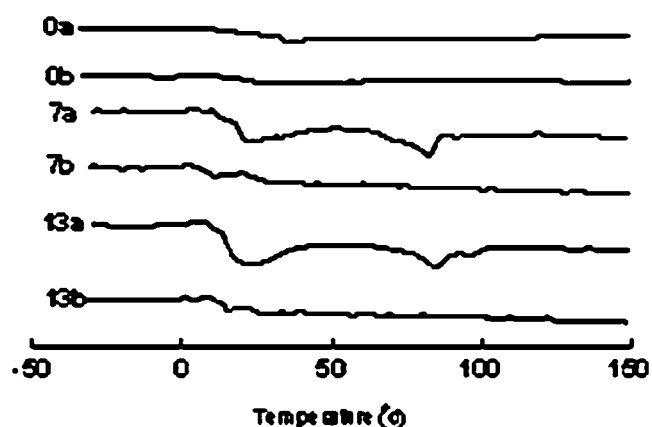

Figure 6 DSC thermograms of Cop2 after 0, 7, and 13 weeks' degradation. [(a) and (b) represents the first and second runs, respectively].

tallization of degradation products. The PCL component appears the most resistant to degradation, and the PGA, the most degradable according to compositional changes. It is concluded that these low T_g terpolymers are of great interest for biomedical applications, especially in the field of controlled drug delivery.

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