Synthesis, Characterization, and Enzymatic Degradation of Copolymers Prepared from ϵ-Caprolactone and β-Butyrolactone

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ABSTRACT: A series of copolymers was prepared by ring-opening polymerization of ϵ-caprolactone and (*R*,*S*)-β-butyrolactone using zinc lactate as catalyst. The resulting PCL/PBL copolymers were characterized by various analytical techniques such as NMR, SEC, DSC, and X-ray diffraction. The CL/BL ratio was higher in the copolymers than in the feeds, indicating a higher reactivity of ϵ-caprolactone with respect to β-butyrolactone. The copolymers appeared to be semicrystalline, the crystalline structure being of the PCL-type. Compression-molded polymer films were allowed to degrade in a pH = 7.0 phosphate buffer containing *Pseudomonas* lipase. Data showed that the copolymers degraded faster than the PCL homopolymer. Various soluble degradation products were detected in the degradation medium. ESEM micrographs confirmed the enzymatic degradation occurred by surface erosion.

Introduction

Biodegradable polymers are of growing interest in the field of temporary therapeutic applications such as sustained drug delivery systems, surgical sutures, osteosynthetic devices, scaffolds for tissue repair and regeneration, etc.[1−3] These materials are also attractive in the field of environmental applications because of the problems related to plastic waste accumulation.[4,5] In fact, biodegradable polymers can replace conventional plastics in applications such as mulch film in agriculture or packaging material, which can contribute to reducing environmental pollution.

Poly(ϵ-caprolactone) (PCL) is one of the most promising synthetic polymers that can degrade in an aqueous medium or in contact with microorganisms and thus can be used to make compostable polymeric devices.[6−8] Its excellent thermal properties, i.e., low melting temperature ($T_m \approx 65$ °C) and high decomposition temperature ($T_d \approx 350$ °C), provide a wide processing range. The enzymatic degradation of PCL polymers has been largely investigated, especially in the presence of lipase-type enzymes.[9−12] Three kinds of lipase were reported to significantly accelerate the degradation of PCL, i.e., *Rhizopus delemer* lipase,[9] *Rhizopus arrhizus* lipase,[10] and *Pseudomonas* lipase.[11,12] Highly crystalline PCL can be totally degraded in several days in the presence of *Pseudomonas* lipase,[11,12] in contrast to hydrolytic degradation which lasts several years.[1]

Poly(β-hydroxybutyrate) (PHB) is a naturally occurring polyester produced by bacteria as intracellular storage material of carbon and energy.[13,14] PHB can also be obtained by ring-opening polymerization of β-butyrolactone.[15,16] PHB and its copolymers with other hydroxyalkanoates are known as biodegradable materials which are of great interest in the field of environmental applications. In fact, a remarkable property of these polymers is their biodegradability in the environment.[17−19] Aerobic and anaerobic PHB-degrading bacteria and fungi have been isolated from various environments. The microorganisms excrete extracellular PHB depolymerases to degrade PHB and utilize the decomposed compounds as nutrients. However, PHB is a brittle material with poor processing and mechanical properties.

Various copolymers have been prepared by ring-opening polymerization of β-butyrolactone and ϵ-caprolactone in order to improve the properties with respect to homopolymers.[20−23] Hori et al. reported the synthesis of various copolymers of (*R*)-β-butyrolactone with ϵ-caprolactone and other lactones catalyzed by distannoxane complexes.[20] Abe et al. prepared block copolymers by sequential ring-opening polymerization of (*R*,*S*)-β-butyrolactone and ϵ-caprolactone using $Zn(C_2H_5)_2/H_2O$ as catalyst.[21] Later on, the same authors reported the synthesis of random copolymers of (*R*)-β-butyrolactone and ϵ-caprolactone.[22] The enzymatic degradation of copolymer films was carried out at 25 °C in 0.1 M phosphate buffer (pH = 7.4) containing PHB depolymerase purified from *Alcaligenes faecalis* or lipase from *Rhizopus delemer.* The highest degradation rate in the presence of lipase was observed for the copolymer with 91% of ϵ-caprolactone. Surprisingly, no degradation was detected for the PCL homopolymer.[22] Kurcok et al. also reported the synthesis of block copolymers of (*R*,*S*)-β-butyrolactone with ϵ-caprolactone.[23] The enzymatic degradation rate in the presence of *Rhizopus arrhizus* lipase strongly depends on the composition of the copolymers and increases with the β-butyrolactone content.

In a series of papers[24−26] we reported the degradation of PCL and its blends with poly(L-lactide) (PLLA) in the presence of *Pseudomonas* lipase or proteinase K[24,25] and the degradation of PCL/PEG block copolymers by *Pseudomonas* lipase.[27] The presence of PLLA strongly reduces the enzymatic degradation of PCL in the blends, while PEG blocks does not alter the degradation behavior of PCL in the copoymers.

In this paper we report on the synthesis, characterization, and enzymatic biodegradation of copolymers of ϵ-caprolactone and (*R*,*S*)-β-butyrolactone (PCLBL) in the presence of *Pseudomonas* lipase with the aim of identifying the effect of incorporation of BL units on the biodegradation characteristics of PCL. The copolymers were processed to films by compression molding. Biodegradation of the copolymers was carried out at 37 °C in a 0.05 M pH = 7.0 phosphate buffer containing *Pseudomonas* lipase in comparison with a PCL homopolymer. Degradation was monitored by weighing to

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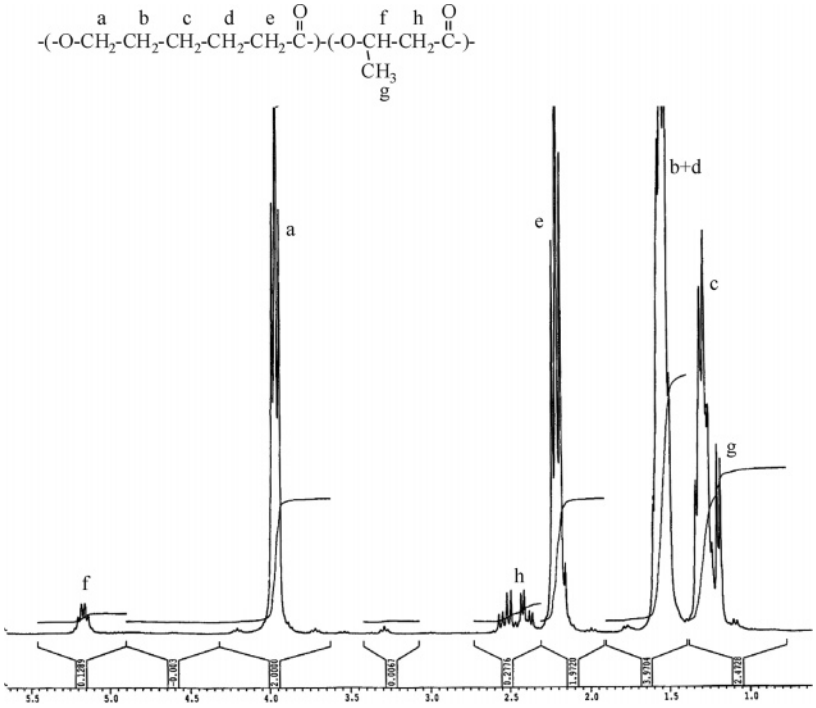

Figure 1. ^{1}H NMR spectrum of a PCLBL copolymer (entry 5).

Table 1. Molecular Characteristics of PCLBL Homo- and Copolymers

sample no.	[CL]/[BL] weight ratio (feed)	[CL]/[BL] molar ratio (feed)	[CL]/[BL] molar ratio (product)[a]	yield (%)	$\bar{M}_n$[b]	$\bar{M}_n/\bar{M}_n$[b]
1	100/0	100/0	100/0	74	29 500	1.8
2	90/10	87/13	97/3	70	19 500	1.9
3	90/10	87/13	94/6	78	20 300	1.5
4	80/20	75/25	92/8	31	28 100	1.4
5	80/20	75/25	88/12	65	22 600	1.5
6	70/30	64/36	84/16	28	15 600	1.3
7	70/30	64/36	82/18	35	15 400	1.3
8	60/40	53/47	87/13	28	15 000	1.4

[a] Determined by ^{1}H NMR. [b] Obtained by SEC with respect to polystyrene standards.

determine weight-loss profiles, differential scanning calorimetry (DSC) to determine thermal property changes, capillary zone electrophoresis (CZE) to evaluate the release of soluble species in the degradation medium, and environmental scanning electron microscopy (ESEM) to examine surface morphology changes.

Experimental Section

Materials. ϵ-Caprolactone and (*R,S*)-β-butyrolactone were supplied by Aldrich and distilled before use. *Pseudomonas cepacia* lipase (40 U/mg) was purchased from Fluka. Hemizinc lactate was obtained from Sigma.

Methods. PCLBL copolymers were synthesized by bulk ring-opening polymerization of ϵ-caprolactone and (*R,S*)-β-butyrolactone using hemizinc lactate (0.1 wt %) as catalyst. The caprolactone/butyrolactone or [CL]/[BL] weight ratio varied from 60/40 to 100/0. A 10 g amount of monomers including caprolactone and butyrolactone and 10 mg of hemizinc lactate were introduced into a flask. After degassing, the flask was sealed under vacuum and polymerization allowed to proceed for 7 days at 120 °C. The obtained polymers were purified by the dissolution/precipitation method using dichloromethane as the solvent and ethanol as the nonsolvent, followed by vacuum-drying up to constant weight.

Enzymatic Degradation. The PCLBL copolymers were compression molded at 75 °C to yield films of 75 mm diameter and ca. 0.4 mm thickness from which samples with initial weights of ca. 30 mg were cut. The samples were placed in vials containing 5 mL of 0.05 M phosphate buffer (pH = 7.0) with 0.75 mg of lipase. At predetermined degradation time intervals up to 69 h three samples were withdrawn from the degradation medium, washed with distilled water, and then vacuum-dried at room temperature for 1 week. For each polymer, control was realized with three specimens allowed to degrade in the absence of enzyme.

Measurements. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Bruker spectrometer operating at 300 MHz using $CDCl_3$ as solvent. Chemical shifts (δ) were given in ppm using tetramethylsilane (TMS) as internal reference. Size-exclusion chromatography (SEC) measurements were performed on a Waters apparatus equipped with a RI detector. THF was used as the mobile phase at a flow rate of 1.0 mL/min. A 20 μL amount of 1.0% (w/v) was injected for each analysis. Calibration was accomplished with polystyrene standards (Polysciences). Differential scanning calorimetry (DSC) thermograms were registered with a Perkin-Elmer instrument DSC 6, the heating rate being 10 °C/min. X-ray diffraction spectra were registered with a Philips diffractometer composed of a Cu Kα (λ = 1.54 Å) source, a quartz monochromator, and a goniometric plate. The surface morphology of the films was examined using a Philips XL30 environmental scanning electron microscope (ESEM), the sample being observed at 5 Torr and 7 °C. Capillary zone electrophoresis (CZE) data were collected using a P/ACE 5000 Beckman instrument equipped with UV absorbance detection at 254 nm and a fused-silica capillary (i.d. 75 μm, length 57 cm) with reverse mode. Identification of the various soluble oligomers was realized using PCL and PBL oligomer standards.[28]

Results and Discussion

PCLBL copolymers were synthesized by bulk ring-opening polymerization of ϵ-caprolactone and (*R,S*)-β-

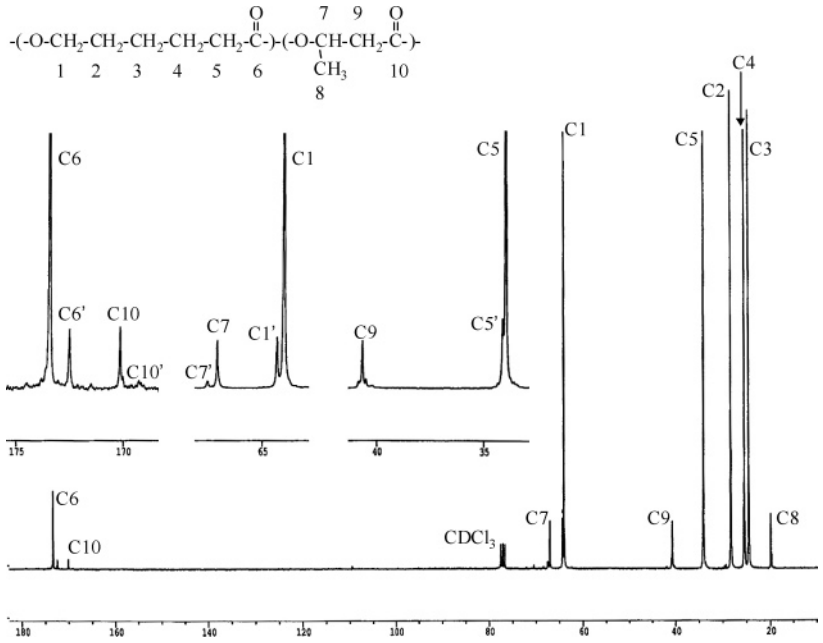

Figure 2. ^{13}C NMR spectrum of a PCLBL copolymer (entry 5).

Table 2. Thermal Properties of PCLBL Homo- and Copolymers

sample no.	1	2	3	4	5	6	7	8
T_m (°C)[a]	65	61	59	60	54 (47)	48	49	57
ΔH_m (J/g)[a]	78	58	56	65	46	45	40	53
T_g (°C)[b]	−69	−68	−65	−65	−63	−59	−8	−62
T_c (°C)[b]	−59	−55	−52	−53	−45	−35	−33	−44

[a] Obtained from the first heating. [b] Obtained from the second heating.

butyrolactone using hemizinc lactate (0.1 wt %) as catalyst. The initial [CL]/[BL] weight ratio ranged from 60/40 to 100/0. Table 1 presents the molecular characteristics of the various copolymers. The [CL]/[BL] molar ratio obtained by ^{1}H NMR is higher than the initial ratio in the feed, especially for copolymers with high initial BL contents. The yield also decreases with increasing BL content. These results show that the reactivity of butyrolactone monomer is lower than that of caprolactone. The number-average molecular weight ($\bar{M}_n$) obtained by SEC ranges from 15 000 to 30 000, and the polydispersity index ($I_p = \bar{M}_w/\bar{M}_n$) is in the range from 1.3 to 1.9. Duplicate polymers were obtained for initial [CL]/[BL] weight ratios of 90/10, 80/20, and 70/30. Differences between them were observed concerning the final [CL]/[BL] molar ratio, yield, and molecular weight, probably because of the rather low reactivities of the monomers.

Figure 1 shows a typical ^{1}H NMR spectrum of the copolymers (entry 5). The signals characteristic of PCL (protons a−e) and PBL (protons f−h) are observed as reported in the literature.[23] Interestingly, the resonance of the methylene protons (h) of PBL is split into two groups of two doublets. This is due to the fact that the two hydrogen atoms (h) are sterically nonequivalent and each hydrogen interacts with the methine hydrogen, thus resulting in four doublets. The integrations of signals a and f are used to calculate the CL/BL ratios of the copolymers.

The microstructure of the copolymer chains was examined by ^{13}C NMR spectra as shown in Figure 2. Signals corresponding to PCL (carbons 1−6) and PBL (carbons 7−10) components are detected as reported in the literature.[21−23] Besides the signal of C1 at 64.0 ppm

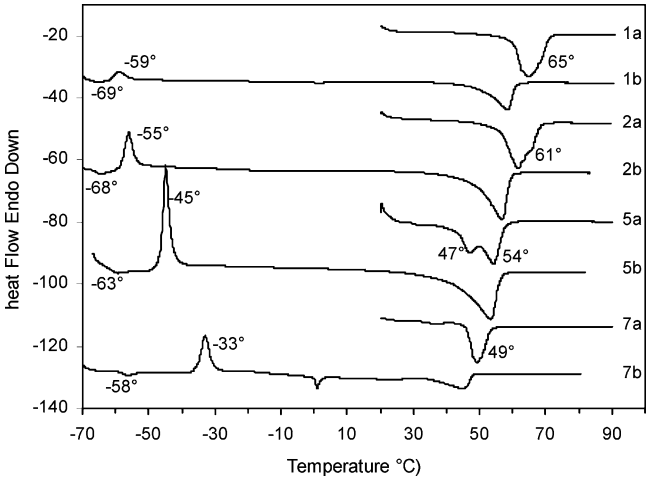

Figure 3. DSC thermograms of PCLBL copolymers (entries 1, 2, 5, and 7): (a) first heating; (b) second heating.

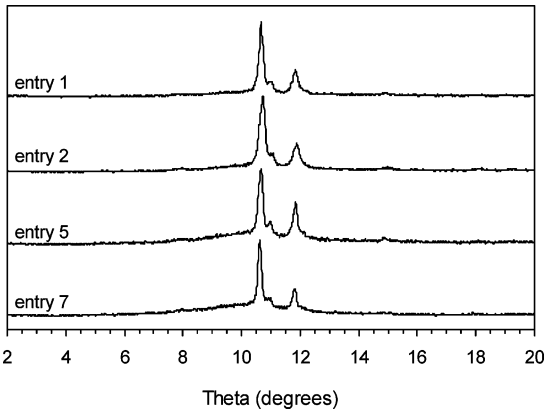

Figure 4. X-ray diffraction spectra of PCLBL copolymers (entries 1, 2, 5, and 7).

belonging to CL−CL diads, a smaller downfield signal C1′ is detected at 64.3 ppm. This signal is assigned to the C1 carbon of BL−CL diads. The intensity difference between C1 and C1′ signals shows that CL units mainly exist in the form of CL−CL diads. Similarly, a slightly downfield signal C5′ is detected at 34.1 ppm besides the

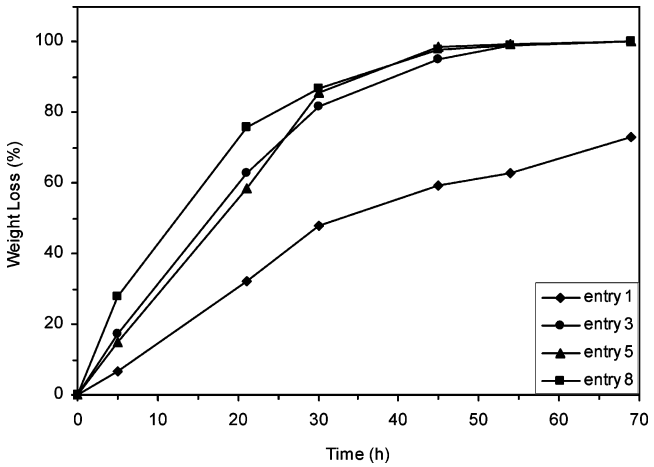

Figure 5. Weight-loss profiles of PCL and PCLBL copolymers (entries 1, 3, 5, and 8) during enzymatic degradation in a pH 7.0 phosphate buffer containing *Pseudomonas* lipase (0.15 mg/mL).

main C5 signal at 34.0 ppm, the former being assigned to CL−BL diads. On the other hand, an upfield signal C6′ (172.5 ppm) is observed besides the carbonyl carbon C6 (173.4) due to of the presence of CL−BL diads.

The situation is different in the case of PBL carbons as the copolymer contains only 12 mol % of BL units. In fact, the signal C10 detected at 170.1 ppm belongs to the carbonyl in the BL−CL diads, while the upfield signal C10′ detected at 169.2 ppm corresponds to BL−BL ones. Similarly, the C7 signal at 67.0 ppm is assigned to the methine carbon in the BL−CL diads and C7′ at 67.4 ppm to BL−BL ones. Concerning the C9 carbon, smaller signals are observed at both sides of the main signal, which might be assigned to the atactic microstructure of PBL blocks. Therefore, BL units mainly exist in the BL−CL diads along the polymer chains. It is of interest to note that in the case of PCLBL block copolymers these junction signals were not detected.[21,23]

The thermal properties of the various polymers were investigated by DSC. After the first heating the melted sample was quenched by immersion in liquid nitrogen, and a second heating was performed so as to observe the glass-transition and crystallization phenomena. Figure 3 shows the DSC curves of PCL and PCLBL copolymers. PCL is a highly crystalline polymer with a melting temperature (T_m) at 65 °C and a melting enthalpy (ΔH_m) of 78 J/g. During the second heating a glass transition (T_g) is observed at −69 °C, followed by a crystallization peak (T_c) at −59 °C and a melting peak. The T_m and ΔH_m values obtained at the second heating are lower than those of the first one because crystallization is not complete during the quenching and second heating processes. T_m and ΔH_m of the copolymer with only 3 mol % of BL units (entry 2) decrease to 61

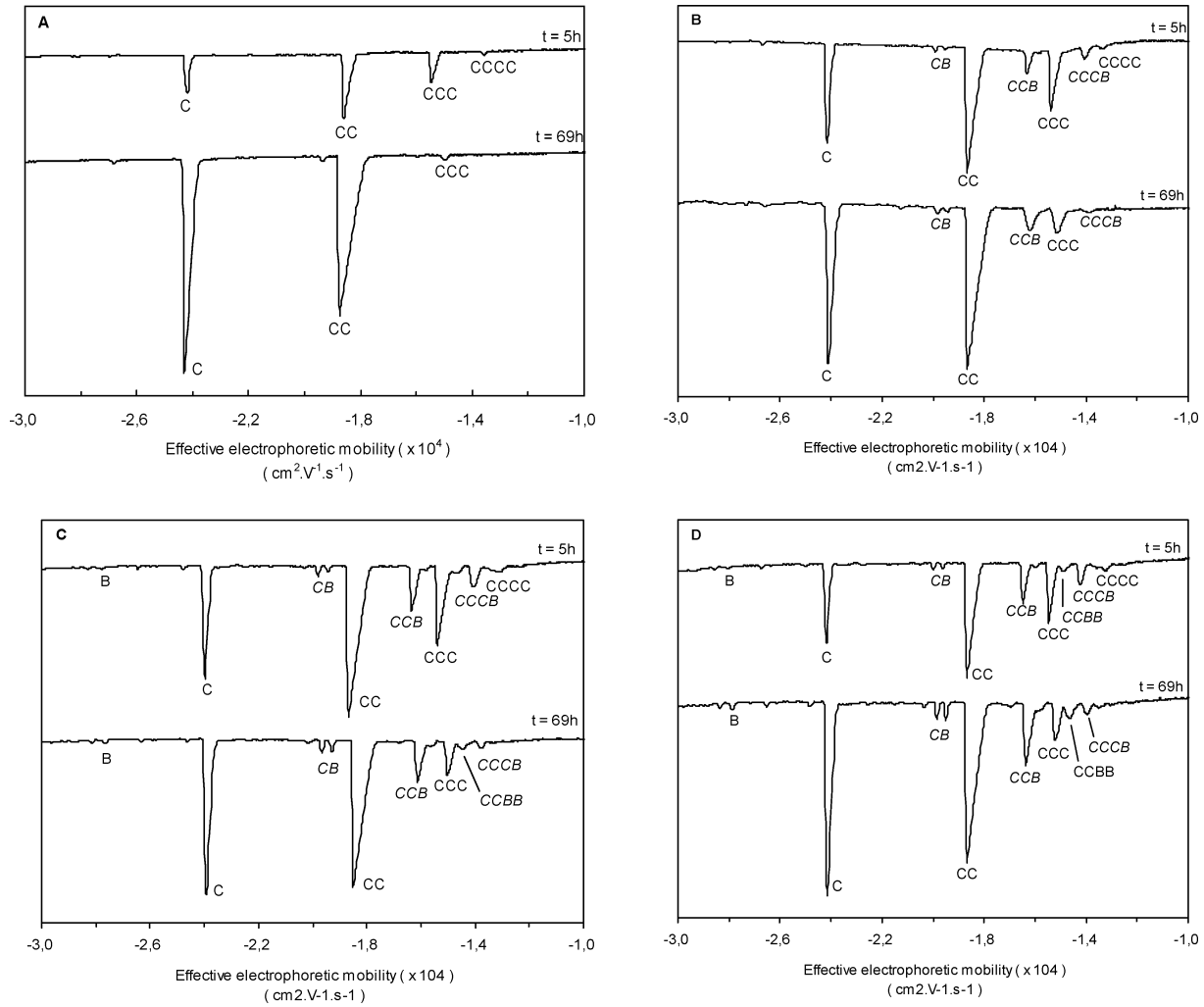

Figure 6. CZE diagrams of the buffer solution containing (A) entry 1, (B) entry 3, (C) entry 5, and (D) entry 7 after 5 and 69 h degradation in the presence of *Pseudomonas* lipase (0.75 mg/mL).

°C and 58 J/g, respectively, showing that introduction of a small amount of BL comonomer can significantly decrease the crystallinity of PCL. On the other hand, T_g and T_c slightly increase to −68 and −55 °C, in agreement with the presence of BL units which disturb the chain regularity and decrease the ability of the chains to crystallize. The same trend continues with the copolymers with 12 and 18 mol % of BL units (entries 5 and 7). The thermal properties of all the polymers are shown in Table 2. It can be concluded that the higher the BL content, the lower the T_m and ΔH_m values and the higher the T_g and T_c.

Figure 4 shows the X-ray diffraction patterns of the copolymers in comparison with PCL homopolymer. PCL shows an intense peak at $\theta = 10.6°$ and two smaller ones at 10.9° and 11.8°. The same pattern is observed for the copolymers, indicating that the crystalline structure of the copolymers corresponds to that of PCL. In other words, only the PCL component can crystallize in the copolymers. This finding can be assigned to the high PCL content. The incorporation of BL units also decreases the crystallinity of the copolymers as deduced from the relative intensity of the diffraction peaks, in agreement with DSC data. Although no quantitative evaluation was performed, comparison of the spectra shows that the area of the diffusion background under the diffraction peaks increases with increasing BL content.

The enzymatic degradation of the copolymer films was investigated in comparison with PCL. Weight-loss data were collected after various degradation time intervals up to 69 h. As shown in Figure 5, the copolymers degrade very fast. After 5 h, the copolymers in entries 3, 5, and 8 lose 17%, 15%, and 28% of their initial weights, respectively. Afterward, weight loss rapidly increases to reach nearly 100% after 45 h. PCL exhibits a slower degradation as compared to the copolymers. It loses 7% and 73% of its initial weight after 5 and 69 h, respectively. This could be assigned to the lower crystallinity of the copolymers, in agreement with DSC data. Thus, it can be concluded that the degradation rate of the copolymers is higher than that of the PCL homopolymer in the presence of *pseudomonas* lipase.

CZE was used to monitor the release of water-soluble species in the buffer solution during degradation. Figure 6A shows the CZE patterns of the solution containing a PCL film after 5 and 69 h degradation in the presence of lipase. The monomer C (6-hydroxyhexanoate), dimer CC, trimer CCC, and tetramer CCCC are detected after 5 h. After 69 h, a large increase is observed in the relative intensity of peaks corresponding to the monomer and dimer while a decrease is detected in the intensity of the trimer peak. The peak of the tetramers, which is initially very weak, becomes undetectable. This shows that most of the PCL material is turned to monomer and dimer and that degradation of trimer and tetramer continues in the solution.

In the case of the copolymer in entry 3 containing 6 mol % of BL units, the monomer C, dimer CC, trimer CCC, and tetramer CCCC are also detected after 5 h. Moreover, three more species appear which are assigned to CB, CCB, and CCCB, respectively, B standing for BL unit (β-hydroxybutyrate). In the case of CB dimer, two peaks of equivalent intensity can be distinguished because of the exitence of CL−BL and BL−CL sequences, although their assignment is not possible at present. The situation could be more complicated for CCB and CCCB species because there are many more possible combinations. Nevertheless, only one peak is detected, showing that the effective electrophoretic mobility values of the different CCB trimer and CCCB tetramer species are very close. After 69 h, the relative intensity of C and CC largely increases while that of CCC decreases and CCCC disappears as in the case of PCL. On the other hand, a peak intensity increase is observed for CB and CCB species and a decrease for CCCB ones.

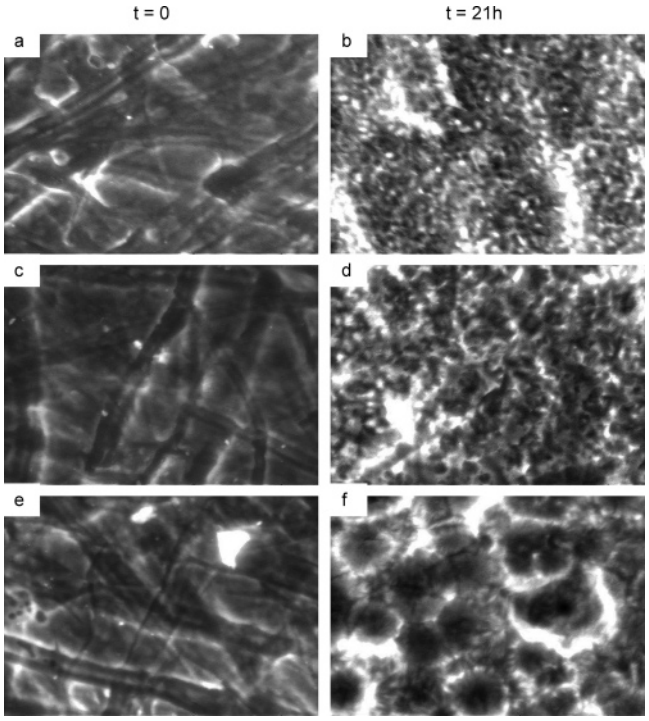

Figure 7. ESEM micrographs of PCL-PBL polymers before and after 21 h degradation by *Pseudomonas* lipase: (a, b) entry 1, (c, d) entry 3, and (e, f) entry 5.

Similar CZE patterns are obtained for the copolymers in entries 5 and 7 containing 12 and 18 mol % of BL units, respectively, as shown in Figure 6C and D. However, the intensity of peaks corresponding to CB, CCB, and CCCB species increases compared to that of entry 3, in agreement with the higher BL contents of entries 5 and 7. Moreover, a very small peak belonging to hydroxybutyrate monomer (B) is detected with an effective electrophoretic mobility of 2.8. A peak assigned to CCBB species was also detected between the peaks of CCC and CCCB.

Surface morphology changes were followed by ESEM, which is a technique of choice to monitor polymer degradation because it does not necessitate high vacuum or gold coating which could result in artifacts. Figure 7A and 7B shows the ESEM micrographs of a PCL film before and after 21 h degradation. The surface initially appears to be more or less rugged due to the surface morphology of the mold used for compression molding. After 21 h degradation by lipase, a homogeneous and porous structure is observed, indicating enzymatic attack at the surface. Similar changes are observed in the case of the copolymers. The copolymer in entry 5 exhibits large pores at the surface after degradation (Figure 7F). Therefore, ESEM micrographs confirm the surface degradation of polymers in the presence of lipase.

Conclusion

PCLBL copolymers were successfully synthesized by ring-opening polymerization of ϵ-caprolactone and β-butyrolactoneusing zinc lactate as catalyst. The CL/BL ratio of the resulting copolymers was higher than that of the monomer feeds because of the reactivity difference between CL and BL monomers. On the other hand, the higher the BL content, the lower the yield. NMR data show that CL units mainly exist in the form of PCL blocks while BL units mainly exist in the CL−BL−CL sequences. All the copolymers are rapidly degraded by *Pseudomonas* lipase, the degradation rate being higher than that of PCL homopolymer. Various water-soluble species issued from the enzymatic degradation of polymers are detected in the buffer solution.

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