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Enzyme-catalyzed polymerization and degradation of copolymers prepared from ϵ-caprolactone and poly(ethylene glycol)

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Abstract

Copolymerizations of ϵ-caprolactone (CL) with monohydroxyl or dihydroxyl poly(ethylene glycol) (PEG) were successfully performed using Novozyme-435 (immobilized lipase B from *Candida antartica*) as catalyst. Diblock and triblock copolymers with different compositions were characterized by ^{1}H NMR, GPC, DSC and X-ray diffraction. The enzymatic copolymerization carried out in toluene presented higher reaction rate and yield than that in bulk. Increasing the [CL]/[EO] feed ratio resulted in increases of molecular weight (M_n) of copolymers. Moreover, the compositions of triblock copolymers were closer to the monomer feed ratios than those of diblock copolymers. The resulting copolymers were all semicrystalline, the crystalline structure being of the PCL type. Solution cast films were allowed to degrade in a pH 7.0 phosphate buffer solution containing *Pseudomonas* lipase. Weight loss data showed that the introduction of PEG segments to the PCL main chain did not alter the enzymatic degradation of PCL significantly.

Keywords: Enzymatic copolymerization; Enzymatic degradation; PCL/PEG copolymer

1. Introduction

Biodegradable polymers have generated an enormous amount of research and interest in the fields of biomedical, agricultural and industrial applications. They are also regarded as solutions aimed at packaging waste management [1]. PCL is one of the most important environmentally biodegradable synthetic polymers due to facile accessibility, variable biodegradability and good mechanical properties. The in vitro enzymatic degradation of PCL polymers has been largely investigated, especially in the presence of lipase-type enzymes. Three kinds of lipase have been found as capable of significantly accelerating the hydrolytic degradation of PCL, i.e. *Rhizopus delemer lipase* [2], *Rhizopus arrhizus lipase* [3], and *Pseudomonas lipase* [4]. Highly crystalline PCL was reported to totally degrade in 4 days in the presence of *Pseudomonas* lipase [4], while simple chemical hydrolysis requires several years [5].

Recently, research on enzymatic polymerization has been receiving increasing attention as a new environmental friendly method of polymer synthesis, in contrast to chemical methods, which generally need harsh conditions and metallic catalysts that must be completely removed especially for medical applications. Up to now, various kinds of biodegradable polymers have been synthesized by enzymatic polymerization, such as polyesters [6–9], polycarbonates [10,11] and polyphosphates [12]. The enzymatic synthesis of PCL has been extensively studied and Novozyme-435 (immobilized lipase B from *Candida antartica*) has been proved an effective catalyst [13]. Although ring-opening copolymerization is a convenient method to synthesize copolymers with different properties acceptable to various applications [14], only a few kinds of copolymers have been prepared by enzymatic ring-opening polymerization, such as polyester [15], poly(ester-co-carbonate)s [16] and polycarbonates [17]. Poly(ethylene glycol) (PEG) has been widely used to form various block copolymers with suitable hydrophilic characters for many

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biomedical and biotechnological applications, such as poly(ethylene glycol)–polyester block copolymers [18–20], due to its hydrophily, good solubility in water and organic solvents, lack of toxicity [21], and absence of antigenicity and immunogenicity [22]. However, the enzymatic copolymerization of PEG with lactone monomers has not been reported yet.

In the present study, we report the enzymatic synthesis, characterization and enzymatic degradation of PCL/PEG diblock and triblock copolymers. The goals of this work were to synthesize the corresponding copolymers and further identify the effect of the different PEG incorporation on the biodegradation characteristics of PCL. PCL/PEG copolymerization was performed using Novozyme-435 as catalyst. The composition and molecular weight of products were studied. In addition, enzymatic degradation tests were performed at 37 °C in 0.05 M pH 7.0 phosphate buffer solution containing *Pseudomonas lipase* (0.2 mg/ml) using solution cast films. Physicochemical property changes of polymers during degradation were monitored by various analytical techniques.

2. Experimental

2.1. Materials

ϵ-Caprolactone (CL) was purchased from Acros and purified by distillation under calcium hydride in vacuo. Novozyme-435 (immobilized lipase B from *Candida antartica*) was purchased from Sigma and used after dried in vacuo for 24 h. Toluene was dried over Na and distillated just before use. Dihydroxyl PEG with molecular weight of 4600 (PEG4600) from Aldrich, zinc lactate from Sigma, monomethyl ether of PEG5000 and *Pseudomonas lipase* (40 U/mg) from Fluka were used as received. Poly(ϵ-caprolactone) (PCL) was synthesized by ring-opening polymerization of CL at 130 °C for 114 h using zinc lactate as catalyst (0.1 wt%). Its M_n and M_w/M_n were 54,200 and 1.7, respectively.

2.2. Measurements

^{1}H nuclear magnetic resonance (NMR, 250 MHz) spectra were recorded on a Bruker spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal reference. Gel permeation chromatography (GPC) measurements were performed on a Waters apparatus equipped with a RI detector using THF as solvent at a flow rate of 1.0 ml/min. 20 μl of 1.0% (w/v) sample solutions were injected for each analysis. Calibration was accomplished with polystyrene standards (Polysciences, USA). DSC thermograms were registered with a Perkin–Elmer instrument DSC 6 operator at 10 °C/min heating rate. The surface morphology of the films was examined using a Philips XL30 ESEM (Environmental Scanning Electron Microscopy) at about 5 Torr and 7 °C. X-ray diffraction spectra were performed on a Philips diffractometer composed of a Cu Kα ($\lambda = 1.54$ Å) source, a quartz monochromator and a goniometric plate.

2.3. Enzymatic copolymerization of ϵ-caprolactone and PEG

Novozyme-435 (1/10 w/w monomers) was dried with anhydrous phosphorus pentoxide as desiccant in vacuo at room temperature for 24 h and then transferred into a thoroughly dried glass flask containing ϵ-caprolactone, PEG and toluene (2:1 v/w of monomers). The flask was then closed with a glass stopper and immersed into an oil bath at 70 °C with stirring for a predetermined time. The resulting copolymers were dissolved in dichloromethane and filtered to remove the enzyme. Then the filtrate was concentrated under reduced pressure to obtain the crude copolymers and further precipitated in methanol as a poor solvent. After being dried in vacuo at room temperature to constant weight, the copolymers obtained were stored under dry conditions (see Table 1).

2.4. Preparation of polymers films

The various films were prepared by solution casting. Typically, a dichloromethane polymer solution (20%) was poured onto a glass plate and the solvent was evaporated under atmospheric pressure at room temperature for 24 h and then in vacuo for 48 h at 40 °C.

2.5. Enzymatic degradation

Enzymatic degradation experiments were carried out at 37 °C in a 0.05 M pH 7.0 phosphate buffer solution. Square samples with dimensions of 5 × 5 × 0.2 mm were cut from solution cast films and placed in vials containing 3 ml of buffer solution with 0.6 mg of enzyme (*Pseudomonas lipase*). The solution was changed everyday. At predetermined degradation time, three specimens were withdrawn from the degradation medium, washed thoroughly with distilled water and then dried under vacuum at room temperature for 7 days.

3. Results and discussion

3.1. Characterization of the copolymers

The resulting copolymers were characterized by ^{1}H NMR, GPC, DSC and X-ray diffraction. ^{1}H NMR spectra confirmed the structures of the copolymers obtained. ^{1}H NMR of copolymers ($CDCl_3$, ppm): $\delta = 1.2–1.8$ (m, $-OCH_2(CH_2)_3$), 2.1–2.4 (t, $COCH_2$), 3.5–3.7 (t, $-OCH_2$ of PEG segments), 3.9–4.2 (t, $-OCH_2$ of PCL segments).

For both of diblock and triblock copolymers, GPC

Table 1
Enzymatic copolymerization of ϵ-CL and PEG

Entry	Type of PEG	Time (h)	Yield (%)	[CL]/[EO] (feed)	[CL]/[EO] (product)[a]	M_n[b]	M_w/M_n[b]
1[c]	HO–PEG–OH (4600)	22	44	2.0	1.3	13,400	1.4
2	HO–PEG–OH (4600)	4	70	2.0	2.1	12,500	1.6
3	HO–PEG–OH (4600)	4	67	4.0	3.9	14,000	1.7
4	HO–PEG–OH (4600)	4	63	5.0	5.3	17,600	1.7
5	HO–PEG–OCH_3 (5000)	4	63	2.0	2.9	16,300	1.7
6	HO–PEG–OCH_3 (5000)	4	66	4.0	5.9	18,900	1.6

Copolymerization conditions: catalyst Novozyme-435 (immobilized lipase B from *Candida antartica*) (1/10 w/w monomers), solvent toluene (2:1 v/w of monomers), 70 °C.
[a] [CL]/[EO] molar ratio determined by ^{1}H NMR.
[b] Data obtained by SEC with respect to polystyrene standards.
[c] Copolymerization reaction carried out in bulk.

chromatograms showed symmetric and narrow molecular weight distributions. There was no peak in the zone of low molecular weights, thus indicating the absence of residual PEG or ϵ-CL.

The X-ray diffraction patterns of the block copolymers were compared with those of PCL and PEG (Fig. 1). PCL showed an intense peak at $\theta = 10.6°$ and two smaller ones at 10.9 and 11.8°, whereas PEG showed two main peaks at $\theta = 9.5$ and 11.5°. There was no PEG diffraction peaks of PEG were detected in the spectra of the copolymers, the crystalline structures of copolymers corresponding to PCL only. Under the conditions of solution casting, only PCL segments could crystallize in the copolymers.

The thermal properties of PCL/PEG copolymers were investigated by DSC in comparison with those of the corresponding PEG and PCL homopolymers. The melting temperature (T_m) and melting enthalpy (ΔH) of various copolymers are shown in Table 2. The different compositions had almost no effect on T_m, which remained very close to PCL T_m. However, ΔH values of the copolymers were higher than that of PCL, but lower than that of PEG.

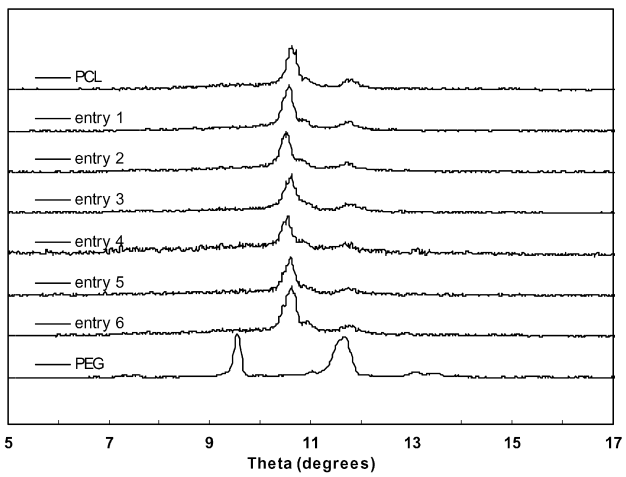

Fig. 1. X-ray diffraction spectra of PCL/PEG copolymers, PCL and PEG.

3.2. Enzymatic copolymerization of ϵ-CL and PEG

The diblock and triblock copolymers of ϵ-CL and PEG were synthesized using Novozyme-435 as catalyst in bulk and in solution (shown in Table 1). Under the same conditions, the CL and PEG were allowed to polymerization in the absence of enzyme for control. After precipitation, no corresponding copolymers could be obtained, which indicate that the lipase enzymes actually catalyze the copolymerization of CL and PEG. The [CL]/[EO] molar ratio of the copolymers obtained was determined from the integrations of methylene bands on the ^{1}H NMR spectra.

The solvent, namely toluene, played a very important role in the copolymerization reaction of PCL and PEG also, in agreement with the previous reports [13]. Bulk polymerization resulted in lower reaction yield and [CL]/[EO] molar ratio in the products up to 22 h. This might be ascribed to two aspects: the higher activity of ϵ-CL in toluene and the higher stability of enzyme in toluene, which resulted in only a short time (4 h) to get a higher yield and a close [CL]/[EO] molar ratio to that of feed.

Along with the increase of the [CL]/[EO] molar feed ratio, the molecular weight of the diblock and triblock copolymers increased. It is also interesting to note the effects of [CL]/[EO] ratio in the feed was different for the diblock and triblock copolymers. The compositions of the triblock copolymers were rather close to those of the feed, while the diblock copolymers exhibited higher difference between the [CL]/[EO] molar ratio in the feed and in the products.

3.3. Enzymatic degradation

Enzymatic degradation of the copolymers was carried out at 37 °C in 0.05 M pH 7.0 phosphate buffer solution with *Pseudomonas lipase* (0.2 mg/ml). Fig. 2 shows the weight loss profiles during various degradation times. The different CL/EO composition of the copolymers had only little effect on the enzymatic degradation in our experiments. Data remained close to that of the PCL homopolymer, except for entry 1, which was richer in EO units. This may be

Table 2
Thermal properties of PCL/PEG homo- and copolymers

Polymer	1	2	3	4	5	6	PCL	PEG5000	PEG4600
T_m (°C)	65	65	66	67	66	66	65	69	67
ΔH (J/g)	61	64	83	71	73	75	45	180	171

explained on the basis of water solubility of PEG and the lower molecular weight of the copolymer. In general, the enzymatic degradation rate of triblock copolymers was slightly higher than that of diblock copolymers.

Entry 6 (diblock copolymer) and entry 3 (triblock copolymer) were employed to monitor the physicochemical properties changes of the copolymers during degradation. Table 3 shows the thermal property changes of PCL and the copolymers after different degradation time. The melting enthalpy of PCL increased from an initial 45–49 J/g after 24 h, to 61 J/g after 48 h, and decreased to 57 J/g after 72 h. These results could be explained by the further crystallization of PCL during degradation. ΔH of both diblock (PCL–PEG) and triblock (PCL–PEG–PCL) copolymers decreased during degradation time up to 72 h. The PEG segments in the copolymers might prevent PCL segments from further crystallization. For PCL homopolymer and copolymers, T_m increased after 24 h, then decreased after 48 and 72 h. Therefore, one can assume that the degradation rate of the polymers had almost no relation to the crystallization of PCL during degradation, because the enzymatic attack eroded both amorphous and crystalline zones of the samples.

Chemical composition changes of the copolymers were followed by ^{1}H NMR. The [CL]/[EO] ratio of both diblock copolymer (entry 6) and triblock copolymer (entry 3) remained unchanged after 48 h degradation. Only PCL segments were degraded by the enzyme. On the other hand, the soluble PEG or PEG-rich segments could also escape from the bulk and dissolve in the degradation medium. These two factors resulted in a relative stability of the chemical compositions of the copolymers during degradation.

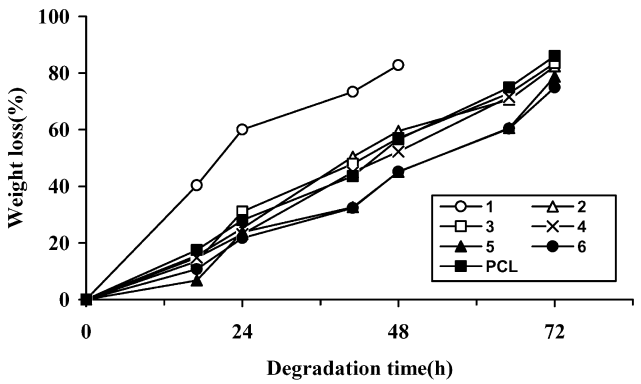

Fig. 2. Weight loss profiles of PCL/PEG copolymers and PCL during enzymatic degradation at 37 °C in a 0.05 M pH 7.0 phosphate buffer solution containing *Pseudomonas* lipase (0.2 mg/ml).

GPC was used to monitor molecular weights changes during enzymatic degradation. Data showed that no significant changes occurred after 72 h enzymatic degradation, despite around 80% weight loss. This can be assigned to the surface erosion by enzyme, in agreement with the absence of hydrolytic degradation in such a short time.

ESEM was used to monitor the film surface morphology changes during degradation, because this technique does not need high vacuum or metal coating that are often source of artifacts. Fig. 3 shows the ESEM micrographs of triblock copolymers (entry 3). It was observed that the two faces of the films had different morphologies as previously reported [18]. The upper face of PCL–PEG–PCL (triblock), which was in contact with air during solvent evaporation, exhibited a lot of 100 μm large spherulites with clearly distinguishable boundaries (Fig. 3a). In contrast, the lower face, which contacted the glass plate, appeared smooth with no spherulites detected (Fig. 3b). After 72 h degradation by lipase, fibrillar structures with distinguishable boundaries were observed on the upper face (Fig. 3c). The lower face became rough and appeared numerous tiny and shallow pores (Fig. 3d). Some little spherulites could also be observed.

In the case of PCL–PEG (diblock) copolymers (entry 6), different morphologies were also observed for the two faces. The upper face was initially full of rather large spherulites of about 150 μm with clearly distinguishable boundaries (Fig. 4a), while the lower face appeared smooth and some spherulites could be observed (Fig. 4b). After 72 h degradation in the lipase-containing buffer solution, some of the spherulites disappeared from the upper face (Fig. 4c),

Table 3
Thermal property changes of PCL and PCL/PEG copolymers during enzymatic degradation

PCL			PCL–PEG (entry6)			PCL-–PEG–PCL (entry3)		
Time (h)	T_m (°C)	ΔH (J/g)	Time (h)	T_m (°C)	ΔH (J/g)	Time (h)	T_m (°C)	ΔH (J/g)
0	65	45	0	66	75	0	66	83
24	67	49	24	68	62	24	69	64
48	66	61	48	66	61	48	65	66
72	65	57	72	65	64	72	63	59

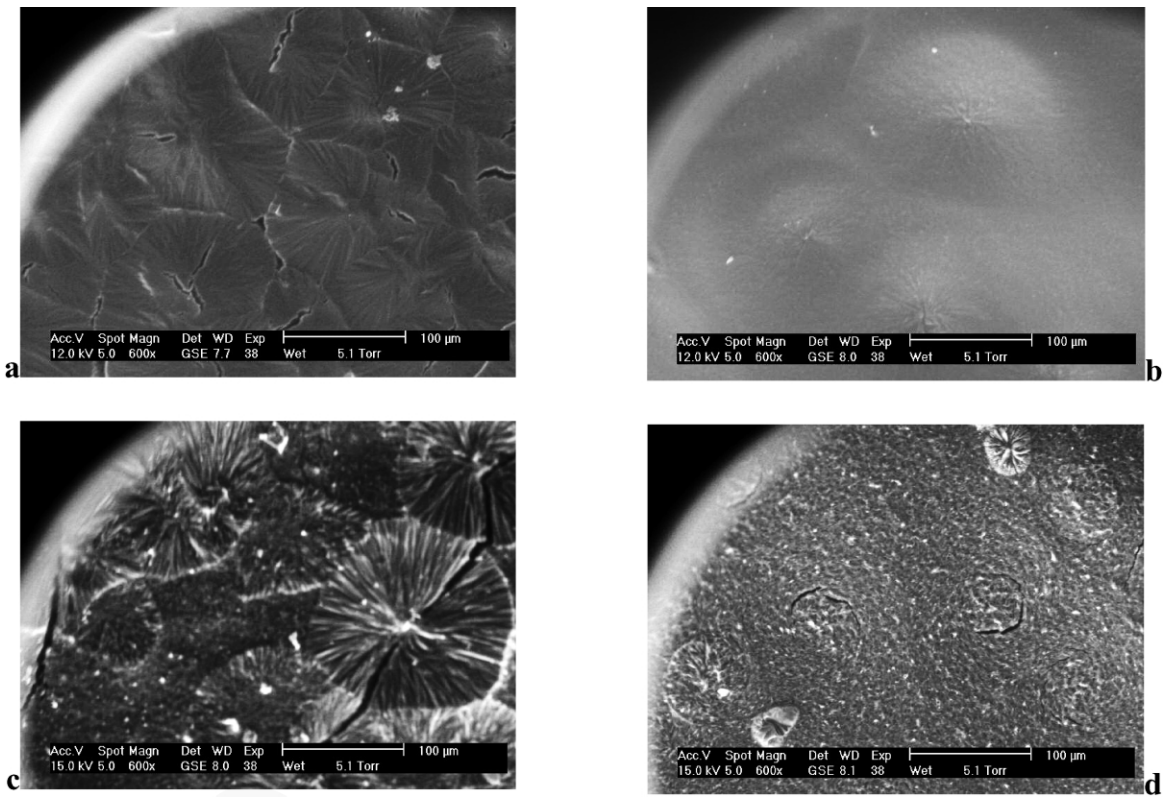

Fig. 3. ESEM micrograph of the PCL-PEG-PCL copolymer (entry 3).

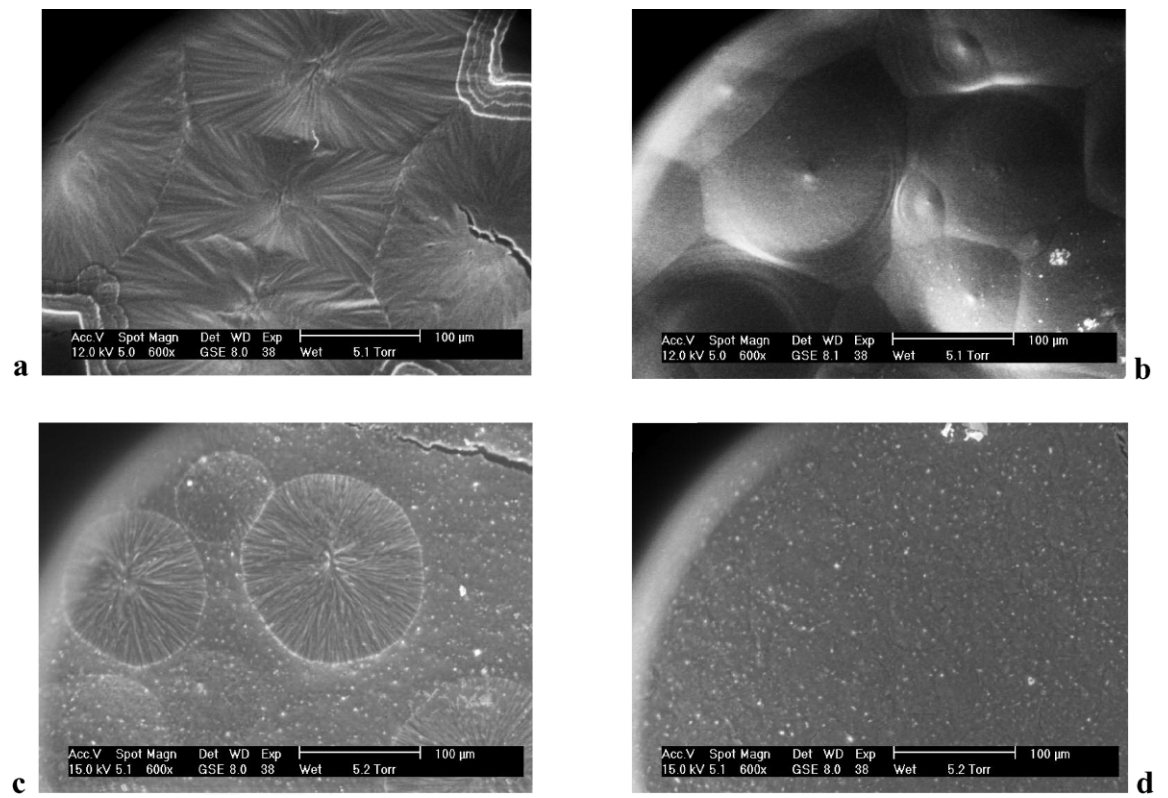

Fig. 4. ESEM micrograph of the PCL-PEG copolymer (entry 6).

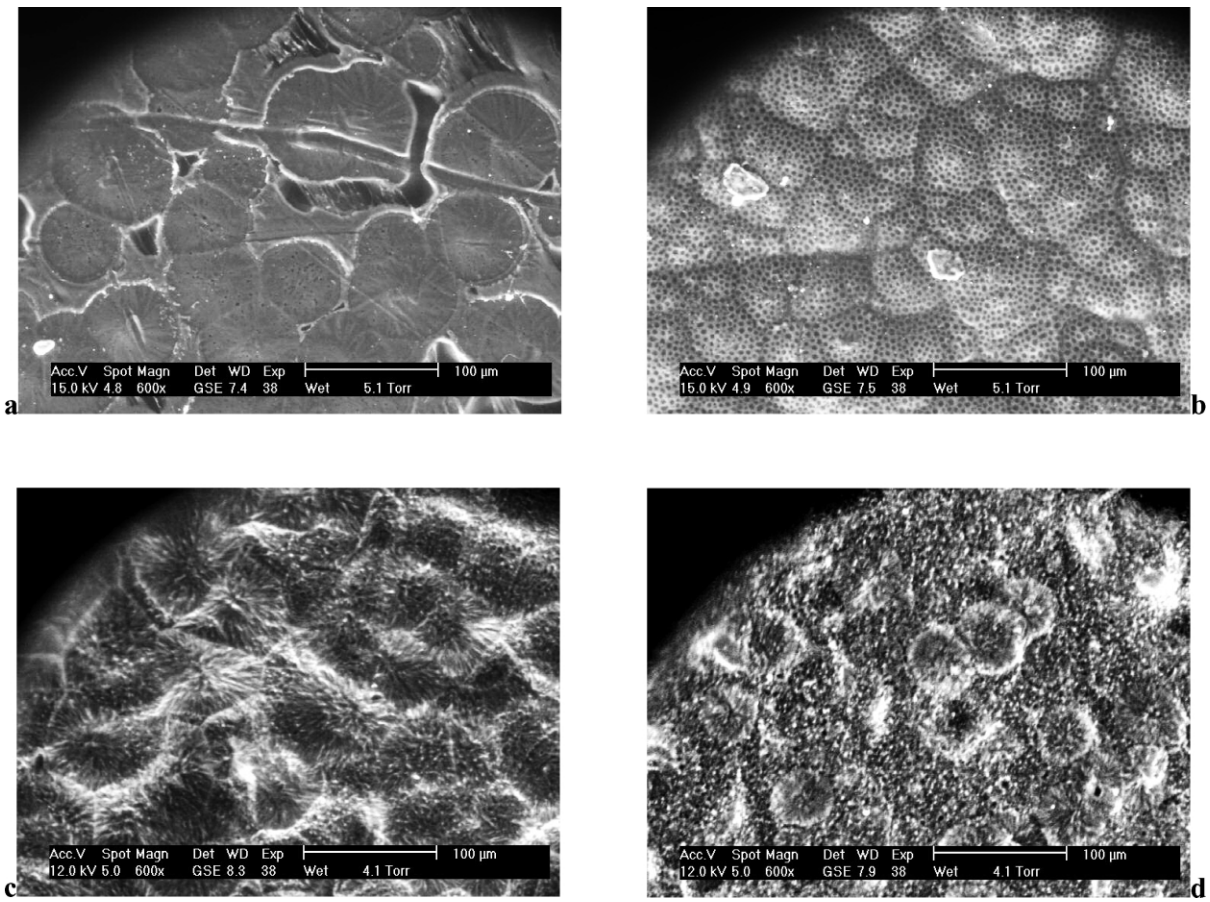

Fig. 5. ESEM micrograph of the PCL homopolymer.

which indicated the spherulites degraded more or less rapidly. The lower face appeared rough (Fig. 4d).

Insofar as PCL homopolymer was concerned, both faces exhibited lots of spherulites (Figs. 5a and 5b). After degradation for 72 h, spongelike structures on the upper face were observed with indistinguishable boundaries (Fig. 5c), while less degraded spherulites were still present on the lower face (Fig. 5d).

4. Conclusions

In this paper, a series of PCL–PEG and PCL–PEG–PCL block copolymers were successfully synthesized for the first time, using Novozyme-435 (immobilized lipase B from *Candida antartica*) as catalyst. Along with the increase of the [CL]/[EO] feed ratio, the molecular weight (M_n) of the copolymers increased for both diblock and triblock copolymers. The block copolymers were all semicrystalline polymers with the PCL type crystalline structure. DSC data showed that PCL continued to crystallize during enzymatic degradation. There was almost no composition and molecular weight changes of copolymers occurred during degradation, due to the higher solubility of PEG and PEG-rich segments, and also to surface erosion phenomenon. In general, enzymatic degradation of PCL was not altered by the incorporation of PEG for both diblock and triblock copolymers, while triblock copolymers seemed be slightly more enzymatically degraded than diblock copolymers.

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