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# Enzyme-catalyzed polymerization and degradation of copolyesters of ε-caprolactone and γ-butyrolactone

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**Abstract**

Copolymers of γ-butyrolactone (γ-BL) and ε-caprolactone (ε-CL) were successfully synthesized by ring-opening polymerization using Novozyme-435 (immobilized lipase B from *Candida antartica*) as catalyst. Copolymers with different compositions were obtained and characterized by $^{1}$H NMR, $^{13}$C NMR, GPC, DSC and X-ray diffraction. Increasing the [BL]/[CL] feed ratio resulted in decreases of molecular weight ($M_n$) of copolymers and reaction yield. Moreover, the BL contents in the copolymers varied according to the feed ratio. The $T_m$ of the copolymers decreased from 58 to 49 °C with increase in BL content from 0 to 14%. The resulting copolymers were all semicrystalline with a PCL-type crystalline structure. Solution cast films were allowed to degrade in a pH 7.0 phosphate buffer solution containing *Pseudomonas* lipase. Weight loss data showed that the degradation rate of copolymers in the presence of *Pseudomonas* lipase decreased with the increase of BL contents.


*Keywords:* Enzymatic copolymerization; Enzymatic degradation; PCL/PBL copolymer

## 1. Introduction

Recently, enzymatic polymerization has been receiving more and more attention as a new environmentally friendly method of polymer synthesis, in contrast to the chemical methods, which generally need harsh conditions and metallic catalysts that must be completely removed especially for medical applications. Furthermore, enzymatic polymerization can offer a novel method to produce polymers that are difficult to be synthesized by conventional polymerization [1]. Among the various polymerization methods, ring-opening polymerization is an important alternative route because leaving groups that can limit monomer conversion or degree of polymerization are not generated during polymerization [2]. Biodegradable polyesters, one of the most important synthetic polymers, are always synthesized by ring-opening polymerization of cyclic lactones.

Till now, enzymes have been applied successfully to synthesize various kinds of biodegradable polymers, such as polyesters and polycarbonates. Among them, enzymatic ring-opening polymerization of lactones has been extensively investigated, such as small-size (4-membered) lactones [3], medium-size (6- and 7-membered) lactones [4,5], and large-size (12-, 13-, 16-membered) lactones [6–8]. The exception is γ-butyrolactone (γ-BL), which has not been yet studied extensively due to its poor polymerizability. However, the use of γ-BL as a monomer for the synthesis of biodegradable polymer materials presents several advantages, such as environmentally friendly characters due to its corresponding polymer produced by microbes, suitable comonomer with other cyclic monomers to modify the characters of homo-polymer, and its low cost [9]. Therefore, the polymerization of γ-BL is of great interest both in the fields of research and product development. Although increasing research on polymerization of γ-BL has been reported, it was found that this monomer could not be polymerized using aluminoxane catalysts [10]. In addition, (co)polymerization using γ-BL as monomer or rich-comonomer led to low molecular weight products, either catalyzed by the enzyme or by the chemical catalyst [9–11]. For the development of the new method of enzymatic polymerization, the relatively low catalytic

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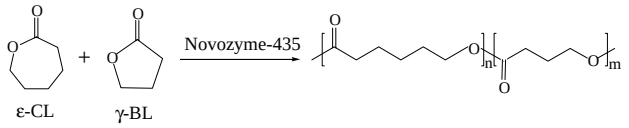


Scheme 1. Enzymatic Ring-opening copolymerization of ε-CL with γ-BL.

activities of enzymes and the productivity of enzymatic catalysts are some of the characteristic drawbacks of this type of polymerization system that should be improved.

Novozyme-435, lipase B from *Candida antartica* immobilized on macroporous acrylic resin, has been proven effective to catalyze the ring-opening polymerization of ε-CL [5]. In our previous work, block copolymers of PEG with PCL had also been synthesized using Novozyme-435 as the catalyst [12]. In the present study, Novozyme-435 was first employed in an attempt to synthesize γ-BL homopolymer and its copolymers with ε-CL (shown in Scheme 1). The results were expected to fill up the gaps in the studies on enzymatic polymerization of lactones with different size, and also to present a novel method to synthesize γ-BL contained biodegradable polymers. The composition and molecular weight of the products were studied. The influence of the presence of γ-BL within PCL-type chains on the enzymatic degradation was also investigated in the presence of *Pseudomonas* lipase. Physicochemical property changes of the polymers during degradation were monitored by various analytical techniques.

## 2. Experimental

### 2.1. Materials

ε-Caprolactone (ε-CL) was purchased from Acros and purified by distillation under calcium hydride in vacuo. Novozyme-435 (immobilized lipase B from *Candida antartica*) was obtained from sigma and used after drying in vacuo for 24 h. Toluene was dried over Na and distilled just before use. γ-Butyrolactone (γ-BL) and zinc lactate from Sigma, and *Pseudomonas* lipase (40 U/mg) from Fluka were used as received. Poly(ε-caprolactone) (PCL) was synthesized by ring-opening polymerisation of ε-CL at 130 °C for 114 h using zinc lactate as the catalyst (0.1 wt%). $M_n$ and $M_w/M_n$ of the resulting polymer were 54,200 and 1.7, respectively.

### 2.2. Measurements

$^1$H nuclear magnetic resonance (NMR, 250 MHz) and $^{13}$C NMR (100 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 rate of 1.0 ml/min. Twenty microliters of 1.0% (w/v) sample solutions were injected for each analysis. Calibration was accomplished with polystyrene standards (Polysciences, USA). Differential scanning calorimetry (DSC) thermograms were registered with a Perkin–Elmer DSC 6 instrument at 10 °C/min heating rate. The surface morphology of the films was examined by using a Philips XL30 ESEM (Environmental Scanning Electron Microscopy) at about 5 Torr and 7 °C. X-ray diffraction measurements 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-CL with γ-BL

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-CL, γ-BL 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 enzymes. 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 were stored under dry conditions. The results are summarized in Table 1.

### 2.4. Preparation of polymer films

The various films were prepared by solution casting. Typically, a dichloromethane solution (20%) of polymer was poured onto a glass plate. 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 $5 \times 5 \times 0.2$ mm$^3$ 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 every 24 h. 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 NMR, GPC and DSC and X-ray diffraction. The $^1$H NMR and $^{13}$C NMR spectra of the copolymers are shown in Figs. 1 and 2, which agree well with the copolymers synthesized using chemical catalysts [9]. $^1$H NMR of copolymers ($CDCl_3$, ppm): $\delta = 1.31$ (m, $-OCH_2CH_2CH_2$ of CL), 1.58 (m, $-OCH_2CH_2$ and $OCH_2CH_2CH_2CH_2$ of CL), 1.89 (m, $-OCH_2CH_2$ of BL),

Table 1
Enzymatic copolymerization of ε-CL with γ-BL

| Entry | BL/CL (feed) | Time (h) | Yield (%) | BL/CL (product)[a] | $M_n$[b] | $M_w/M_n$[b] |
|---|---|---|---|---|---|---|
| 1[c] | 4:3 | 4 | 7 | 7:93 | 9600 | 1.4 |
| 2 | 4:3 | 4 | 23 | 9:91 | 9900 | 1.5 |
| 3 | 4:3 | 24 | 26 | 10:90 | 10,600 | 1.5 |
| 4 | 1:1 | 24 | 41 | 10:90 | 12,000 | 1.4 |
| 5 | 1:1 | 48 | 31 | 11:89 | 13,000 | 1.4 |
| 6 | 1:9 | 24 | 62 | 2:98 | 16,100 | 1.8 |
| 7 | 1:4 | 24 | 49 | 3:97 | 14,900 | 1.7 |
| 8 | 1:2 | 24 | 41 | 8:92 | 12,800 | 1.6 |
| 9 | 2:1 | 24 | 12 | 14:86 | 9900 | 1.4 |
| 10[d] | 1:0 | 48 | – | – | – | – |

Copolymerization conditions: Novozyme-435 (immobilized lipase B from *Candida antartica*) (1/10 w/w monomers); solvent toluene (2:1 v/w of monomers); 70 °C.
[a] The [BL]/[CL] molar ratio determined by $^1$H NMR.
[b] Data obtained by GPC with respect to polystyrene standards.
[c] The copolymerization reaction carried out in bulk.
[d] After precipitation no corresponding polymer could be obtained.

2.26 (t, –COC$H_2$ of CL), 2.32 (t, –COC$H_2$ of BL), 4.0 (t, –OC$H_2$ of CL), 4.05 (t, –OC$H_2$ of BL). $^{13}$C NMR of copolymers ($CDCl_3$, ppm): 173.93 (–*C*=O of CL), 173.22 (–*C*=O of BL), 64.74 (–O*C*H$_2$ of CL), 64.51 (–O*C*H$_2$ of BL), 34.48 (–CO*C*H$_2$ of CL), 31.31 (–CO*C*H$_2$ of BL), 28.71 (–OCH$_2$*C*H$_2$ of CL), 25.89 (–OCH$_2$CH$_2$*C*H$_2$ of CL), 25.06 (–COCH$_2$*C*H$_2$ of CL), 24.90 (–COCH$_2$*C*H$_2$ of BL). Most of signals in the $^{13}$C NMR spectrum were split to the major and minor peaks due to the existence of diad homo and hetero sequence. The major signals of CL were from the diad homo sequence (CL–CL) and the minor ones were from the diad hetero sequence (*CL*–BL and BL–*CL*). On the other hand, the major signals of BL were assigned to the diad hetero sequence (*BL*–CL and CL–*BL*) because they had similar intensity to the minor ones of CL, whereas the minor signals of BL were due to the diad homo sequence (BL–BL). Therefore, the resulting copolymers were random copolymers.

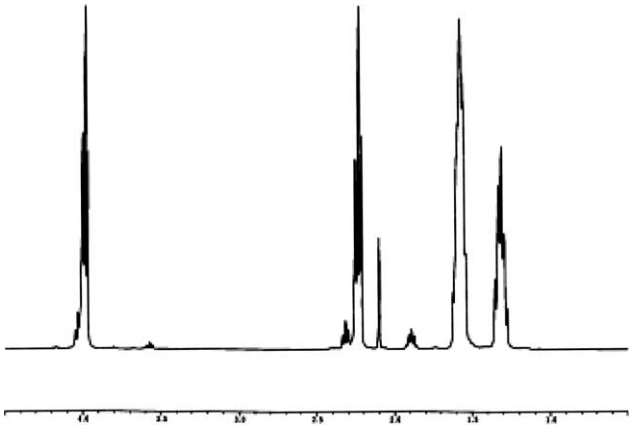

Fig. 1. $^1$H NMR spectrum of copolymer (entry 8).

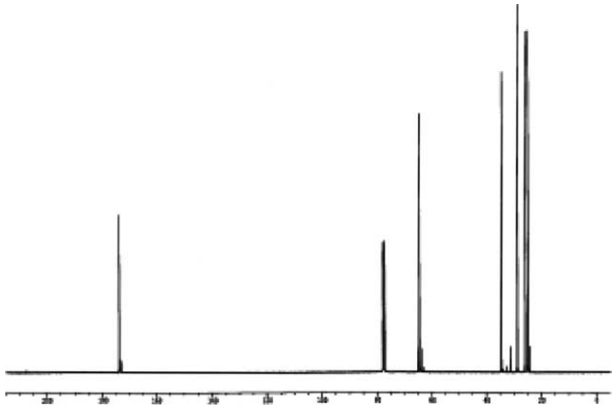

Fig. 2. $^{13}$C NMR spectrum of copolymer (entry 8).

GPC chromatograms of the copolymers showed one symmetric and narrow molecular weight distribution. There was no peak of low molecular weight, thus indicating the absence of residual ε-CL or γ-BL.

The X-ray diffraction patterns of the copolymers in comparison with PCL are shown in Fig. 3. PCL showed an intense peak at $\theta = 10.6°$ and two smaller ones at 10.9 and 11.8°. The same features were observed for the copolymers. Therefore, the crystalline structure of the copolymers corresponded to that of PCL, probably due to low γ-BL contents.

Thermal properties of the copolymers obtained were investigated by DSC in comparison with that of PCL homopolymer. The melting temperature ($T_m$) and melting enthalpy ($\Delta H$) of various copolymers are shown in Table 2. Only one endothermic peak with a $T_m$ lower than that of PCL could be observed. $T_m$ varied according to the copolymers

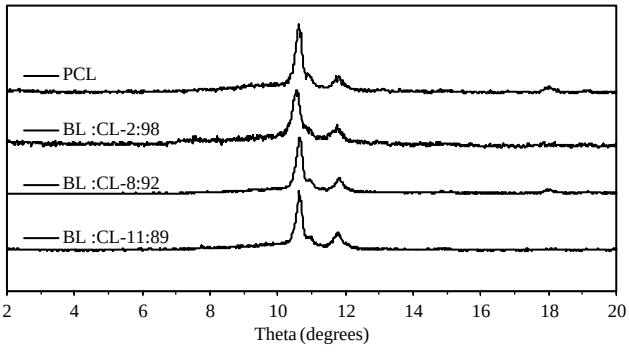


Fig. 3. X-ray diffraction spectra of copolymers and PCL.

Table 2
Thermal properties of copolymers and PCL homopolymer

| Polymer | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | PCL |
|---|---|---|---|---|---|---|---|---|---|---|
| $T_m$ (°C) | 54 | 54 | 52 | 53 | 53 | 58 | 56 | 55 | 49 | 58 |
| $\Delta H$ (J/g) | 73 | 76 | 63 | 69 | 63 | 57 | 64 | 70 | 63 | 37 |

Second heating scan (DSC).

composition. Along with increasing γ-BL contents from 0 to 14%, the $T_m$ of copolymers decreased from 58 to 49 °C.

### 3.2. Enzymatic copolymerization of ε-CL with γ-BL

Copolymers of ε-CL with γ-BL were successfully synthesized using Novozyme-435 as the catalyst in bulk and in solution. Different reaction conditions were studied, such as monomer feed ratio ([BL]/[CL]), reaction time and solvent (shown in Table 1). Under the same conditions, ε-CL and γ-BL (molar ratio, 1:1) were allowed to polymerize 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 ε-CL and γ-BL. The [BL]/[CL] molar ratio of the copolymers obtained was determined from the integrations of methylene bands on the $^1$H NMR spectra according to the method of Nakayama [9].

The solvent (toluene), played a very important role in the copolymerization reaction of ε-CL and γ-BL, in agreement with previous reports [5,12]. Bulk polymerization resulted in lower reaction yield and [BL]/[CL] molar ratio in the products. The reaction time was another important influencing factor. Along with the increase of reaction time from 4 to 24 h, the molecular weight, reaction yield, and BL content in the products, slightly increased. When the reaction time was prolonged to 48 h, the reaction yield decreased rapidly, although the $M_n$ and BL content still increased slightly. This finding could be assigned to the low activity of the enzyme when it stayed in organic solvent at high temperature for the long time and also maybe to lipase-catalyzed degradation. Therefore, in the following experiments, the reactions were carried out in toluene for 24 h.

The BL contents in precipitated copolymers varied according to the feed ratio; 67% of BL feed ratio resulted in 14% of BL in the copolymer. In the case of high BL feed ratio, the yield was less than 15% and the molecular weight was no more than 10,000. The enzymatic homopolymerization of BL was also studied (Table 1, entry 10). After precipitation from dichloromethane solution to methanol, no corresponding polymer was obtained, while the γ-BL monomer could be found according to the flat-bed chromatogram. γ-BL has low ring strain energy of 36.4–36.8 kJ/mol [13], whereas ε-CL has higher ring strain energy of 44.7 kJ/mol that makes it more active than γ-BL for ring-opening reactions [14]. According to literature [15], the polyester chain growth in enzymatic polymerization system most probably involves the ring-opening of the monomer by nucleophilic attack of the serine residue of lipase, followed by the hydrolysis of the acyl-enzyme intermediate or its esterification with low molecular weight alcohol or hydroxyl-terminated polyester chain. Thus, enzymatic ring-opening of γ-BL is more difficult, not only because of its lower ring strain energy, but also mainly because of its lower hydrophobicity, which makes it more difficult to be recognized by the enzyme [15,16]. The complex of BL-lipase is likely to be more difficult to form in comparison with that of CL, thus explaining that only small amount of γ-BL were found in the copolymers, even in the presence of BL-rich feed.

### 3.3. Enzymatic degradation of copolymers

The enzymatic degradation of copolymers was carried out at 37 °C in a 0.05 M pH 7.0 phosphate buffer solution with *Pseudomonas lipase* (0.2 mg/ml). Fig. 4 shows the weight loss profiles during degradation.

Till now, three kinds of lipases have been found as capable of significantly accelerating the hydrolytic degradation of PCL, i.e., *Rhizopus delemer lipase* [17], *Rhizopus arrhizus lipase* [18], and *Pseudomonas lipase* [19]. It was also reported that the BL-rich PCL–BL copolymers were easily hydrolyzed and enzymatically hydrolyzed in the presence of *Rhizopus arrhizus lipase* and *Rhizopus delemer lipase* [9]. It is very interesting to note that in our tests, the degradation rate of copolymers decreased along with the increase of BL contents. This feature can be assigned to the differences in the reactivities of lipase from different origins. *Pseudomonas lipase* was preferred to catalyze the degradation of CL units.

Three samples with different compositions, entries 5, 6 and 8 were employed to monitor the physicochemical property changes of the copolymers during degradation. Table 3 shows the thermal property changes of PCL and copolymers with degradation. The $T_m$ remained almost stable during 72 h degradation. The melting enthalpy of PCL increased from an initial 37 to 40 J/g after 24 h, to 42 J/g after 48 h, and to 43 J/g

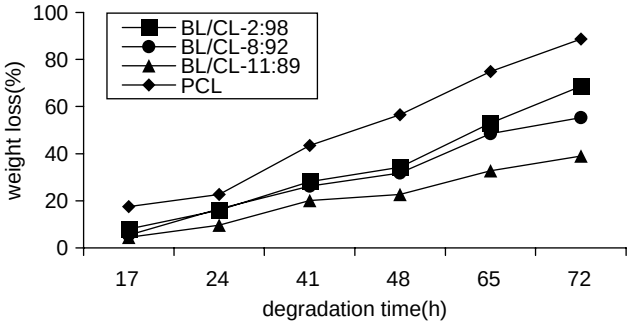


Fig. 4. Weight loss profiles of copolymers and PCL during enzymatic degradation at 37 °C in a 0.05 M pH 7.0 phosphate buffer solution containing *Pseudomonas lipase* (0.2 mg/ml).

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

| PCL | | | Copolymer (entry 5) | | | Copolymer (entry 6) | | | Copolymer(entry 8) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (h) | $T_m$ (°C) | $\Delta H$ (J/g) | Time (h) | $T_m$ (°C) | $\Delta H$ (J/g) | Time (h) | $T_m$ (°C) | $\Delta H$ (J/g) | Time (h) | $T_m$ (°C) | °$H$ (J/g) |
| 0 | 58 | 37 | 0 | 53 | 63 | 0 | 58 | 57 | 0 | 55 | 70 |
| 24 | 60 | 40 | 24 | 52 | 53 | 24 | 57 | 58 | 24 | 55 | 58 |
| 48 | 59 | 42 | 48 | 52 | 54 | 48 | 57 | 64 | 48 | 54 | 57 |
| 72 | 59 | 43 | 72 | 52 | 54 | 72 | 56 | 65 | 72 | 54 | 56 |

Second heating scan (DSC).

after 72 h. The CL-richer copolymer (entry 6) presented the same tendency. In the case of the other two samples with higher BL contents, $\Delta H$ decreased obviously after 24 h degradation and then remained constant. Therefore, although the introduction of γ-BL into PCL chains influenced the crystallization of PCL, the selected degradation of CL units of *Pseudomonas lipase* dominated the enzymatic degradation rate of the copolymers.

Chemical composition changes of the copolymers were followed by $^1$H NMR. During the enzymatic degradation, the compositional changes were not significant. After 72 h degradation, the [BL]/[CL] ratio in the copolymers slightly increased from 11:89 (entry 5), 2:98 (entry 6) and 8:92 (entry 8) to 13:87, 4:96 and 10:90, respectively.

GPC was used to monitor the molecular weight changes during enzymatic degradation. Data showed that almost no changes occurred after 72 h enzymatic degradation. The molecular weight ($M_n$) of entry 5, 6 and 8 were initially 13, 000, 16,100 and 12,800, and then retained 12,400, 15,600 and 12,100, respectively. This can be assigned to the accelerated degradation catalyzed by enzyme, in agreement with the absence of hydrolytic degradation during 72 h. At the same time, the polydispersity of the copolymers presented little increase during degradation, due to the selected degradation of CL units by *Pseudomonas lipase*.

ESEM was used to monitor the film surface morphology changes during degradation, because this technique does not require high vacuum or gold coating that often results in artifacts. Fig. 5 shows the ESEM micrographs of the copolymer with [BL]/[CL] molar ratio of 2:98 (entry 6). Two faces of the film appeared smooth initially (Fig. 5a,b), in agreement with the fact that PCL crystallized very quickly. After 72 h degradation by lipase, the upper face presented fibrillar structures with some distinguishable boundaries (Fig. 5c), while the lower face was eroded with a sponge-like structure (Fig. 5d).

In the case of the copolymer with [BL]/[CL] molar ratio of 8:92, different morphologies on the two faces of the film could also be observed. The upper face, which was in contact with air during solvent evaporation, was initially full of vague spherulites of about 75 μm (Fig. 6a) according to fast evaporation of the solvent at the surface and thus fast crystallization. In contrast, the lower face, which contacted with the glass plate, appeared smooth with the presence of

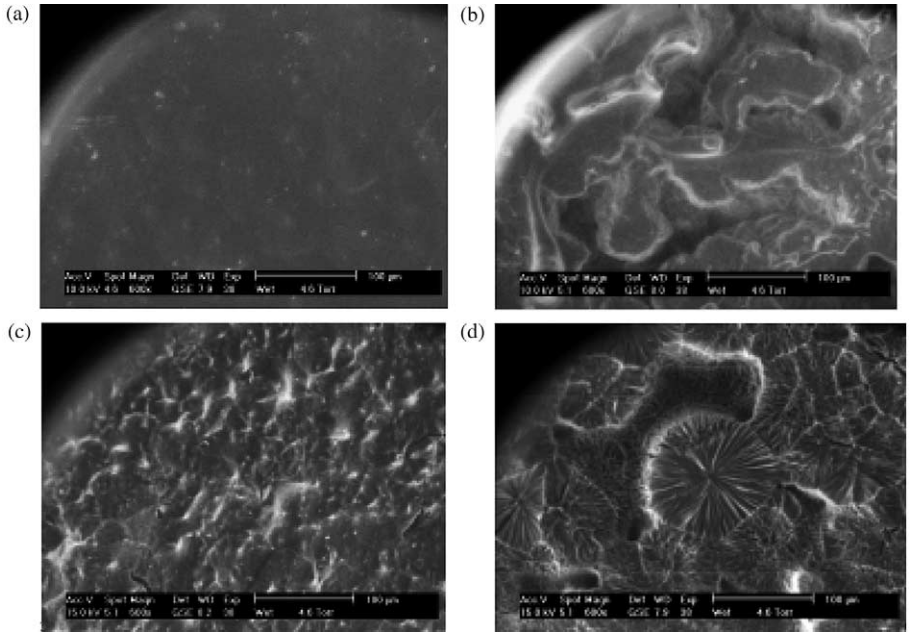


Fig. 5. ESEM micrograph of the copolymer (entry 6).

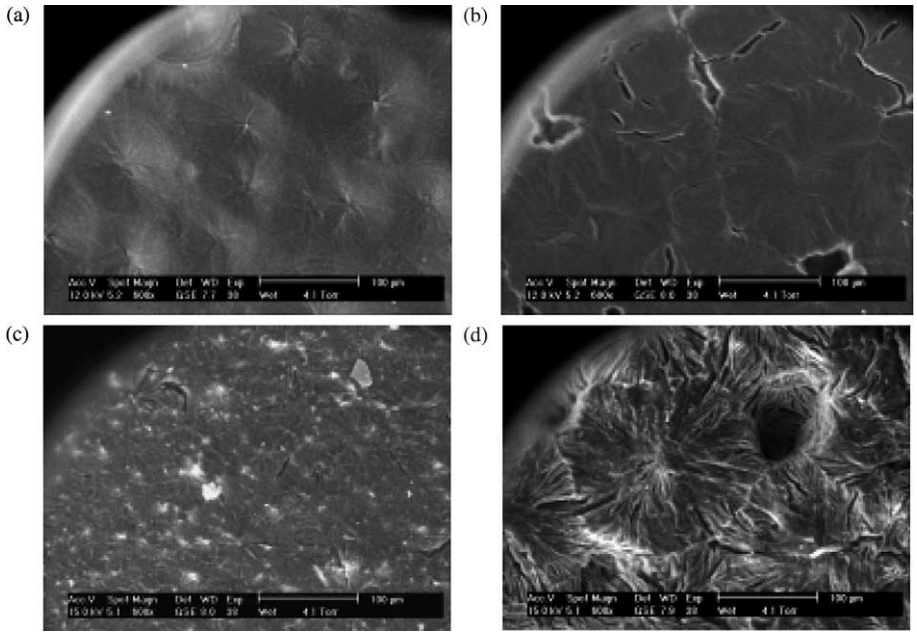


Fig. 6. ESEM micrograph of the copolymer (entry 8).

rather large spherulites of about 150 μm (Fig. 6b) according to slow solvent evaporation and formation of crystallites. After 72 h degradation in the lipase-containing buffer solution, the upper face was degraded and no spherulites could be observed (Fig. 6c), while the lower face presented rather large spherulites together with some fibrillar structures and holes (Fig. 6d).

Figure 7 shows the ESEM micrographs of the copolymer [BL]/[CL] molar ratio of 11:89. In this case, it was also observed that the two faces of the film had different morphologies. The upper face was initially full of spherulites of about 50 μm (Fig. 7a), while the lower face appeared smooth with some cracks formed during film casting (Fig. 7b). After 72 h degradation by lipase, both faces were full of fibrillar structures, and boundaries between the spherulites almost disappeared (Fig. 7c,d).

Insofar as PCL homopolymer was concerned, both faces exhibited lots of spherulites as reported in a previous paper [12]. After degradation for 72 h, the sponge-like structures on the upper face were observed with indistinguishable boundaries, while less degraded spherulites were still present on the lower face.

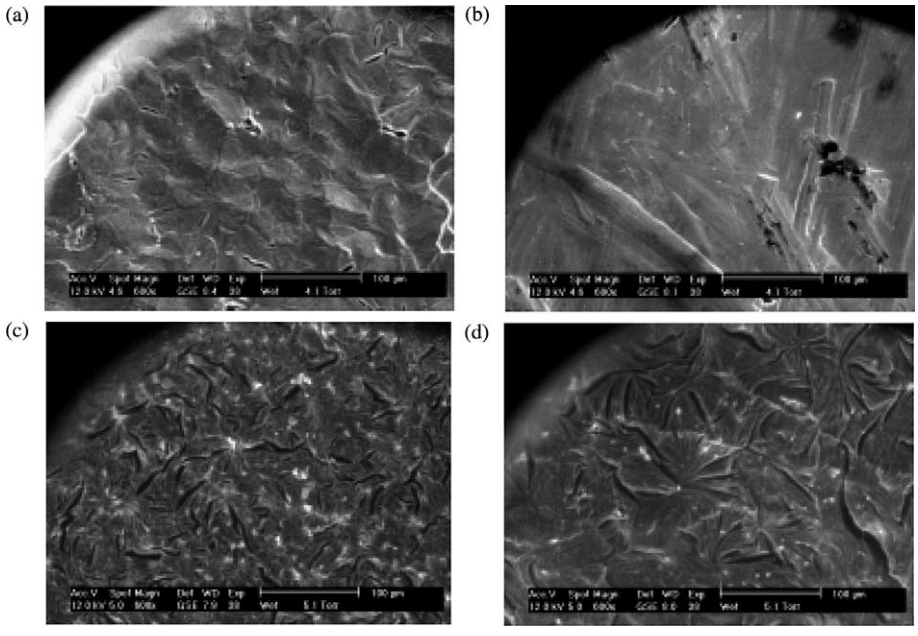


Fig. 7. ESEM micrograph of the copolymer (entry 5).

## 4. Conclusions

In this paper, a series of copolymers of γ-butyrolactone (γ-BL) and ε-caprolactone (ε-CL) were successfully synthesized for the first time, using Novozyme-435 (immobilized lipase B from *Candida antartica*) as catalyst. The BL content in the copolymers varied according to the feed ratio. Along with the increase of the [BL]/[CL] feed ratio, the molecular weight ($M_n$) of the copolymers and the reaction yield decreased. Moreover, the $T_m$ of the copolymers decreased from 58 to 49 °C with increasing the BL contents from 0 to 14%. The resulting copolymers were all semicrystalline, the crystalline structure being of the PCL type. During degradation in the presence of *Pseudomonas lipase*, the molecular weight ($M_n$) of the copolymers were almost no change, while the [BL]/[CL] ratio in the copolymers slightly increased, in agreement with the weight loss data and enzymatic degradation surface. *Pseudomonas lipase* degraded preferentially the CL units in the copolymers, in contrast to *Rhizopus arrhizus lipase* and *Rhizopus delemer lipase* according to the previous reference.

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