Effect of the Remaining Lanthanide Catalysts on the Hydrolytic and Enzymatic Degradation of Poly-(ε-caprolactone)

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Summary: Poly-(ε-caprolactone) is a biodegradable polymer, which can be used for both medical and environmental applications.
Due to its multiple applications the synthesis of such a polymer has been attracting an increasing attention in the past few decades.
In our work, the polymers were synthesised by bulk polymerisation, using different lanthanide halides as initiators. The lanthanide derivatives are known as very active catalysts in the ring-opening polymerisation of cyclic esters. Moreover, they are not toxic in comparison of catalysts, which are usually used for this synthesis.
In this paper, the influence of the lanthanides on both the hydrolytic and enzymatic degradation of the PCL obtained by ring-opening polymerization of ε-caprolactone with different lanthanide-based catalysts such as: lanthane chloride ($LaCl_3$), ytterbium chloride ($YbCl_3$) and samarium chloride ($SmCl_3$) was assessed.
Samarium seems to slightly accelerate the hydrolytic degradation of the polymer and to slow down or inhibit its enzymatic degradation, mainly when the molecular weight of the polymer is high. The behaviour of PCL containing another lanthanide like lanthane is dependent on the nature of the metallic ion. Complete degradation, by the Lipase PS from *Pseudomonas cepacia*, is achieved only with Ytterbium.

Keywords: biodegradation; hydrolyse; lipase PS; polycaprolactone, rare earth

Introduction

Biodegradable polymers can find wide application in medical field and environment aspect as prosthesis, bandages, packaging materials, or controlled drug release matrix for active principles (drugs, pesticides). Most of the currently available biodegradable polymers are used for either of the two purposes, but some of them like starch, chitosan, poly -(L-lactide) (PLLA) and poly -(ε-caprolactone) (PCL) are applicable for both.[1]

In accordance with their applications areas, biodegradable polymers with corresponding biodegradation rates should be synthesised. For example, a drug-delivery system with an

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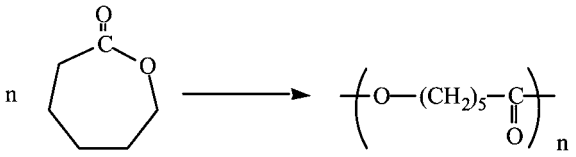
implantable period of one year requires a biodegradable polymer with a slow degradation rate, such as PCL, under the trade name Capronor. On the other hand, the release of pesticides in soil should be very rapid because plants need the active principle to be delivered in a short period. Therefore PCL with desired degradation rates should be synthesised and the degradation rate should be determined by an effective method.

During the recent decades, synthesis of PCL has received a great attention. The polymer is prepared by catalysed ring-opening polymerisation of ε-caprolactone, a 6-member ring cyclic ester, through anionic, cationic or co-ordinate polymerisation, according to the following scheme:



A wide variety of catalysts can be used for this synthesis.[2-5] Among these catalysts, lanthanide-based catalytic systems proved to be very active in ring-opening polymerization.[6-9] Moreover, these lanthanide-based catalysts are non-toxic in comparison of classic catalysts like stannous octanoate or aluminium-and zinc-alkoxides which are usually used in the ring-opening polymerisation of ε-caprolactone. Mortality studies have revealed that rare earth is not highly toxic.[10] For instance, LD_{50} of samarium chloride ($SmCl_3$) is the same as that of NaCl.

Enzymatic degradation of PCL was studied by many authors.[11-16] It was reported that two kinds of enzymes, lipases and esterases, can degrade PCL. Among the numerous lipases being able to degrade this polymer, Gan *et al.* showed that the Lipase PS from *Pseudomonas cepacia* was the most efficient one, as a PCL film could degrade rapidly and completely within 4 days in a phosphate buffer solution containing this enzyme.[13] It was also indicated that the enzymatic biodegradation happens mainly on the surface, because it is difficult for a hydrophilic enzyme to diffuse into a hydrophobic polymer.[16]

In this paper, the influence of the lanthanides on both the hydrolytic and enzymatic degradation of the PCL obtained by ring-opening polymerization of ε-caprolactone with different lanthanide-

based catalysts: lanthane chloride (La), ytterbium chloride (Yb) and samarium chloride (Sm) was assessed. The importance of the degradations was evaluated by weight loss. The evolution of crystallinity and molecular weight of PCL were followed all over the degradations. The degradation behaviour of PCL of different molecular weight with and without samarium was also compared. Finally, the degradation mechanism of PCL is discussed.

Experimental

Materials

ε-Caprolactone (Acros) containing ca 0.1 % water (measured by Karl Fischer analysis) was used without further purification. Commercial samples of lanthanide halides: $SmBr_3$.$6H_2O$; $SmCl_3$.$6H_2O$; $YbCl_3$.$6H_2O$; $LaCl_3$.$7H_2O$ were purchased from Aldrich, THF adducts: $SmCl_3$ .3THF were synthesised according to the published methods[7]. Lipase PS from Pseudomonas cepacia was purchased from Sigma. One unit of the lipase (U) corresponds to the amount of enzyme which liberates one μmol oleic acid from triolein per min at pH 8.0 and 40°C.

Thermal synthesis

The reactions were performed by conventional heating under conditions described in table 1. The reactions were carried out in 100mL flasks, equipped with condenser and magnetic stirrer. The flasks were placed in a thermostated oil bath previously heated at the required temperature. Temperature was kept constant and measured inside the reaction mixture.

Table 1. Characteristics of PCL obtained by ring-opening polymerisation

Run	Catalyst 1%	Temperature °C	Time Hours	Mn g/mol	PDI Mw/Mn	Crystallinity %
PCL 6	$SmBr_3$ .6 H_2O	200	45	6 500	1.2	70
PCL 16	$SmCl_3$ .3 THF	200	45	16 200	1.2	77
PCL 65	$SmCl_3$ .3 THF	200	90	64 600	1.8	66
PCL Sm	$SmCl_3$ .6 H_2O	230	25	4 500	2.0	82
PCL La	$LaCl_3$ .7 H_2O	230	20	5 800	1.5	81
PCL Yb	$YbCl_3$ .6 H_2O	230	20	6 000	1.6	80

Two series of PCL were obtained: one presenting different molecular weight from 6 500 to 64 600 (PCL 6, 16 and 65) to evaluate the influence of this parameter, the other with the same molecular weight (about 6 000) but synthesised from different lanthanide-based catalysts (PCL Sm, La and Yb).

Hydrolytic and enzymatic degradation

Substrates were ground and the fractions comprised between 0.1 and 1 mm used for hydrolytic and enzymatic degradations tests. A part of each sample was dissolved in dichloromethane and precipitated in methanol to eliminate the lanthanide catalysts. Polymers were then vacuum dried at room temperature for 48 hours before degradation tests. The effect of lanthanide on the hydrolytic and enzymatic degradation of poly-(ε-caprolactone) can thus be evaluated. Hydrolytic degradation of PCL (200 mg) was carried out in 10mL of pH 7 phosphate buffer solution (Sigma), containing 0.02% NaN_3 to prevent micro-organism growth. Enzymatic degradation was performed with the same amount of PCL but with 5mL of phosphate buffer solution (with 0.02% NaN_3) and 5mL of enzymatic solution (2.5 U/mg polymer) in distilled water (2mg/mL). In the substrate control, the enzyme was omitted from the reaction mixture, while in the enzyme control, the substrate was omitted. Degradations were conducted in 16mL tubes incubated at 37°C and 100 rpm. During the degradations, a tube was periodically withdrawn from the incubator (Fisher Bioblock Scientific) and its content entirely filtered through a Whatman filter (0.5 μm pore size) to separate PCL powder from the degradation medium. Remaining PCL powders were washed with distilled water and vacuum dried at room temperature to constant weight. Hydrolytic and enzymatic degradations are expressed as percentage weight loss:

$$\text{Weight loss (\%)} = [(Wo - Wi) / Wo] \times 100$$

where Wo and Wi are weight of powders before and after degradation tests, respectively.

PCL powders and degradation media were kept at room temperature and –20°C for further analysis.

Characterization

The polymers were characterized before and during degradations by several techniques:

Size Exclusion Chromatography (SEC) analyses were carried out in THF solution (10mg/mL, 20°C, flow rate 1mL/min) on a Spectra System P1000 apparatus, equipped with two 5µm mixed-C PL gel columns and a Shodex RI71 refractive index detector. Polystyrene standards were used for column calibration and Mark-Houwink corrections were performed for determination of the average molecular weights.

Differential Scanning Calorimetry (DSC) was performed by using a TA Instruments 2920 apparatus, at a heating rate of 10°C/min under nitrogen atmosphere. To evaluate the crystallinity of PCL, ΔHf of PCL completely crystalline estimated to 139.5 J/g was used, according to the literature.

Nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded on a Bruker Avance 300 spectrometer. As degradations were conducted in aqueous media, spectra were recorded using pre-saturation sequence to eliminate the water signal. 10 to 20% of D_2O were added in the tubes for locking.

Results and Discussion

Influence of the molecular weight

The degradation study of three PCL samples of molecular weight varying from 6 000 to 65 000 with and without lanthanides was performed in hydrolytic (figure 1) and enzymatic (figure 2) media.

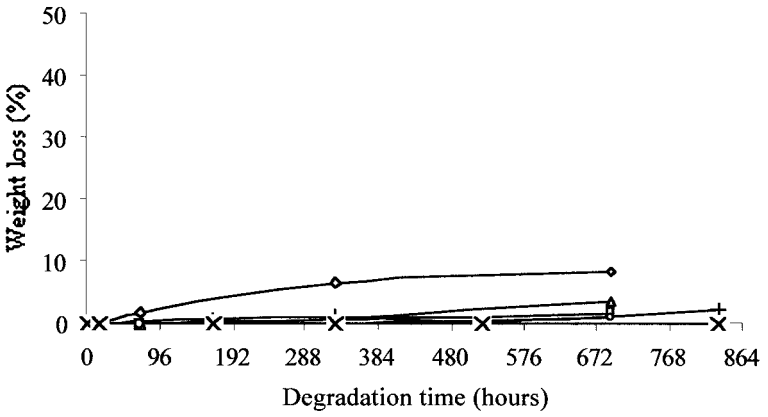


Figure 1. Weight loss during the hydrolytic degradation of three PCL samples of different molecular weight with samarium (◊ PCL 6, △ PCL 16, + PCL 65) and without samarium (□ PCL 6, ○ PCL 16, × PCL 65).

The measured weight losses were very low during the hydrolytic degradation (figure 1): PCL 6 with remaining samarium shows a weight loss of 10% after 700 hours against 2% for the same sample without samarium. This seems to indicate that the presence of the lanthanide derivative weakly accelerates the hydrolytic degradation of PCL. When the molecular weight of the polymer is larger, the hydrolytic degradation is too low to observe any significant difference between PCL with and without Sm.

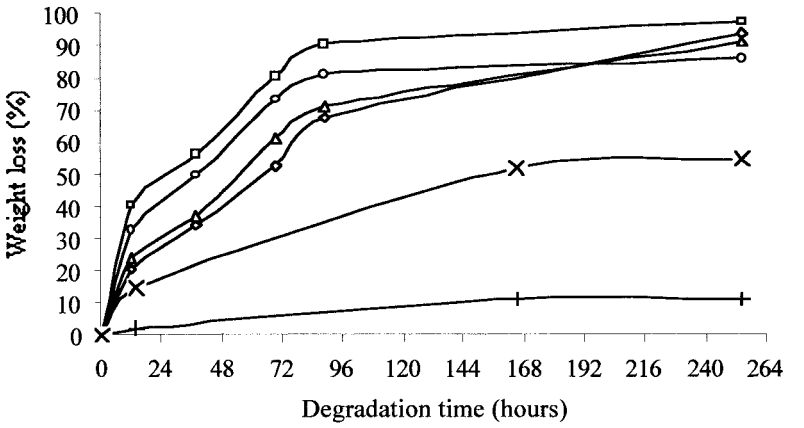


Figure 2. Weight loss during the enzymatic degradation of three PCL samples of different molecular weight with samarium (◊ PCL 6, △ PCL 16, + PCL 65) and without samarium (□ PCL 6, ○ PCL 16, × PCL 65).

The enzymatic degradation is much more important and rapid than the hydrolytic one as the samples of low molecular weight are degraded beyond 85% in less than 250 hours. Concerning PCL 6, more than 90% of the polymer is degraded in 250 hours when the sample was not purified and it was completely degraded by the Lipase in only 96 hours when the samarium was eliminated. This suggests that the presence of the metal slows down the enzymatic degradation. The same phenomenon seems to exist for PCL 16 even if its importance is lower. Nevertheless, the presence of samarium does not prevent the degradation of PCL of low average molecular weight.

At the same time, the weight loss is much less important for PCL 65 when it contains the lanthanide. Effectively, only 11% of Sm containing PCL was degraded by lipase whereas 55% of this polymer without Sm disappeared. In this case, the samarium complex apparently inhibited the activity of the Lipase.

Influence of the lanthanide on the degradation of PCL

The degradation study of three PCL samples of the same molecular weight (about 6 000) but obtained from different lanthanides (samarium, lanthane or ytterbium) was performed in enzymatic medium (figure 3).

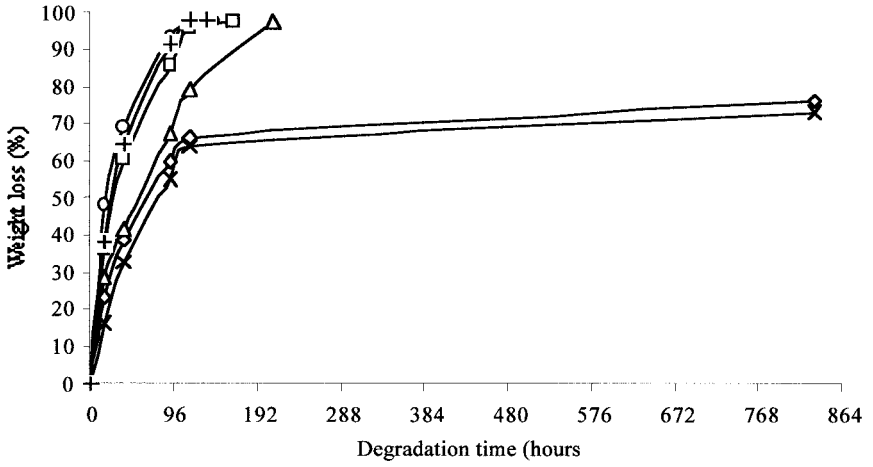


Figure 3. Weight loss during the enzymatic degradation of three PCL samples of molecular weight of about 6 000 containing ◊ La, △ Yb, ✕ Sm, and not containing □ La, ○ Yb, + Sm remaining residues of catalysts.

The enzymatic degradation of PCL purified from lanthanide residues (samarium, ytterbium or lanthane) is the same for the three samples. The polymer was completely degraded by the Lipase PS in less than 96 hours without any significant difference between the different samples.

In presence of the rare earth, the degradation behavior was different depending on the lanthanide used for the synthesis. Effectively, the enzymatic degradation with ytterbium is complete after 192 hours (8 days) whereas only 76.5 and 73.6 percent of the polymer degraded after 840 hours (35 days) for PCL containing lanthane and samarium, respectively. This confirms that the presence of these lanthanides slows down or inhibits the enzymatic degradation of PCL.

Complementary work is now undertaken in our laboratory to determine how the inhibition of the Lipase by the metal could proceed.

Evolution of the characteristics of PCL during the degradations

Evolution of the crystallinity and the molecular weight of the polymer was also followed during all degradation tests. As an example, the results obtained for PCL 6 with and without Sm residues in hydrolytic and enzymatic media are presented tables 2 and 3, respectively.

Table 2. Evolution of molecular weight and crystallinity of PCL 6 with and without Sm residues during the hydrolytic degradation.

Hydrolytic	Mn (g/mol)		PDI (Mw/Mn)		Crystallinity (%)	
Time (hours)	PCL 6 (Sm)	PCL 6	PCL 6 (Sm)	PCL 6	PCL 6 (Sm)	PCL 6
17	6 400	6 450	1.2	1.2	70	87
68	5 940	6 430	1.3	1.2	82	82
327	5 860	6 300	1.2	1.2	78	78
688	5 750	6 180	1.3	1.3	81	81

Table 3. Evolution of molecular weight and crystallinity of PCL 6 with and without Sm residues during the enzymatic degradation.

Enzymatic	Mn (g/mol)		PDI (Mw/Mn)		Critallinity (%)	
Time (hours)	PCL 6 (Sm)	PCL 6	PCL 6 (Sm)	PCL 6	PCL 6 (Sm)	PCL 6
12	5 920	6 240	1.2	1.3	74	76
37	5 850	6 200	1.2	1.3	75	74
69	5 840	6 250	1.2	1.3	75	72
88	5 780	6 260	1.3	1.2	74	70

Before degradation, the percentage of crystallinity for PCL 6 was 70%, its molecular weight 6 500 g/mol and its polydispersity index 1.21 (Table 1).

Initially, the crystallinity increased during the hydrolytic degradation indicating the preferential degradation of the amorphous phase [16]. The decrease of the number average molecular weight was more important for PCL 6 with Sm (11%) than the same sample without the lanthanide

(5%). This result confirms that the presence of Sm in PCL accelerates slightly its hydrolytic degradation. Finally, this slight increase of crystallinity and decrease of molecular weight could be explained by water that penetrates to some extent within the amorphous phase.

Concerning the enzymatic degradation, crystallinity increased to 76% after only 12 hours for PCL without samarium, then decreased to 70% at the end of the test, which seems to indicate that crystalline phase is attacked. For PCL containing samarium, crystallinity increased to 75% after 12 hours and stay constant all over the degradation. In this case, because of the inhibition of the Lipase by the metal, the degradation of the polymer could be slow down which could explain that its crystallinity is stable after 88 hours.

No significant variation of Mn and PDI is observed between the hydrolytic and enzymatic degradation, which seems to demonstrate that the degradation occurs almost exclusively at the polymer surface.

Determination of the degradation by-products by ^{1}H-NMR

^{1}H-NMR analysis was used to determine the by-products of PCL degradation. An example of spectrum obtained after an enzymatic degradation test is given in figure 4.

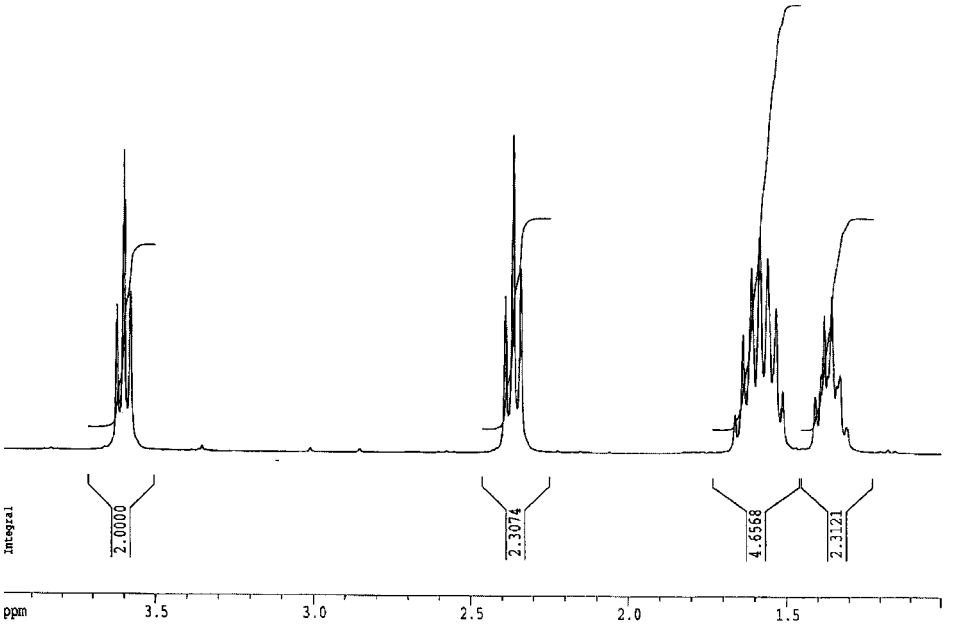


Figure 4. typical ^{1}H-NMR spectrum obtained during an enzymatic degradation.

Chemical shifts are characteristic from hydroxycaproic acid, specially the resonance of CH_2-O which appears at 3.6 ppm instead of 4 ppm as for poly-(ε-caprolactone). All the degradation media were tested and the only product of degradation identified was hydroxycaproic acid. This result is in agreement with the literature which indicates that hydroxycaproic acid is a pharmacologically inactive biodegradation product from PCL which can be absorbed by body or removed by metabolism.[17]

Conclusion

This investigation confirms that the enzymatic degradation of PCL is much more rapid and important than the hydrolytic one.

Samarium, used as initiator of polymerisation for PCL, seems slightly accelerate the hydrolytic degradation of the polymer and to slow down or inhibit its enzymatic degradation, mainly when the molecular weight of the polymer is high. The behavior of PCL samples containing another

lanthanide like lanthane confirms that the enzymatic degradation is highly slowed down or inhibited by the rare earth. Complete degradation by the Lipase PS from *Pseudomonas cepacia* is achieved only with ytterbium

Finally, enzymatic degradation of the polymer gives only hydroxycaproic acid as product of degradation.

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