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# Synthesis of an X-ray opaque biodegradable copolyester by chemical modification of poly ( $\epsilon$ -caprolactone)

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## Abstract

Poly ( $\epsilon$ -caprolactone-*co*- $\alpha$ -iodo- $\epsilon$ -caprolactone) was synthesized by binding iodine to PCL chain bearing carbanionic site on  $\alpha$ -position of carbonyl groups using lithium diisopropylamide. Copolyesters containing from 10% to 25% of iodo-units were thus obtained. Viscoelastic properties (modulus, loss angle), thermal properties ( $T_m$ ,  $T_c$ ,  $\Delta H_m$ ), crystallinity and in vitro degradability of this new type of copolymers were measured. Their opacity to X-rays was assessed, and appeared high enough to be of interest for biomedical applications.

*Keywords:* Polycaprolactone; Copolymer; Radio-opacity; Degradation

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## 1. Introduction

Polymeric biomaterials featuring intrinsic radio-opacity continue to attract considerable scientific attention since it is highly desirable to visualize an implant or an embolization device via routine X-rays radioscopy. However polymeric materials are transparent to X-rays and when a polymeric biomaterial has to be implanted in the human body, it is often made radio-opaque by addition of an X-ray absorbing additive. Most of these radio-opaque additives are mineral compounds such as barium salts or zirconium dioxide [1,2]. Sometimes an appropriate ligand was added to chelate the opacifier [3]. However physical, physico-chemical and mechanical properties are often affected by the incorporation of these additives [1,4]. The most important drawbacks are the following: (i) radio-opaque fillers have to be added to rather high concentrations, (ii) metal are not covalently bound to the polymer and thus they can leach into the body fluids making radio-opacity a temporary phenomenon, and leading to potential systemic toxicity [5,6], and (iii) inorganic salts and polymer do not mix homogeneously. It was found on commercial

dental composites that only 31% exhibit adequate X-ray opacity [7].

Another approach to make a polymer radio-opaque is to covalently link a radiocontrast dye, typically containing an iodine moiety, to the polymeric backbone. Some iodine-containing methacrylates were synthesized and copolymerized with monomers such as methyl methacrylate (MMA) or 2-hydroxy-ethyl methacrylate (HEMA) [7–10]. Radio-opaque polyurethanes were also synthesized, starting either from iodine-containing monomers like diatrizoic acid derivatives [11] or from commercially available medical grade aliphatic polyurethane in which amino groups of the polymer chain were coupled to a iodine-containing molecule [12]. Finally it is noteworthy that biocompatible cellulose and cellulose acetate were also evaluated as radio-opaque materials after coupling of iodine-containing moieties [13].

Poly ( $\epsilon$ -caprolactone) (PCL) is a biocompatible aliphatic polyester of interest for biomedical and pharmaceutical applications, which has been proposed to make sustained release drug delivery systems because of its high permeability to drugs and despite its relatively low degradation rate in vivo. We recently proposed a simple versatile method to chemically modify aliphatic polyesters chains, especially PCL [14]. This method is based on the anionic

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activation of the PCL chain by the removal of a proton of the methylene group in  $\alpha$  position of the ester carbonyl, using a non-nucleophilic strong base such as lithium diisopropyl amide (LDA). In a second step an electrophilic reagent is added to the generated macropoly-carbanion (Scheme 1).

This rapid easy and versatile method yields a substituted PCL with substitution ratios in the 10–20% range. In this paper we wish to report the formation of a new iodo-PCL (I-PCL) copolymer obtained by substitution of iodine on the PCL backbone. Some physico-chemical and viscoelastic properties as well as radio-opacity of the I-PCL were investigated.

## 2. Materials and methods

PCL ( $\overline{M}_n = 42,500$ ;  $\overline{M}_w = 80,000$ ) and LDA (2 M/L in THF/*n*-heptane/ethylbenzene) were obtained from Aldrich (St. Quentin Fallavier, France) and used without further purification.

NH<sub>4</sub>Cl was obtained from Fluka (St. Quentin Fallavier, France), anhydrous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> from Acros Organics (Noisy-le-Grand, France), MgSO<sub>4</sub> from Prolabo (Paris, France), CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>OH from Riedel de Haën (St. Quentin Fallavier, France) and were used as received.

THF was obtained from Acros Organics (Noisy-le-Grand, France) and distilled on benzophenone/sodium until a deep blue color was obtained.

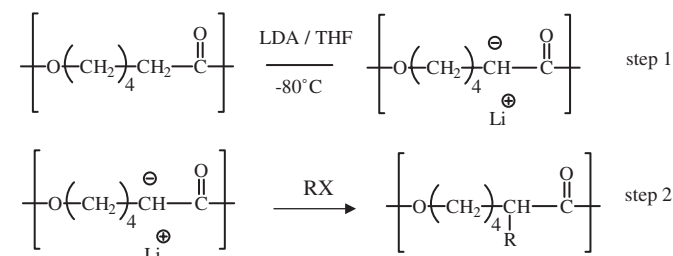
Iodine was obtained from Aldrich (St. Quentin Fallavier, France) and was used without further purification.

### 2.1. Substitution reaction

Typically, PCL (0.035 mol, 4 g) was dissolved in anhydrous THF (100 cm<sup>3</sup>) in a reactor equipped with a mechanical stirrer and kept at –78 °C under argon atmosphere. A solution of LDA (0.035 mol, 17.5 cm<sup>3</sup>) was injected with a syringe through a septum and the mixture was kept at –78 °C for 30 min. Typically iodine (0.035 mol, 8.9 g) was dissolved in a few cm<sup>3</sup> of THF before addition and the reaction was carried on for 30–120 min depending on the trial. The mixture was then hydrolyzed with 200 cm<sup>3</sup> of a 2 M aqueous solution of NH<sub>4</sub>Cl (20 g NH<sub>4</sub>Cl/dm<sup>3</sup>). pH was adjusted to  $\approx 7$  by using HCl 37%. The copolymer was extracted with dichloromethane (2  $\times$  100 cm<sup>3</sup>). The combined organic layers were washed three times with a solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (3  $\times$  100 cm<sup>3</sup>, 3 eq./I<sub>2</sub>), dried on anhydrous MgSO<sub>4</sub> and filtered. The solvent was partially evaporated under reduced pressure. The concentrated copolymer solution was treated with an excess of methyl alcohol. The precipitated copolymer was washed with methyl alcohol until the solution was clear. The copolymer was then dried under vacuum for several hours.

### 2.2. Methods

Molecular weights were determined by size exclusion chromatography (SEC) using a Waters equipment fitted with a 60 cm long 5  $\mu$ m mixed C



Scheme 1. General scheme for PCL anionic modification.

PLgel column as the stationary phase, THF at 1 cm<sup>3</sup>/min flow rate as the mobile phase, and a Waters 410 refractometric detector, as reference to polystyrene standards. Typically, polymer (10 mg) was dissolved in 2 cm<sup>3</sup> of THF and the resulting solution was filtered on a 0.45  $\mu$ m Millipore filter before injection of 20  $\mu$ L of sample solution.  $\overline{M}_n$  and  $\overline{M}_w$  are expressed according to calibration using polystyrene standards. For degradation investigations a Waters PDA 2996 photodiode array detector was added.

<sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) spectra were recorded at room temperature using a AMX300 Bruker spectrometer operating at 300 MHz. Deuterated chloroform or dimethylsulfoxide was used as solvents, and chemical shifts were expressed in ppm with respect to tetramethylsilane (TMS).

Infrared (IR) spectra were recorded with a Perkin–Elmer 1760 FTIR spectrometer, polymers being cast on NaCl plates from solvent solution.

Viscoelastic properties were investigated on a Metravig Viscoelastimeter (Limonest, France) at 27 and 38 °C using a cyclic stress: compression and traction frequencies were in the range (15.2–500) Hz and the imposed deformation length was in the range (0.4–4)  $\mu$ m.

Radio-opacity was determined by using a Prostyle intra/Planmeca system using the following irradiation parameters: 63 kV/8 mA/0.02 s.

## 3. Results and discussion

### 3.1. Synthesis of I-PCL

The substitution reaction corresponded to the second step in Scheme 1 with iodine as the electrophilic species as shown in Scheme 2.

Four copolymers, I-PCL1 to I-PCL4, were synthesized using the various experimental conditions shown in Table 1.

### 3.2. Characterization of I-PCL

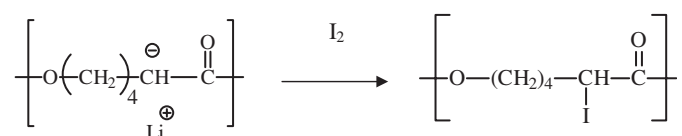
#### 3.2.1. Iodine

The extent of substitution was calculated from the <sup>1</sup>H NMR ratio of the integration of peaks at 4.5 ppm (methyne of CH<sub>2</sub>–CHI) or 2 ppm (methylene of CH<sub>2</sub>–CHI) versus integration of the peak at 4.1 ppm (methylene of O–CH<sub>2</sub>) (Fig. 1).

The binding of iodine was confirmed by solid state <sup>13</sup>C NMR on the I-PCL gel obtained in the first run, where peaks corresponding to the carbon atoms of iodated PCL appeared as shoulders of the main peaks (Fig. 2). It is noteworthy that in run I-PCL1, carried out at higher reaction temperature, a higher iodine content was obtained, leading to a partially cured structure. It was assumed that intermolecular iodine elimination can occur in presence of the carbanionic charges.

#### 3.3. Molar masses

Chromatograms of I-PCL obtained in runs 2–4 (corresponding to polymers soluble in THF) are shown in Fig. 3:



Scheme 2. Synthesis scheme of formation of I-PCL.

Table 1  
Main result of I-PCL synthesis via anionic substitution reaction

Run	$t$ (min)	$T$ (°C)	Yield (%)	Iodine (%)	$M_n$ (g/mol)	$M_w$ (g/mol)	PdI
Initial PCL				0	35,500	56,800	1, 6
I-PCL1	50	[-50; -45]	30	25	Gel <sup>a</sup>	/	/
I-PCL2	30	[-70; -65]	83	10	9500	45,600	4.8
I-PCL3	50	[-70; -65]	66	14	7000	19,600	2.8
I-PCL4	120	[-70; -65]	74	11	6000	21,600	3.6

<sup>a</sup>Copolymer was insoluble in organic solvents, only an important swelling was observed.

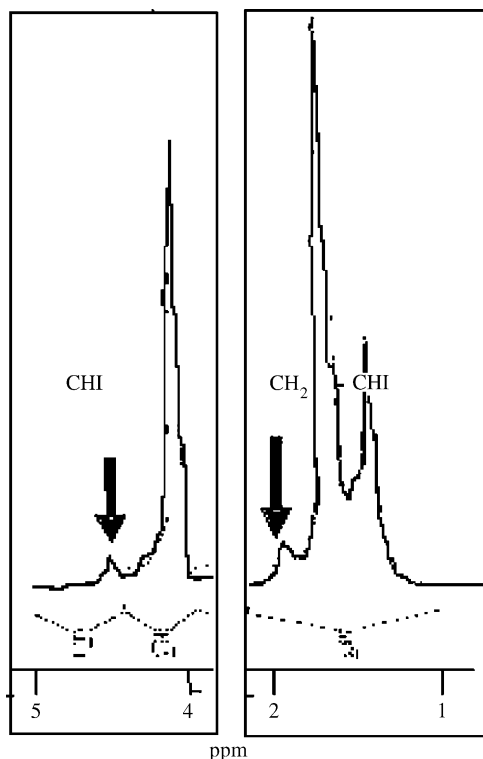


Fig. 1. <sup>1</sup>H NMR of I-PCL run 1 in CDCl<sub>3</sub>.

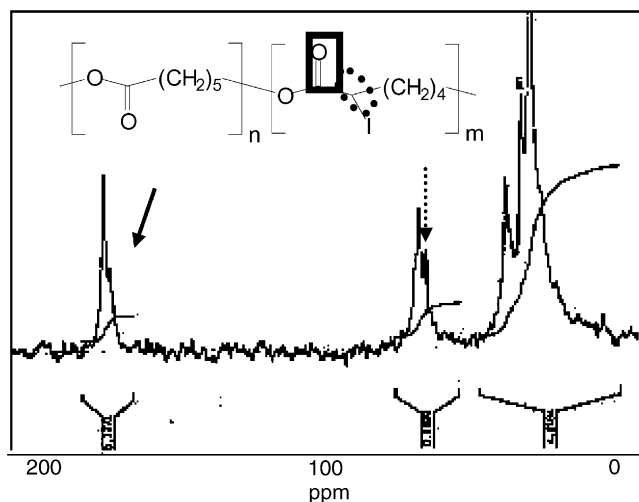


Fig. 2. <sup>13</sup>C NMR of I-PCL run 1 in CDCl<sub>3</sub>.

It can be shown that chain breakings are occurring during the substitution reaction, in agreement with previous results [9]. All chromatograms were monomodal and a continuous decrease of  $\overline{M}_n$  occurred as a function of reaction time in the second step, as shown in Fig. 4. The decrease of  $\overline{M}_n$  is dramatic during the first minutes and then becomes slower. Polydispersity is high, between 2.8 and 4.8, due to the occurrence of chain breakings and intermolecular bonds. Chain scissions occurring during the substitution reaction lead to the formation of shorter copolymers end-capped with a carboxylic acid chain end as previously described [14].

### 3.4. Crystallinity and thermal properties

Thermal characteristics (melting point  $T_m$ , crystallization temperature  $T_c$ , and melting enthalpy  $\Delta H_m$ ) of I-PCL2 and I-PCL3 were evaluated by DSC. Results are shown in Table 2 and in Fig. 5. All values were obtained during the second thermal run of DSC.

The binding of iodine on PCL chain led to changes in thermal characteristics. Melting and crystallization occur at lower temperatures ( $\Delta T \approx 15^\circ\text{C}$ ). Iodo copolymer was less crystalline than PCL, as shown by the lower value of  $\Delta H_m$ . It was confirmed by X-ray diffraction data that show a crystallinity of 50% for the PCL and 42% for the I-PCL. Crystallinity degree was obtained from X-ray diffractogram as the ratio: area of the crystalline peak on area of the amorphous diffuse region.

### 3.5. Viscoelastic properties

Viscoelastic properties were investigated at 27 °C (room temperature) and 38 °C ( $\approx$  physiological temperature) on polymer plates submitted to cyclic stress. Compression and traction frequencies were ranging between 15.2 and 500 Hz and the imposed deformation length was in the range 0.4–4  $\mu\text{m}$ . Each run was done in triplicate. Plates were prepared by molding samples, commercial PCL and 14% substituted I-PCL, at 100 °C under pressure. This dynamic experiment gave access to the dynamic modulus of the polymer which corresponded to the Young modulus provided the dynamic stress applied stays in the range of the elastic domain.

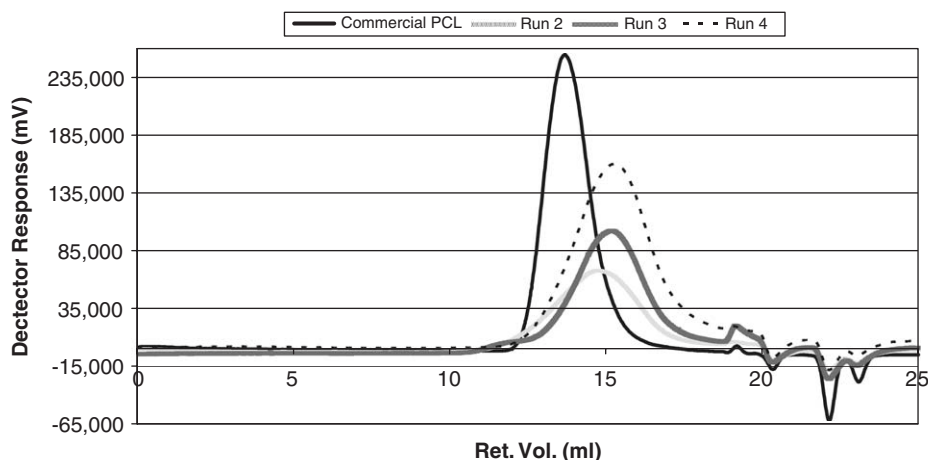


Fig. 3. Chromatograms of THF solutions of PCL, and I-PCL.

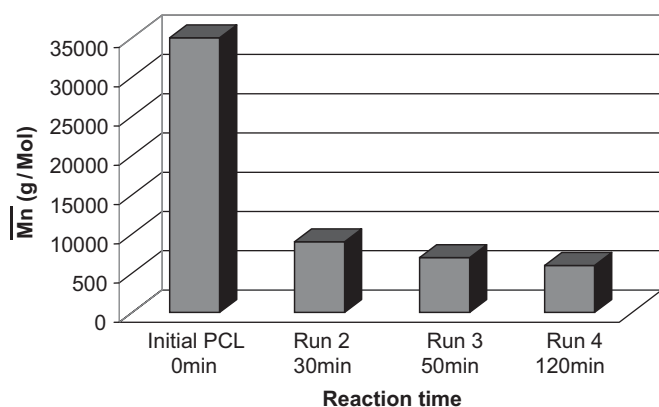


Fig. 4. Evolution of I-PCL  $\overline{M}_n$  as a function of the reaction time.

Table 2  
Thermal characteristics of PCL and I-PCL

Sample	$T_m$ (°C)	$T_c$ (°C)	$\Delta H_m$ (J/g)
PCL	60	23	68
I-PCL 2	47	8	30
I-PCL 3	46	10	32

It can be seen in Fig. 5 that the dynamic modulus of I-PCL (13 MPa) was about five times lower than the PCL one (60 MPa). In the same time, loss angle remained quite low for both samples, with typical values of 1–10°, showing an elastic behavior of the material.

Fig. 6 shows the effect of temperature on the mechanical properties of the 14% I-PCL plate. The same plate was used at the two temperatures. When temperature rose from 25 to 38 °C the Young modulus was divided by a factor 1.5–2.

### 3.6. Radio-opacity

Radio-opacity was investigated on 100 mg polymer disks obtained by dissolution of polymer in chloroform and

evaporation of the solvent. Disks were 11 mm diameter, the center of the disks was 370  $\mu\text{m}$  thick ( $e$ ) and the border 2 mm thick ( $E$ ) (Scheme 3). Fig. 7 presents pictures of disks taken with a Prostyle intra/Planmeca system using the following irradiation parameters: 63 kV/8 mA/0.02 s.

It is observed that PCL is totally transparent while a detectable radio-opacity is obtained even with limited thickness and iodine content. A 10% iodine-substituted PCL is corresponding to 11 wt%. It is reported that materials containing 15 and even 8 weight percentages are well detected with comparable radio-opacity to that of a 2 mm aluminum wedge [8,12]. Direct substitution of iodine on the polyester backbone gave an appropriate radio-opacity. This was not the case for cellulosic compounds in which the use of aromatic iodo-compound was necessary to improve X-ray opacity [13]. This PCL-based material can offer many applications as radio-opaque biodegradable construction material or drug release systems.

### 3.7. Hydrolytic degradation

Hydrolytic degradation was followed on I-PCL plates obtained by evaporation of a solution of I-PCL in  $\text{CHCl}_3$ . Plate area was  $\approx 1 \text{ cm}^2$  and thickness  $\approx 610 \mu\text{m}$ . Plates were placed in a pH = 7.4 phosphate buffer at 37 °C to mimic in vivo conditions. After 174 days, the degradation level was evaluated using:

- The water absorption, defined as  $m_{d(t)} - m_{vd(t)}/m_{d(t)}$ ,  $m_{d(t)}$  standing for the copolymer mass at time  $t$ , and  $m_{vd(t)}$  standing for the copolymer mass at time  $t$  after a vacuum drying at  $10^{-3}$  bar. Excess of water was removed by gently sweeping the plate surface with an absorbent tissue. PCL absorbed only 0.5% of water after 60 weeks [15]. No water absorption was found in I-PCL after 25 weeks and 3% after 70 weeks.
- The weight loss of the samples, reflecting the release of soluble oligomers formed during degradation, which is

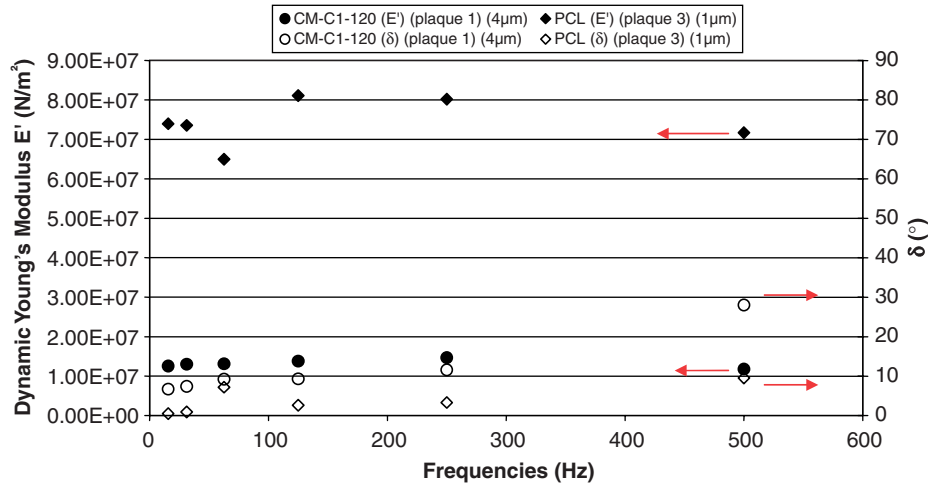


Fig. 5. Young modulus and loss angle of PCL and I-PCL run 3.

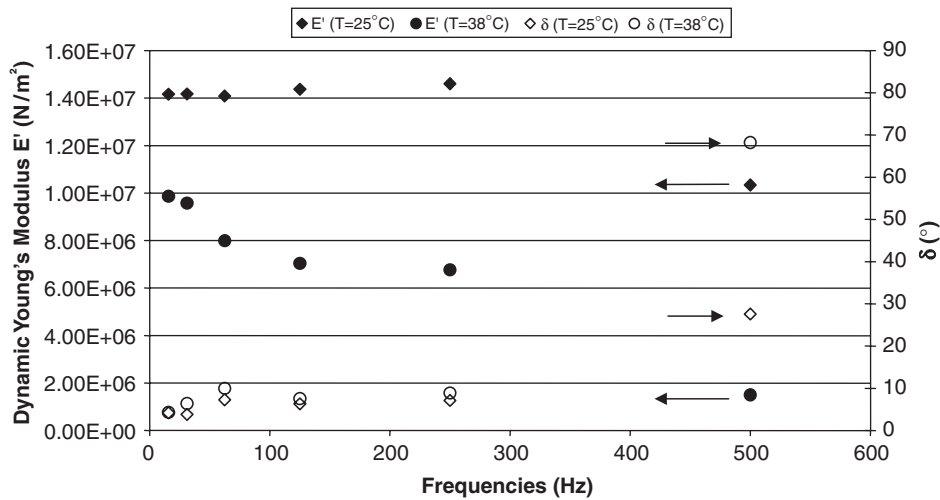
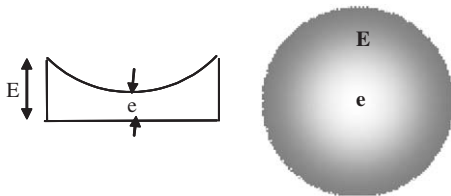


Fig. 6. Influence of temperature on Young modulus and loss angle of I-PCL run 3.



Scheme 3. I-PCL disks aspects: thickness  $E = 2$  mm, thickness  $e = 370 \mu\text{m}$ .

defined as follow:  $m_{vd(t)} - m_{vd(t)}/m_{vd(t0)}$ ,  $m_{vd(t0)}$  standing for the copolymer mass before degradation. No significant weight loss was detected in PCL during a degradation period of 60 weeks [15], while an 8% weight loss was found in I-PCL samples after 25 weeks and 13% after 70 weeks. This is reflecting a greater sensitivity of I-PCL to hydrolytic degradation, due to both the lower crystallinity ratio and the lower molar mass.

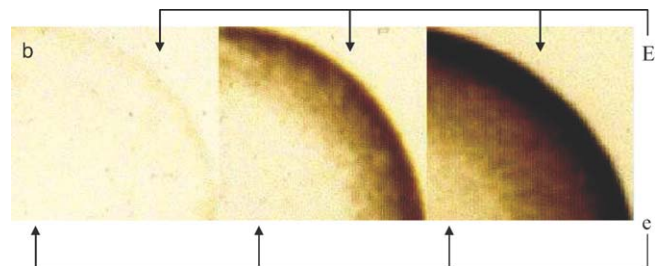


Fig. 7. From left to right: PCL transparent, mixture PCL/I-PCL 50/50 w/w, I-PCL 10 mol% iodine.

Water soluble degradation byproducts were studied by capillary zone electrophoresis (CZE). Signals corresponding to iodide and/or iodate were detected. These compounds can result either from iodine trapped into the polymer chains, which is rapidly dismuted in CZE eluent, or from the breaking of the carbon-iodine bond during

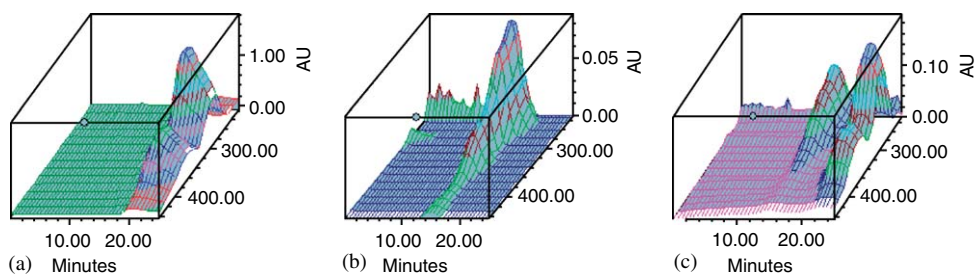


Fig. 8. GPC chromatograms of (a) THF solution of  $I_2$ ; (b) freshly prepared THF solution of I-PCL 0 min; and (c) 24 h THF solution of I-PCL.

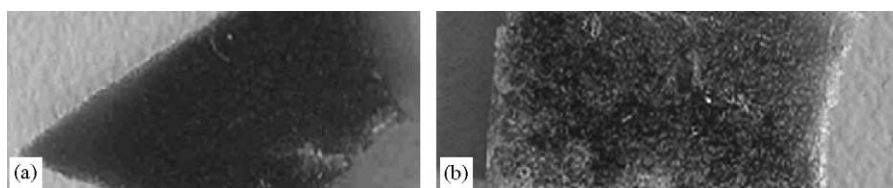


Fig. 9. (a) Surface of I-PCL at  $t_0$  and (b) surface of I-PCL after 25 weeks degradation.

degradation. The last assumption was confirmed by GPC using a diode array detector. The chromatogram of a freshly prepared polymer solution exhibited a single peak at  $\approx 14$  min corresponding to the I-PCL, while an additional peak at  $\approx 20$  min, whose spectrum is characteristic of iodine, appeared in the aged solution (Fig. 8).

DSC thermograms of degraded I-PCL showed no significant changes in  $T_m$ ,  $T_c$  and  $\Delta H_m$  values as compared to the original I-PCL.

Composition of the degraded copolymer, determined from  $^1H$  NMR spectra, showed also a tiny decrease of the proportion of iodine (substitution ratio decreased from  $\approx 11\%$  to  $9.5\%$ ).

### 3.8. Visual aspect of I-PCL

Pictures of degraded I-PCL plates were taken after a 25 weeks degradation time. Pictures in Fig. 9 show the modification of the roughness during the degradation process. Holes, porosities and decrease of the intensity of the brownish color could be observed. These changes were not detected in genuine PCL plates.

## 4. Conclusion

Covalent binding of an iodine atom on a PCL backbone was achieved by a rapid and easy two steps one pot substitution reaction of iodine on the macropolycarbanion formed by addition of LDA on PCL. Substitution degrees up to 25% were obtained. I-PCL is a radio-opaque degradable aliphatic copolyester containing no additional radio-opaque filler, which has potential applications in temporary reconstructing material or drug delivery system.

This new PCL-based copolymer exhibits interesting properties in term of mechanical and degradation

properties and radio-opacity. On one hand, a lower dynamic Young's modulus was found compared to PCL, with a typical value of 13–14 MPa at room temperature. On the other hand, degradation rate of the copolymer is higher than the one of PCL, an 8% weight loss being observed in the I-PCL after 25 weeks. At last an I-PCL containing a 10% iodine weight ratio showed an easily detectable radio-opacity, even when mixed with a 50% weight ratio radio-transparent PCL.

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