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SYNTHESIS OF [^{3}H]-LABELLED POLY(ε-CAPROLACTONE)

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SUMMARY

Tritiated poly(ε-caprolactone), where part of the methylene-type protons close to the ester carbonyl groups are replaced by tritium, was synthesized. The substitution was based on the elimination of some of the hydrogen atoms by a poorly nucleophilic strong base, namely lithium diisopropylamide (LDA). This first step led to the formation of carbanionic sites on the main chain of the polymer. The second step consisted of an attack on the carbanion by tritiated water (HTO) which resulted in $^1H \rightarrow ^3H$ exchange along the polymer chains. The specific activity of the final compound was 5.63 MBq/g (152 μCi/g) equivalent to 2% substitution reaction. The radiolysis ratio of the tritiated polymer was rather low (0.35 % per month). The method is very versatile and paves the road to deuterated and tritiated poly(ε-caprolactone)s.

Key words : Poly(ε-caprolactone), Lithium diisopropylamide, Polymer modification, Tritium labelling, Biodegradable polymers.

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INTRODUCTION

Poly (ε-caprolactone) (PCL) is a biocompatible and degradable polyester derived from ε-caprolactone, which itself is a monomer that can also be combined with other cyclic monomers such as lactide and glycolide to generate a great number of potentially degradable and biodegradable aliphatic polyesters (1). PCL and its copolymers have potential application in the controlled drug delivery area (2, 3), for pleural and pericardia post-operating adhesions (4) and in orthopaedics e.g. as prosthetic devices (5). Under some conditions PCL-based compounds degrade due to contact with animal or human tissues or organisms. In the field of environmental applications, PCL and its copolymers are being used as such or as blends with starch to make devices degradable and even bioassimilable by outdoor organisms (6). For all these applications, monitoring the fate of degradation products *in vivo* is one of the critical steps in evaluating degradation characteristics and extending the biocompatibility criterion to degradation products. Because of the complexity of living systems, the degradable polymer chains to be traced have to be labelled. Two major methods can be used : fluorescent-labelling and radio-labelling. Fluorescent labelled copolymers have been developed to investigate the biocompatibility and degradation products of polyesterurethanes based on poly((R)-3-hydroxybutyric acid) (PHB) and PCL/PGA copolymers (7). However it was necessary to modify the composition of the copolymers in order to introduce a suitable functional group that can then be reacted with fluorescent dyes. The structure of the obtained copolymers was thus notably different from that of the parent copolymers. Radio-labelling with foreign nuclides such as $^{125/131}$iodine raises the same risk of artefacts. Therefore radio-labelling using the isotopes of carbon or hydrogen was regarded as preferable. In this way the polymers undergo minimal modification.

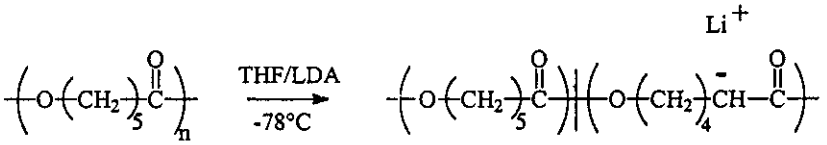
So far, low molecular weight radio-labelled PCL has been mentioned in the literature although the experimental conditions for its polymerization were

not described (8, 9). [^{3}H]-labelled PLA has been previously used (10) and recently the tritiation of lactide followed by the polymerization of the [^{3}H]-labelled cyclic dimer was reported (11, 12). Radio-labelled lactides with specific activities ranging from 111-148 GBq/mmol (3-4 Ci/mmol) were obtained and the percentage of recovered lactide was in the range 50-60 %. Tritiated lactide, diluted with normal cold lactide, was polymerised using stannous octoate or Zn lactate to synthesise highly tritiated PLA polymers. The recovered tritiated compounds had specific activities ranging from 29.6 to 44.4 MBq/mg (0.8 to 1.2 mCi/mg). Polystyrene-related weight average molecular weights $\overline{M_w}$ were in the range of 25000 to 60000, $\overline{M_w}/\overline{M_n}$ being lower than 2.5.

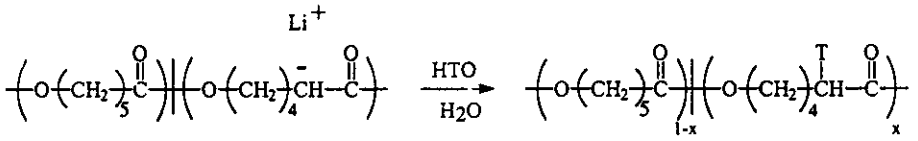
In this paper we report a simple and versatile process of making [^{3}H]-labelled high molecular weight PCL by substituting some of the main chain hydrogens after carbanion formation using LDA (13)

RESULTS AND DISCUSSION

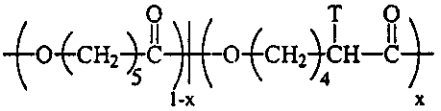
Reagents such as LDA or BuLi are capable of abstracting an activated proton from an organic molecule (13). Such activated protons can be found in the α-position of a carbonyl group as is the case for PCL macromolecules. We have shown recently that LDA can be used at low temperature to abstract a proton from PCL polymer chains dissolved in an organic solvent, and thus to generate a carbanion in the α-position with respect to the carbonyl group according to the following reaction :



The resulting carbanionic site is extremely reactive, and can react with many electrophiles and lead to the introduction of functional groups in the PCL macromolecules according to the general reaction :



Despite partial degradation caused by intrachain reactions involving the active sites, the strategy was successfully used to synthesise various functionalised PCLs (14). It was thus applied to label the PCL chain with tritium by using HTO as the electrophile in order to yield isotopic copolymers :



PCL(^{1}H,^{3}H) or PCL(H,T)

To select the most convenient protocol and to optimise the reaction in terms of main-chain preservation, yield and substitution ratio, the isotopic modification was firstly carried out with D_2O as electrophile reagent. Accordingly a PCL-LDA mixture at a 1M/1M LDA/monomer unit ratio was allowed to react for 30 min before an excess of D_2O was added and allowed to react for 30 more minutes. The temperature was kept at –78°C during the reaction. The substitution ratio was determined from ^{1}H NMR spectra by comparing the area of the resonance peak corresponding to protons of the unmodified CH_2CO group (b) to the areas either of the resonance peak generated by the OCH_2 group (a) or of the peaks generated by the $(CH_2)_3$ group (c and d) that reflected both modified and unmodified units (Fig. 1).

The yield of polymer recovered by precipitation from methyl alcohol was 80%. The substitution ratio was estimated at 14 % and the polystyrene-related number average molecular weight $\overline{M_n}$ was 23,200 and $\overline{M_w}/\overline{M_n}$ ratio was 2.1 as estimated by size exclusion chromatography (SEC).

This preliminary assay showed that $^1H \rightarrow ^2H$ exchange was feasible and is a fast one pot reaction which does not require any polymerization reaction and can yield high molecular weight deuterated PCL.

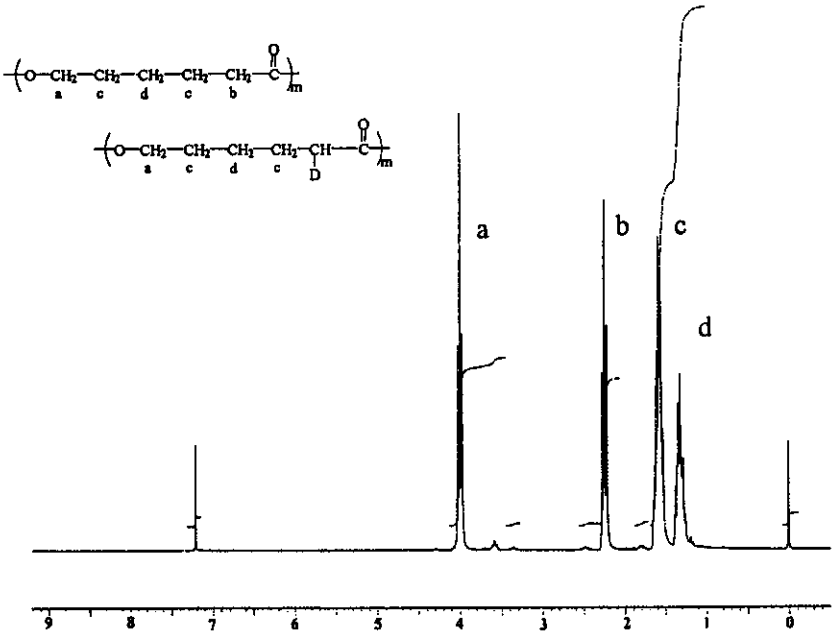


Figure 1 : Typical 250MHz ^{1}H NMR spectrum of deuterated-PCL in deuterated chloroform

The same general protocol was retained for the [^{3}H]-labelling, using tritiated water at a low concentration in LDA (0.25 equivalent/monomeric unit). The amount of LDA was reduced in order to decrease the degree of substitution ratio and end up with higher molecular weight products. Under these conditions, the yield of recovered [^{3}H]-poly(ε-caprolactone) was 60% and the polymer had the following charateristics ($\overline{M_n}$ =28,000 and $\overline{M_w}/\overline{M_n}$ =1.9) as determined by SEC using a refractometric detector. Data showed that the chain length was not dramatically affected by the reaction (Fig. 2). A flow scintillation detector was also used as detector. For the sake of comparison, the corresponding SEC chromatogram which reflects only the radioactive species is also presented in Figure 2. From the comparison, one can conclude that the polymer was tritiated, the retention times being almost the same for the two detection systems. Moreover there were no radioactive low molecular weight compounds present.

The specific activity of the [^{3}H]-labelled-PCL was 5.63 MBq/g (152 μCi/g), a value which was considered to be high enough to allow significant

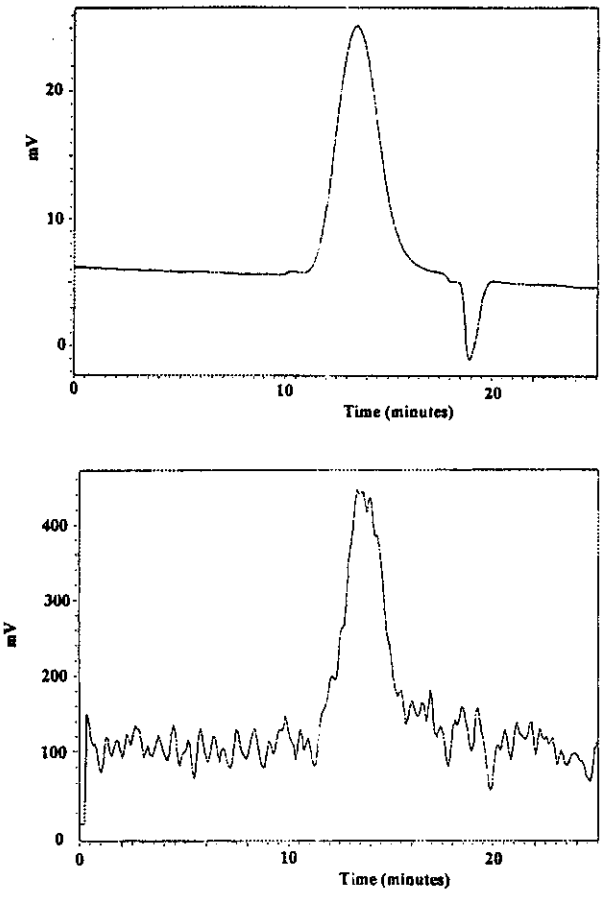


Figure 2 : SEC chromatograms of tritiated-PCL in THF after refractometric detection (top) and flow scintillation detection (bottom).

detection during degradation studies under *in vitro* or *in vivo* conditions. Based on the specific activity of HTO (3.7GBq/g ; 100 mCi/g), and on the fact that both the hydrogen and the tritium atoms of HTO could react identically, the value of the substitution ratio was evaluated as 2 %.

One of the main problems related to the use of radio-labelled compounds as tracers is radiolysis. It can be the source of radioactive degradation by-products which can be mistaken from the radiolabelled compound itself. From the flow scintillation-detected SEC chromatogram, which did not show any radioactive low molecular weight compound, we could conclude that the radio-labelled PCL polyester did not contain any significant radioactive low molar

weight polluting species originating from the synthesis. After 7.5 months storage at 4-6 °C, the radio-labelled PCL lost no more than 4.4 % of its initial radioactivity (Fig. 3).

The loss of radioactivity from the labelled-PCL stored at 4°C was also checked over a shorter period of time, e.g. 113 days. During this period, the radiolysis was c.a. 0.35 %/month. When plotted as a function of time, the data led to linear extrapolation to day zero thus confirming the presence of 1.8% radioactive by-products in the recently synthesised [^{3}H]-labelled PCL. In conclusion after 7.5 months storage 2.6 % of the initial radioactivity was lost as a result of radiolysis.

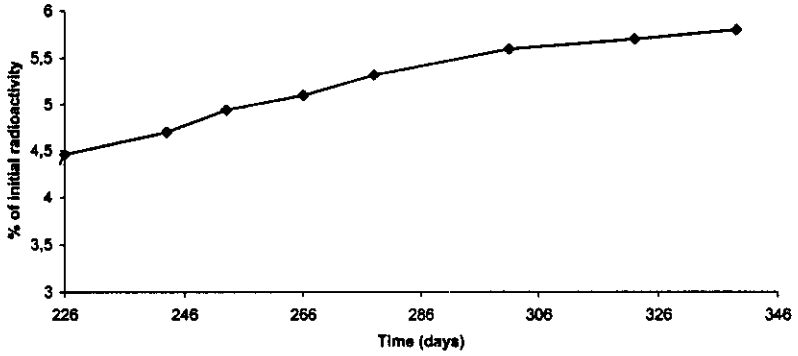


Figure 3. Plot of the cumulative loss of radioactivity versus time for the tritiated polymer.

EXPERIMENTAL

Chemicals

PCL ($\overline{M_n}$ =53700; $\overline{M_w}$ =80000) was obtained from Aldrich (Milwaukee, USA), LDA (Lithium Diisopropylamide : 2M in THF/n-heptane) was purchased from ACROS Organics (Geel, Belgium) and deuterium oxide from Euriso-Top (Groupe CEA C.E. Saclay, France). All were used without further purification.

HTO, specific activity : 3.7 GBq/g (100mCi/g) was purchased from NEN Life Science Products (Boston, USA).

Methods

Tritiation procedure

A solution of PCL (5.7g, 0.05 mole of monomer unit) in 200 ml of anhydrous THF was introduced into a 500 ml three-necked round-bottomed flask, previously flame dried and kept under a flow of argon dried through three gas-drying units filled with silicagel, sodium hydroxide pellets and molecular sieves respectively. The solution was magnetically stirred and the temperature kept at –78°C using a dry ice/acetone mixture. A commercial solution of LDA, 2M in THF/n-heptane (6.25 ml, 0.0125 mole: 0.25 equivalent/monomeric unit) was then introduced with a syringe through a septum. The mixture was kept at – 78°C with stirring for 30 minutes. 250 µl of HTO in 5 ml of THF (0.0139 mole HTO: 66.6 MBq/mmole ≡ 1.8 mCi/mmole) was introduced into the reaction flask through a septum and the stirred reaction solution kept for a further 30 minutes at –78°C. After coming to room temperature an aqueous solution of ammonium chloride (10g of NH_4Cl in 200 ml water) was then added to the reaction mixture and the whole stirred. The alkaline mixture (pH ≈9) was acidified with a 37% aqueous HCL solution. The tritiated polymer was extracted with dichloromethane (2x100 ml). The combined organic phases were washed with distilled water (2x100 ml) and dried over anhydrous $MgSO_4$. After filtration of $MgSO_4$ the solvent was partially evaporated under reduced pressure and the polymer precipitated by addition of methyl alcohol. The coacervate which formed immediately was washed with methyl alcohol until the washing solution was clear. The tritiated polymer was then dried under vacuum for several hours.

Deuteration procedure

The protocol was basically the same as the one used for the tritiation. However the following conditions were applied : PCL (0.01 mole : 1.14g), LDA (0.01 mole : 1 equivalent/monomer unit), and D_2O (0.5 ml) instead of HTO.

Radiolysis assessment

The powdered tritiated polymer was stored in a refrigerator at 4°C for several months. The radioactivity was measured at different time intervals in the following manner: Typically 11 mg of the radioactive PCL were dissolved in 1 ml of THF. A 50 µl aliquot of the sample solution was diluted in 7 ml of Ultima Flo™ AP, a solvent based LSC-cocktail, and counted using a Liquid Scintillation Analyzer, Tri-Carb 2100 TR Packard. The rest of the initial solution was distilled under vacuum and collected by trapping in a liquid nitrogen trap. Aliquots of the collected THF were assayed for the presence of any radioactive volatile which could have been removed in the distillation procedure.

Radioactivity measurement

The specific activity was determined using a Liquid Scintillation Analyzer, Tri-Carb 2100 TR Packard. Typically, 10 mg of polymer was dissolved in 1 ml of THF and 50 µl of the sample solution was diluted in 7 ml of Ultima Flo™ AP, and counted.

Nuclear Magnetic Resonance (NMR)

The substitution ratio of the deuterated PCL in deuterated chloroform was determined by ^{1}H NMR using a Bruker spectrometer operating at 250 MHz.

Size exclusion chromatography (SEC)

Molecular weights were determined by SEC using Waters equipment fitted with a 60 cm long column using 5µm mixed C PLgel as the stationary phase, THF at 1 cm^3/min flow rate as the mobile phase, and a Waters 410 refractometric detector, or a flow scintillation analyzer, Radiomatic Flo-one™ Beta Packard for detection. Typically, 10 mg of polymer was dissolved in 1 ml of THF and the resulting solution was filtered on a 0.45 µm Millipore filter before injection of 20 µl of sample solution. $\overline{M_n}$ and $\overline{M_w}$ data were referred to polystyrene standards.

CONCLUSION

An efficient method of labelling PCL with tritium, based on the use of LDA at low temperature, has been developed. This new method is based on the $^{1}H \rightarrow ^{3}H$ exchange carried out directly on the polymer in solution at low temperature. The specific activity of the product was 152 μCi/g and the molecular weight $\overline{M_n}$ = 28000. The method should be applicable to other polyesters or polymers provided the corresponding polymeric chains contain activated proton atoms where carbanionic sites can be created.

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