Enzyme-Catalyzed Ring-Opening Polymerization of Seven-Membered Ring Lactones Leading to Terminal-Functionalized and Triblock Polyesters

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ABSTRACT: Terminal-functionalized polyesters and triblock polyesters were synthesized by lipase-CA-catalyzed ring-opening polymerization of seven-membered ring lactones, i.e., 1,5-dioxepan-2-one (DXO) and ϵ-caprolactone (CL), in the bulk in the presence of an appropriate alcohol that acts as an initiator. To introduce a double bond at the chain end, 4-pentene-2-ol was used to initiate the polymerization of the lactones. The unsaturation introduced at the chain end in this way is a useful approach for synthesizing comb polymers. Two different dihydroxyl compounds, viz. poly(caprolactone diol) and poly(ethylene glycol), were used as macro-initiators. Triblock copolymers were synthesized in this way, where the macro-initiator formed the middle block. Polymers having different degrees of polymerization were synthesized by varying the molar feed ratio of monomer to initiator. DXO and CL showed significant differences in reactivity toward lipase-CA-catalyzed polymerization initiated by different alcohols as initiators. The polymers were characterized by FTIR, NMR, SEC, optical microscopy, and DSC techniques.

Introduction

Functionalized polyesters are of interest in various industrial and biomedical applications where the functional groups can be used for covalently attaching a pro-drug; for preparing comb, graft, and network polymers; and for varying the hydrophilicity of the polymers. Such polyesters have also been synthesized by the lipase-catalyzed ring-opening polymerization (ROP) of lactones in the presence of a functional alcohol. The lipase-catalyzed ROP of lactones, e.g., ϵ-caprolactone (CL) and dodecalactone (DDL), initiated by a functionalized alcohol such as 5-hexen-1-ol, 5-hexyn-1-ol, or 2-hydroxyethyl methacrylate to introduce unsaturation at the polymer terminal has been reported.[1] A single-step production of methacryl-type polyester macromonomer by the lipase-catalyzed polymerization of DDL in the presence of vinyl methacrylate was performed where the vinyl ester acted as a terminator during the polymerization, and the method was therefore called the "terminator method" to form terminal-functionalized polymers.[2−4] Lipase could also be utilized to selectively functionalize carbohydrates and thus avoid the time-consuming protection−deprotection chemistry usually involved in the selective functionalization of carbohydrates. Regiospecific acylation of the primary hydroxyl group of methyl β-D-glucopyranoside was combined with the ROP of CL in the presence of lipase to give methyl 6-O-poly(ϵ-caprolactone)-β-D-glucopyranoside.[5] A multi-arm heteroblock star copolymer consisting of poly(ϵ-caprolactone) (PCL) and poly(lactic acid) (PLA) chain segments was synthesized using 1-ethyl glucopyranoside as the initiator and porcine pancreatic lipase as the catalyst.[6] PCL amphiphilic macromonomers were prepared by first using di- to hexahydroxyl group initiators for the regioselective ROP of CL, followed by selective functionalization of the PCL hydroxyl end groups.[7] The hydroxyl compounds used as initiators in this approach included hexahydroxy-functional dendrimer, benzyl ester of 2,2′-bis(hydroxymethyl)propionic acid, and ethyl β-D-glucopyranoside. In another study, lipases from different sources were screened to determine their relative abilities to catalyze the polymerization of ω-pentadecalactone (PDL) using 2-hydroxyethyl methacrylate and ω-hydroxyl-ω'-methacrylate poly(ethylene glycol) as initiators.[8] Brush copolymers could be synthesized from these macromers using an azo-initiator. Block copolymers of CL or PDL with monohydroxyl-terminated polybutadiene were prepared via lipase-catalyzed polymerization.[9] Copolymerization of CL with mono- or dihydroxyl-terminated PEG was performed to yield di- or triblock copolymers using lipase as the catalyst.[10] Thiol-end-functionalized PCL has also been synthesized using 2-mercaptoethanol as the initiator.[11]

Our interest in poly(1,5-dioxepan-2-one) (PDXO) is due to the versatility of this polymer as a component in random and block copolymers and in cross-linked systems suitable for biomedical and pharmaceutical applications. The lipase-catalyzed ROP of DXO has recently been reported.[12] The objective of the present studies was to synthesize end-functionalized or triblock copolymers using different alcohols as initiators via lipase-CA-catalyzed ROP of seven-membered ring lactones such as 1,5-dioxepan-2-one (DXO) and CL. 4-Pentene-2-ol was used as the initiator to introduce unsaturation at the polymer chain end, which was useful for tandem reactions, such as the formation of comblike polymers or hydrophilic/hydrophobic grafts in polyvinyls. Dihydroxyl-terminated macro-initiators such as poly(caprolactone diol) (PCL-diol) or poly(ethylene glycol) (PEG) of different molecular weights were used to form triblock copolymers with DXO or CL.

Experimental Section

Materials. Tetrahydro-4H-pyrane-4-one (Maybridge Chemical, U.K.), *m*-chloroperbenzoic acid (Acros, Belgium), dichloromethane, $MgSO_4$, $NaHSO_3$, $NaHCO_3$, and diethyl ether (Labora, Sweden) were used as received. ϵ-Caprolactone (Aldrich, Germany) was dried and distilled over CaH_2 at reduced pressure prior to use. 4-Pentene-2-ol (Aldrich, Germany), poly(caprolactonediol) (PCL-diol, mol·wt 1250 and 2000 g/mol; Aldrich, Germany), poly(ethylene glycol) (PEG, mol·wt 1000 and 2000 g/mol; Aldrich,

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Germany) were used as received. Novozyme 435 (activity approximately 10 000 PLU/g according to the supplier) was donated by Novozyme Inc., Denmark. Novozyme 435 (lipase-CA) is a lipase (B lipase) from *Candida antarctica* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism absorbed on a microporous resin. It consists of bead-shaped particles with a diameter in the range of 0.3−0.9 mm. The bulk density of Novozyme 435 is approximately 0.43 g/cm^3, and its moisture content is 1−2% w/w. The enzyme, Novozyme 435, was dried over P_2O_5 (Merck, Germany) at 0.1 mmHg for 42 h before use. DXO was synthesized in the laboratory.

Synthesis of 1,5-Dioxepan-2-one (DXO). DXO was synthesized from tetrahydro-4H-pyran-4-one through Bayer−Villiger oxidation according to the method reported by Mathisen et al.[13] The DXO obtained was purified by recrystallization from diethyl ether and two subsequent distillations under reduced pressure. Finally the monomer was dried over CaH_2 overnight and distilled under reduced pressure. ^{1}H NMR δ (ppm): 4.2 (t, 2H, $-CH_2$–OCO−), 3.8 (t, 2H, $-CH_2$–CH$_2$–OCO−), 3.7 (t, 2H, $-CH_2$–CH$_2$–COO−), 2.6 (t, 2H, $-CH_2$–COO−).

Polymerization of DXO in the Presence of Alcohol as an Initiator. All glassware was silanized, flame-dried, and stored under a nitrogen atmosphere at 0 ppm moisture in a glovebox. In a typical polymerization, the lactone, enzyme (5.0 wt % of monomer), and alcohol were weighed in a round-bottom flask inside the glovebox. The flask was sealed and immersed in an oil bath at 60 °C for 2 h under continuous stirring. After 2 h, when the viscosity of the reaction mixture became very high and the magnetic bar stopped stirring, the flask was removed from the oil bath, and a sample of the reaction mixture was taken for ^{1}H NMR analysis to determine the percentage monomer conversion. The polymer was obtained by dissolving the reaction mixture in a small amount of chloroform and then precipitating it in excess cold hexane. The polymer was dried under vacuum before analysis by NMR spectroscopy, SEC, DSC, FTIR spectroscopy, and optical microscopy. Different series of polymers with different degrees of polymerization were synthesized with each initiator.

Polymerization of CL in the Presence of Alcohol as an Initiator. A procedure similar to that described above for DXO was used to polymerize CL with different alcohols as initiators, except that the polymerization was performed for 4 h at 60 °C. The percentage monomer conversion was determined by ^{1}H NMR analysis of the crude reaction mixture. The polymer was obtained by dissolving the reaction mixture in a small amount of chloroform and then precipitating it in excess cold hexane. The polymer was dried under vacuum before characterization.

Characterization

NMR Spectroscopy. The percentage monomer conversion and the degree of polymerization were determined by ^{1}H NMR spectroscopy, and the monomer sequence in block copolymers was evaluated using ^{13}C NMR spectroscopy. A Bruker Avance 400 MHz NMR instrument operating at 400.13 and 100.62 MHz for ^{1}H and ^{13}C, respectively, was used for this purpose. $CDCl_3$ was used as the solvent as well as the internal standard (δ = 7.26 and 77.0 ppm).

SEC. The molecular weights of the polymers were determined by size-exclusion chromatography (SEC) using dimethylformamide (DMF) as the eluent at a flow rate of 1.0 mL/min. The injection volume was 50 μL. A Waters 717 plus autosampler and a Waters model M-6000A solvent pump equipped with a PL-EMD 960 light-scattering evaporative detector, two PL gel 10-mm mixed B columns (300 × 7.5 mm) from Polymer Laboratories, and one Ultrahydrogel linear column (300 × 7.8 mm) from Waters and connected to an IBM-compatible PC were used. Narrow-molecular-weight polystyrene standards were used for calibration. Millenium version 3.20 software was used to process the data.

DSC. The thermal properties of the polymers were determined by differential scanning calorimetry (DSC) using a Mettler-Toledo DSC 820 module under a nitrogen atmosphere (nitrogen flow rate of 80 mL/min) with a sample mass of 5 ± 1 mg and a heating rate of 5 °C/min. The samples were subjected to a heating−cooling−heating cycle from −70 to 100 °C, and the analysis was performed on the second heating plot.

The relative crystallinity of the PCL segment of different samples was calculated according to equation 1

$$w_c = \frac{\Delta H_f}{\Delta H_f^0} \times 100 \quad (1)$$

where w_c is the crystallinity, ΔH_f is the heat of fusion of the sample, and ΔH_f^0 is the heat of fusion of 100% crystalline PCL. The value of ΔH_f^0 used for the calculations was 139.5 J/g.[14]

FTIR Spectroscopy. FTIR measurements were performed on a Perkin-Elmer 2000× FTIR spectrometer, equipped with a golden-gate single-reflection ATR unit with a diamond crystal. The spectra were taken as averages of 30 scans at a resolution of 4 cm^{-1}.

Optical Microscopy. A polarized optical microscope (Leitz Ortholux POL-BK II) was used to examine the morphology of the samples.

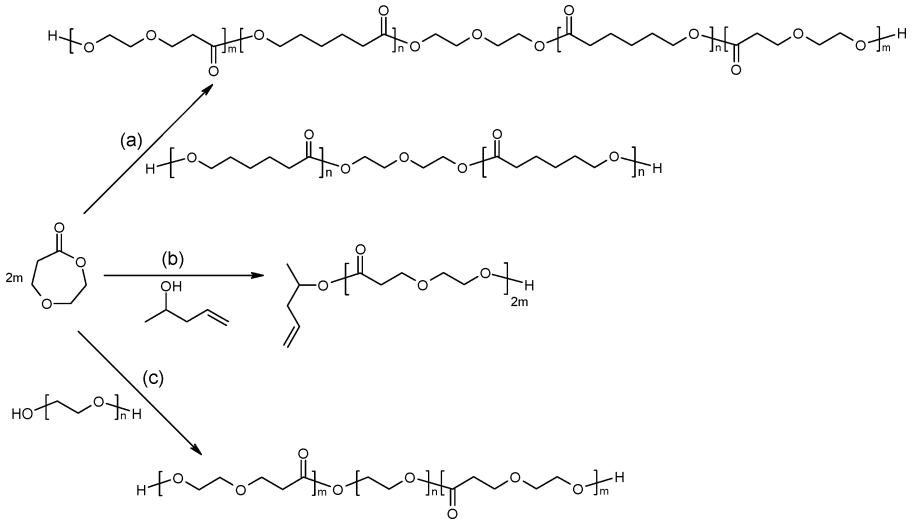
Results and Discussion

Novozyme 435 (lipase-CA) has a high polymerization activity for lactones. It also has the merit of being a metal-free catalyst that can easily be removed from the final product by filtration and does not release any toxic metallic residues. When it was used as a catalyst for the ROP of DXO or CL in the bulk at 60 °C, the reaction mixture could be described as a suspension of enzyme beads in the monomer. An increase in viscosity was observed with time, which is a typical characteristic of bulk polymerization. In an earlier publication, we reported the bulk polymerization of DXO with lipase-CA without drying the enzyme or rigorously drying the glassware used to carry out the polymerization.[12] Under such conditions, the amount of water present in the enzyme could act as an initiator. In the present studies, the enzyme was dried over P_2O_5 under reduced pressure, and the glass apparatus was silanized and flame-dried before use. The polymerization was therefore initiated only by the hydroxyl group of the added alcohol.

During the bulk polymerization of DXO with lipase-CA at 60 °C in our previous study, an increase in viscosity of the reaction mixture was observed in 1 h.[12] After 4 h, the magnetic bar stopped stirring because of the very high viscosity of the reaction mixture, and no sample could be withdrawn for kinetic measurements. In the present study, when DXO was bulk polymerized with lipase-CA at 60 °C in the presence of different alcohols as initiators, an increase in viscosity was observed within 30 min in all cases. The polymerization of DXO was therefore carried out only for 2 h, at which point the viscosity of the reaction mixture became very high and the magnetic bar stopped stirring. In the case of CL, the polymerizations were carried out for 4 h, as the increase in viscosity was not observed until after 2 h. This shows the difference in reactivity of DXO and CL toward lipase-catalyzed polymerization. This difference in the behavior of these two lactones indicates a difference in their specificities toward lipase catalysis. CL might have acted as a poor substrate for the lipase-catalyzed polymerization because of its weak binding at the enzyme's active surface. Similar behavior was observed in our previous investigations when water was allowed to act as the initiator.[12]

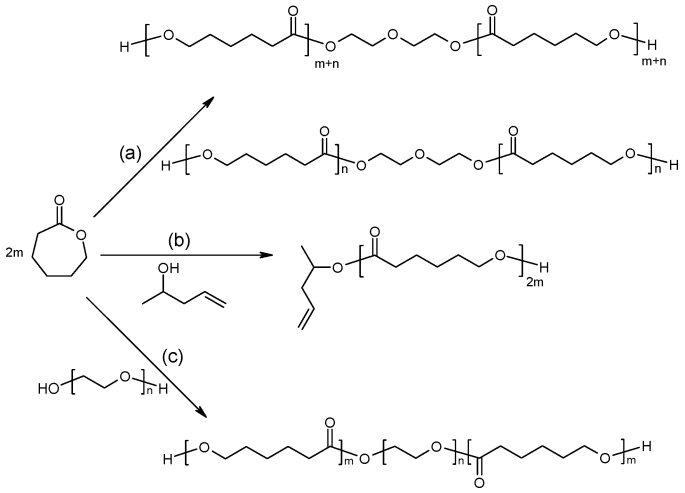
4-Pentene-2-ol was used as an initiator to synthesize PDXO or PCL macromers having a double bond at one chain end. Dihydroxyl-terminated macro-initiators such as PCL-diol or PEG were used to synthesize triblock copolymers with DXO or CL. The hydroxyl group at the two ends of the macro-initiator

Scheme 1. Enzymatic Synthesis of End-Functionalized or Triblock Polyesters Based on DXO Using Different Alcohols as Initiators[a]



[a] (a) PCL-diol forming the middle block of the triblock copolymer, (b) 4-pentene-2-ol introducing terminal unsaturation, (c) PEG forming the middle block of the triblock copolymer.

Scheme 2. Enzymatic Synthesis of End-Functionalized or Triblock Polyesters Based on CL Using Different Alcohols as Initiators[a]



[a] (a) PCL-diol acting as a chain extender to form PCL homopolymer, (b) 4-pentene-2-ol introducing terminal unsaturation, (c) PEG forming the middle block of the triblock copolymer.

chain initiated the polymerization generating a triblock copolymer with the macro-initiator as the middle block. The reaction pathways for the synthesis of homo- and copolymers of DXO or CL with these initiators are shown in Schemes 1 and 2, respectively.

The percentage monomer conversion in each of the polymers was determined from the ^{1}H NMR spectrum of the crude reaction mixture of each sample by taking the ratio of peak intensities due to polymer and monomer. For DXO-based polymers, the ratio of the intensities of oxymethylene protons of PDXO at 4.1 ppm (t, 2H, $-CH_2-$OCO$-$) to those of DXO at 4.2 ppm (t, 2H, $-CH_2-$OCO$-$) was used to calculate the percentage monomer conversion. In the case of CL, the ratio of peak intensities used to obtain the percentage monomer conversion was based on the oxymethylene protons at 4.0 ppm due to PCL (t, 2H, $-CH_2-$OCO$-$) and at 4.1 ppm due to CL (t, 2H, $-CH_2-$OCO$-$).

Use of 4-Pentene-2-ol as the Initiator. 4-Pentene-2-ol was used as an initiator to introduce terminal functionalization in PDXO or PCL. Polymers having different degrees of polymerization (DP) were synthesized by varying the molar ratio of monomer to initiator. Three different polymers of theoretical DP values of 30, 55, and 100 were synthesized with DXO as shown in Table 1. The percentage monomer conversion was determined from the ^{1}H NMR spectrum of the crude reaction mixture in all cases, and it was found to decrease with increasing molar feed ratio of monomer to initiator (Table 1). This was because the polymerization was allowed to run for a limited period of time. With an increase in the monomer-to-initiator molar ratio, the number of growing chains at any given time was less. Because the polymerization was performed in the bulk, the viscosity increased with time, so that the accessibility of

Table 1. Lipase-CA-Catalyzed ROP of DXO in the Presence of 4-Pentene-2-ol as an Initiator

entry	M/In[a]	conversion[b] (%)	DP[c]	DP[d]	M_n[d]	M_n[e]	M_w[e]	PDI[e]	T_g[f] (°C)
1	30	92.6	28	29	3400	7500	13 000	1.7	−40.6
2	55	78.7	43	44	5200	9900	20 000	2.0	−39.5
3	100	63.0	63	63	7400	15300	33 900	2.2	−38.4

[a] Monomer-to-initiator molar feed ratio. [b] Determined by ^{1}H NMR spectroscopy of the crude reaction mixture. [c] DP calculated by feed composition and monomer conversion using the formula. DP = (M/In) × (conversion/100). [d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer. [e] Obtained by SEC analysis with polystyrene standards. [f] Obtained by DSC analysis.

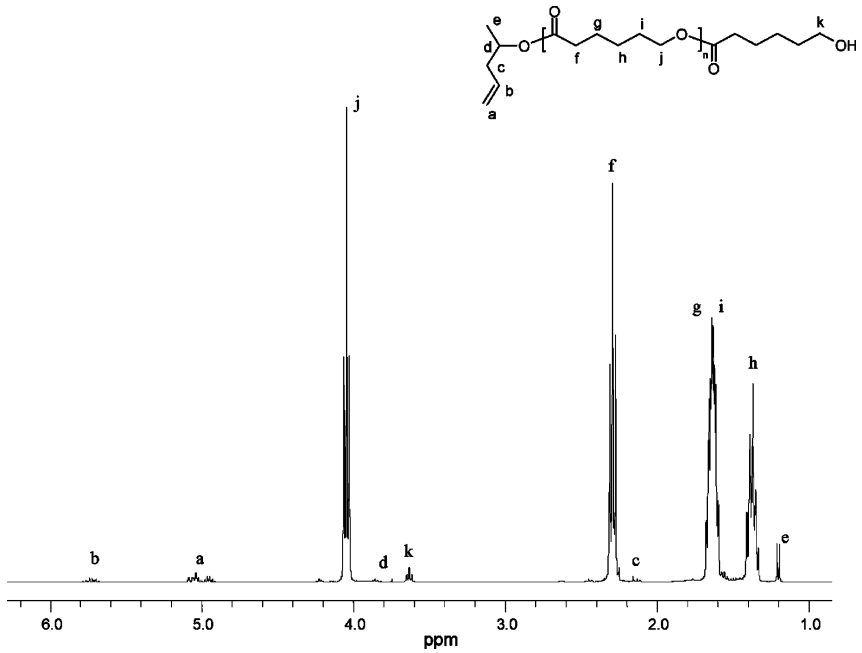


Figure 1. ^{1}H NMR spectrum of PCL synthesized by the lipase-CA-catalyzed ROP of CL in the presence of 4-pentene-2-ol as an initiator (entry 1, Table 2).

monomer to the growing polymer chains was limited. Therefore, a high viscosity and fewer growing centers led to low monomer conversion.

The DP values of different PDXO samples were determined by ^{1}H NMR spectroscopy by taking the ratio of the intensity of oxymethylene protons at 4.2 ppm to that of the terminal methylene protons at 3.6 ppm. As can be seen in Table 1, the DP increased with increasing molar feed ratio of monomer to initiator, which confirmed that the alcohol had acted as an initiator. The number-average molecular weight (M_n) of each sample was calculated from these DP values, and the results are also presented in Table 1. Another way to calculate the DP of the polymers obtained is to take into account the monomer-to-initiator molar feed ratio (M/In) and the monomer conversion by using eq 2. This is on the assumption that the polymerization has the characteristics of living polymerization. If so, all of the chains are initiated at the same time, and equal numbers of monomer units are therefore attached to each growing polymer chain.

$$\mathrm{DP} = (\mathrm{M/In}) \times (\mathrm{conversion}/100) \qquad (2)$$

The DP values calculated in these two ways were very close to each other, as can be seen in columns 4 and 5 of Table 1. This shows that almost equal numbers of monomers were indeed incorporated into each growing polymer chain. These results thus indicate that lipase-CA-catalyzed polymerization has the characteristics of living polymerization. This also confirmed the role of the added alcoholic moiety as an initiator.

An increase in molecular weight was observed in SEC chromatograms with an increase in the molar ratio of monomer to initiator. This further supports the role of added alcohol as an initiator. The PDI values increased from 1.7 to 2.2 with increasing monomer-to-initiator molar feed ratio. This could be due to the fast polymerization kinetics of DXO, which led to a high viscosity of the reaction mixture in a short period of time. The longer chains generated at the high monomer-to-initiator ratio have a high probability of undergoing transesterification reactions under such conditions of polymerization. The increase in PDI was therefore due to the diffusion-controlled nature of the polymerization at higher conversion, which resulted in the occurrence of transesterification reactions and, in turn, increased M_w without changing M_n.

Table 2. Lipase-CA-Catalyzed ROP of CL in the Presence of 4-Pentene-2-ol as the Initiator

entry	M/In[a]	conversion[b] (%)	DP[c]	DP[d]	M_n[d]	M_n[e]	M_w[e]	PDI[e]	W_c[f] (%)
1	30	93.6	28	26	3000	6600	9500	1.4	57.6
2	65	84.8	55	55	6400	12 000	17 500	1.5	51.8
3	130	66.7	87	87	10000	22 000	31 500	1.4	47.8

[a] Monomer-to-initiator molar feed ratio. [b] Determined by ^{1}H NMR spectroscopy of the crude reaction mixture. [c] DP calculated by feed composition and monomer conversion using the formula. DP = (M/In) × (conversion/100). [d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer. [e] Obtained by SEC analysis with polystyrene standards. [f] Obtained by DSC analysis.

The thermal properties of the polymers were determined by DSC. The glass transition temperature (T_g) of the PDXO samples increased with increasing molecular weight of the polymer, as shown in Table 1.

Three different polymers of theoretical DP values of 30, 65, and 130 were synthesized with CL, and the results are presented in Table 2. The monomer conversion decreased with increasing molar feed ratio of monomer to initiator, and this can be explained on the basis of reasons similar to those discussed before for DXO. The incorporation of the pentene moiety was confirmed by the presence of respective proton signals in the ^{1}H NMR spectra. Figure 1 shows a representative ^{1}H NMR spectrum for PCL having an olefin moiety at one chain end.

The ratio of oxymethylene protons at 4.0 ppm to terminal methylene protons at 3.6 ppm was used to calculate the DP values of different 4-pentene-2-ol-initiated PCL samples, and these values were found to increase with increasing monomer-to-initiator molar feed ratio. The M_n values calculated from these DP results are presented in Table 2. The DP for each sample was also calculated using eq 2, and the values determined by the two methods were found to be quite close to each other, as shown in Table 2. This again shows that the lipase-CA-catalyzed polymerization of CL in the presence of 4-pentene-2-ol as an initiator has the characteristics of living polymerization.

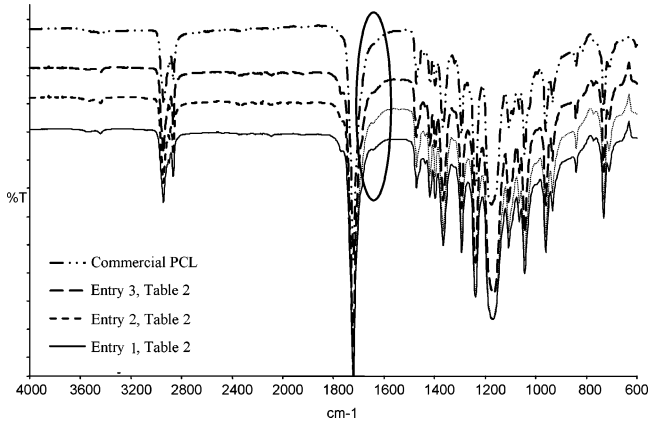


Figure 2. FTIR spectra of PCL samples having different degrees of polymerization (entries 1−3 in Table 2) synthesized by the lipase-CA-catalyzed ROP of CL in the presence of 4-pentene-2-ol as an initiator; the peak at ∼1645 cm^{-1} confirms the presence of unsaturation at the polymer chain end, and the intensity of the peak decreases with increasing degree of polymerization of PCL.

The role of the added alcohol as an initiator was also confirmed by the increase in molecular weight with increasing molar ratio of monomer to initiator observed in the SEC chromatograms. No significant change in PDI was observed with increasing monomer-to-initiator ratio, indicating that no change in the extent of transesterification reactions occurred when the feed ratio was changed. All of these values are presented in Table 2.

No clear glass transition of PCL could be observed in the DSC thermograms as the heating began at −70 °C, which is very close to the expected T_g of the polymer. The relative crystallinity of PCL homopolymers was calculated from the melting peak (heat of fusion) at about 60 °C. A decrease in crystallinity was observed with increasing chain length of the PCL homopolymers, as shown in Table 2. This could be due to a more regular packing of small chains generating more regular crystals. The short polymer chains crystallized in extended chains or in once- or twice-folded crystals with a very small proportion of amorphous material. On the other hand, the long chains could not as effectively be accommodated into the crystals and had a large proportion of statistical chains in the amorphous phase, resulting in reduced crystallinity.

Table 3. Lipase-CA-catalyzed ROP of DXO in the Presence of PCL-diol as the Macroinitiator

entry	initiator	M/In[a]	conversion[b] (%)	DP[c]	DP[d]	M_n[d]	M_n[e]	M_w[e]	PDI[e]	T_g[f] (°C)	W_c[f] (%)
1	PCL1250	25	89.5	22	21	3700	8700	13 900	1.6	−49.2	0.6
2	PCL1250	40	77.8	31	27	4400	14 400	27 900	1.9	−44.4	0.3
3	PCL1250	60	58.3	35	31	4800	19 700	34 200	1.7	−42.5	0.0
4	PCL2000	25	83.3	21	23	4700	10 000	15 700	1.6	−47.6	17.5
5	PCL2000	40	78.9	32	30	5500	15 000	26 800	1.8	−45.7	1.9
6	PCL2000	60	54.5	33	34	5900	18 800	42 800	2.3	−40.3	1.1

[a] Monomer-to-initiator molar feed ratio. [b] Determined by ^{1}H NMR spectroscopy of the crude reaction mixture. [c] DP calculated by feed composition and monomer conversion using the formula. DP = (M/In) × (conversion/100). [d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer. [e] Data obtained by SEC with polystyrene standards. [f] Data obtained by DSC.

The presence of a carbon−carbon double bond due to the 4-pentene-2-ol initiator in the PDXO or PCL homopolymers was also confirmed by FTIR spectroscopy. The spectra of all three PCL samples are compared with that of commercial PCL in Figure 2. The low-intensity peak at ∼1645 cm^{-1} due to C=C stretching indicates the presence of unsaturation. The relative intensity of this peak decreased with increasing DP of the PCL chain.

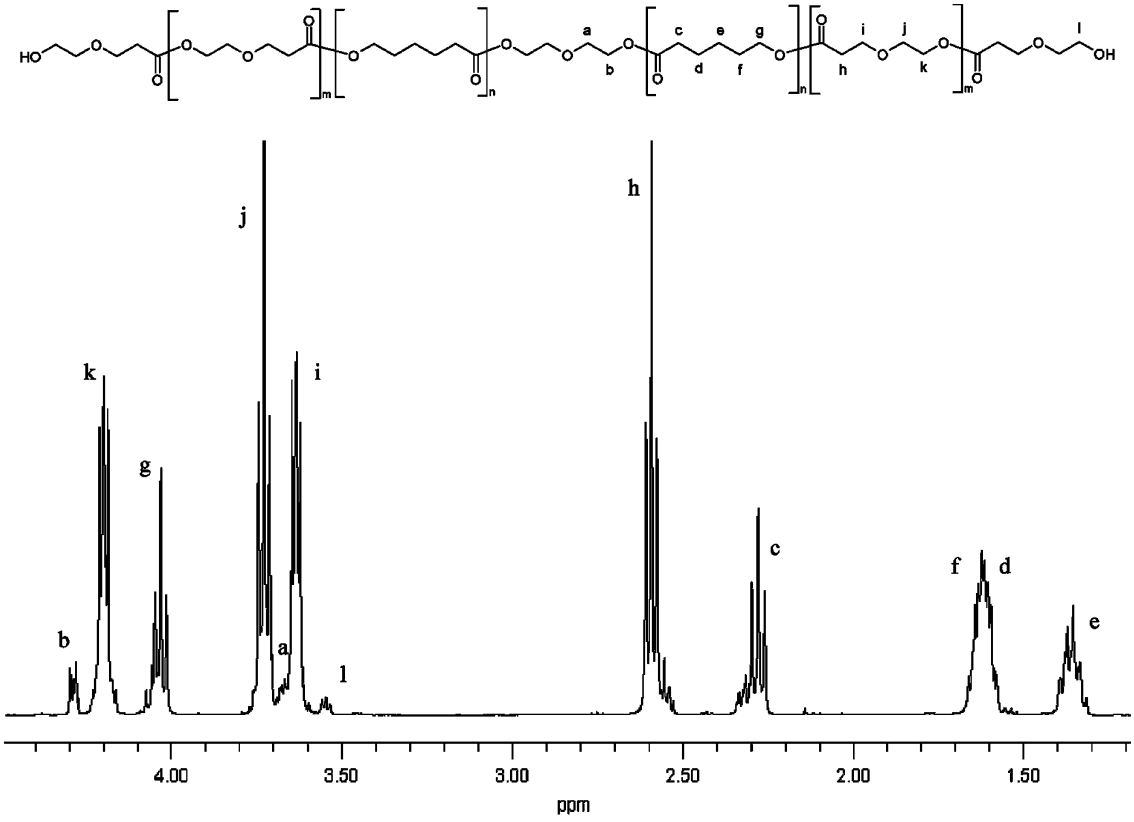


Figure 3. ^{1}H NMR spectrum of poly(DXO-*b*-CL-*b*-DXO) synthesized by the lipase-CA-catalyzed ROP of DXO in the presence of PCL-diol as an initiator (entry 1, Table 3).

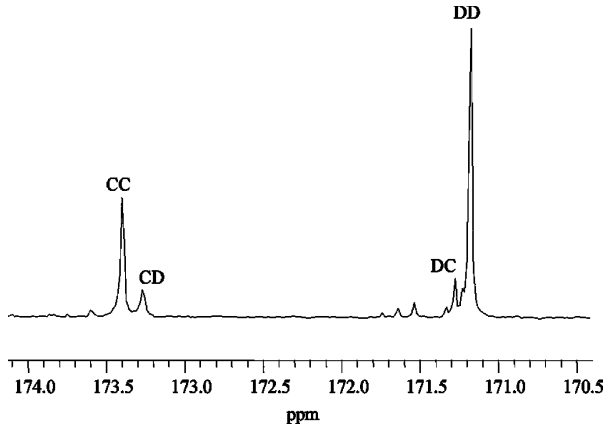


Figure 4. ^{13}C NMR spectrum of poly(DXO-*b*-CL-*b*-DXO) synthesized by the lipase-CA-catalyzed ROP of DXO in the presence of PCL-diol as an initiator (entry 1, Table 3).

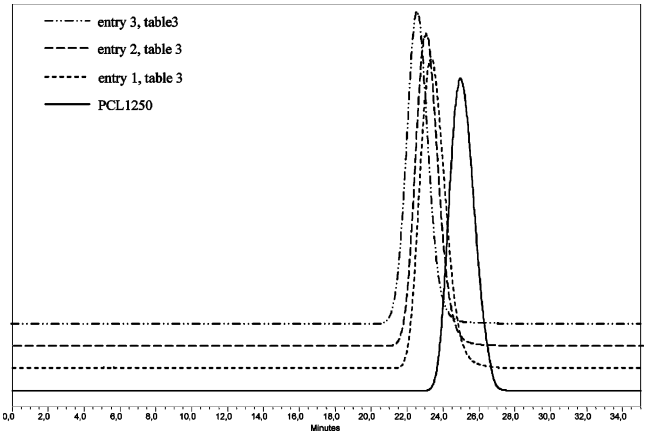


Figure 5. SEC chromatograms depicting the increase in molecular weight of poly(DXO-*b*-CL-*b*-DXO) copolymers (entries 1−3 in Table 3) with increasing monomer-to-initiator ratio in comparison with that of PCL1250.

Use of PCL-diol as a Macro-initiator. Dihydroxyl-terminated PCL-diols of different molecular weights (1250 and 2000 g/mol) were used to initiate the DXO or CL polymerization with lipase-CA. With DXO, the triblock copolymer poly(DXO-*b*-CL-*b*-DXO) was obtained. Six different triblock copolymers of DXO and PCL-diol were synthesized by changing the monomer-to-initiator molar feed ratio from 25 to 60 as shown in Table 3. The percentage monomer conversion and DP were calculated from ^{1}H NMR analyses of the crude reaction mixture and the precipitated polymer, respectively. In both sets of polymers synthesized by PCL1000 or PCL2000, the percentage monomer conversion decreased with increasing monomer-to-initiator ratio. This is for similar reasons as discussed before in the case of 4-pentene-2-ol.

The ratio of the peak intensity of the methylene protons at 2.6 ppm to that of the terminal methylene protons at 3.6 ppm was used to calculate the DP values for different DXO triblock copolymers with PCL-diol. These DP results, included in Table 3, are the overall values and therefore should be divided by 2 to obtain the actual DP of the polymer chain attached to each hydroxyl group of the macro-initiator. For example, the DP = 30 in entry 5 of Table 3 indicates that each hydroxyl group of the macro-initiator has a PDXO chain of DP = 15. A representative ^{1}H NMR spectrum of poly(DXO-*b*-CL-*b*-DXO) is shown in Figure 3. The M_n values calculated from the DP results from ^{1}H NMR spectra for all samples are summarized in Table 3, together with the other results. The DP was also calculated from the monomer-to-initiator molar feed ratio and the percentage monomer conversion using eq 2. The DP values obtained by the two methods were quite close to each other, as shown in columns 5 and 6 of Table 3, indicating the living character of the polymerization.

To further verify the structure of the synthesized polymers, sequence analysis was carried out in the carbonyl region of the ^{13}C NMR spectra because of the greater sensitivity to sequence effects of ^{13}C compared to ^{1}H NMR spectroscopy.[15] The spectrum of poly(DXO-*b*-CL-*b*-DXO) (entry 1 in Table 3) is shown in Figure 4. Four different peaks corresponding to the homopolymer and two dyads, i.e., transition between DXO (D) and CL (C) and vice versa, are assigned in the spectrum. Two additional low-intensity peaks were also seen close to the DXO region arising from possible transesterification reactions of the growing DXO chain. The sequence lengths of the PDXO and PCL blocks were determined using eqs 3 and 4, respectively.

$$\bar{L}_D = \frac{I_{DD}}{I_{DC}} + 1 \tag{3}$$

$$\bar{L}_C = \frac{I_{CC}}{I_{CD}} + 1 \tag{4}$$

A sequence length of 8 was obtained for the PCL block, which was calculated to be 10 using the ^{1}H NMR spectrum, and a sequence length of 16 was obtained for the PDXO segment, which was 21 according to the ^{1}H NMR spectrum.

SEC chromatograms of all of the polymers showed an increase in molecular weight with increasing monomer-to-initiator molar feed ratio, and a slight increase in the PDI value of poly(DXO-*b*-CL-*b*-DXO) was also observed. This can again be explained by the occurrence of transesterification reactions at the higher monomer-to-initiator ratio, where the higher viscosity and longer chains promote such reactions. Figure 5 shows the SEC chromatograms of different poly(DXO-*b*-CL-*b*-DXO) samples (entries 1−3 in Table 3) in comparison to that of PCL1250. No peak at higher retention times was observed, indicating that all of the PCL-diol was involved in initiating the polymerization.

An increase in T_g of PDXO with an increase in the molecular weight of the PDXO block was observed in the DSC thermograms of poly(DXO-*b*-CL-*b*-DXO), as shown in the Table 3. The relative crystallinity of the PCL middle block in the triblock polymer poly(DXO-*b*-CL-*b*-DXO) was found to decrease with increasing block length of the PDXO segments, because of the hindrance created by the PDXO segments to the crystallization of the PCL blocks.

PCL is a semicrystalline polymer with a melting temperature, T_m, of ∼60 °C whereas PDXO is an amorphous polymer. In poly(DXO-*b*-CL-*b*-DXO) triblock copolymers, the PCL middle block showed a tendency to crystallize that was affected by the length of the PDXO blocks. To investigate this effect of the PDXO blocks on the crystallinity of the PCL segment, samples (entries 4−6 in Table 3) were analyzed by optical microscopy. As shown in Figure 6, the presence of crystalline domains due to the PCL segment was significantly reduced as the PDXO block length was increased. As explained before, the longer the PDXO chains, the more hindrance they provide to PCL crystallization.

Poly(DXO-*b*-CL-*b*-DXO) triblock copolymers synthesized in this way can be used as slowly degrading sutures, temporary implants, or drug-delivery systems, because PCL segments exhibit outstanding permeability and biodegradability and the product of PCL degradation, hydroxyhexanoic acid, is nontoxic

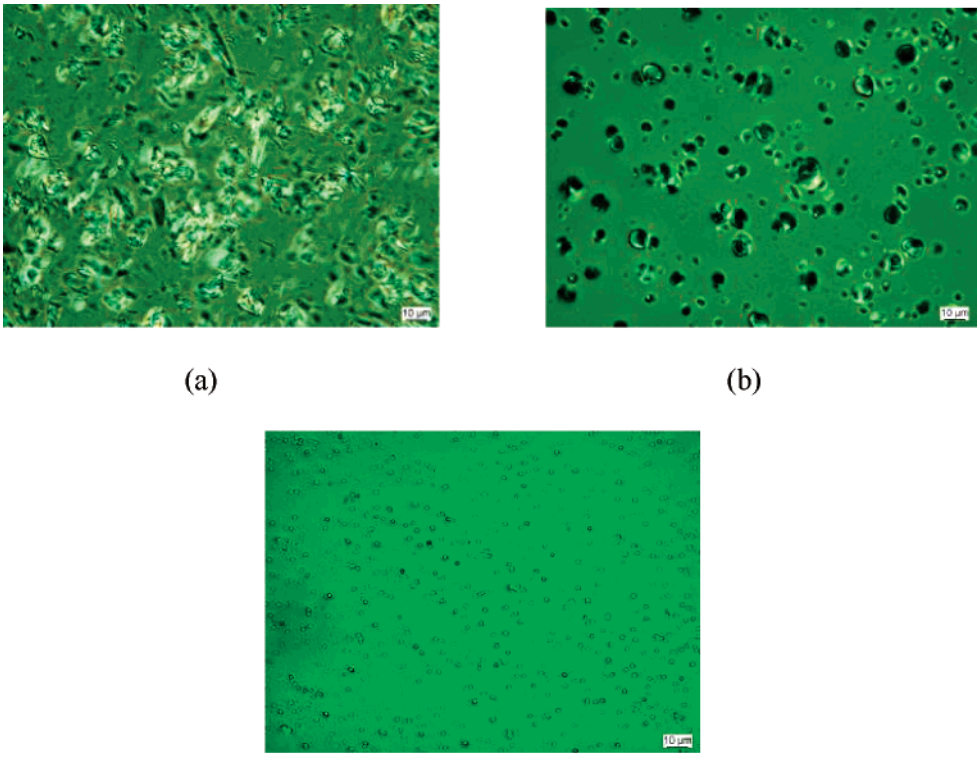


Figure 6. Optical microscope images of poly(DXO-*b*-CL-*b*-DXO) samples having different DP values (entries 4−6 in Table 3) showing the relative change in the degree of crystallinity of the PCL segment of the terpolymer. DP of PDXO block = (a) 23, (b) 30, and (c) 34.

Table 4. Lipase-CA-catalyzed ROP of CL in the Presence of PCL-diol as Macroinitiator

entry	initiator	M/In[a]	conversion[b] (%)	DP[c]	DP[d]	M_n[d]	M_n[e]	M_w[e]	PDI[e]	W_c[f] (%)
1	PCL1250	25	84.7	21	20	3500	11 100	14 900	1.3	54.0
2	PCL1250	40	76.3	31	32	4900	16 400	21 200	1.3	50.4
3	PCL1250	70	66.2	46	46	6500	23 100	29 100	1.3	50.0
4	PCL2000	25	77.8	19	16	3800	9400	13 200	1.4	50.7
5	PCL2000	40	70.9	28	26	5000	13 900	18 600	1.3	45.3
6	PCL2000	70	58.6	41	43	6900	20 000	26 500	1.3	43.1

[a] Monomer-to-initiator molar feed ratio. [b] Determined by ^{1}H NMR spectroscopy of the crude reaction mixture. [c] DP calculated by feed composition and monomer conversion using the formula. DP = (M/In) × (conversion/100). [d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer. [e] Data obtained by SEC with polystyrene standards. [f] Data obtained by DSC.

Table 5. Lipase-CA-catalyzed ROP of DXO in the Presence of PEG as Macroinitiator

entry	initiator	M/In[a]	conversion[b] (%)	DP[c]	DP[d]	M_n[d]	M_n[e]	M_w[e]	PDI[e]	T_g[f] (°C)
1	PEG1000	25	91.4	23	20	3300	8800	13 200	1.5	−47.0
2	PEG1000	45	80.9	36	33	4800	14 300	30 200	2.1	−46.8
3	PEG1000	90	67.7	61	56	7500	21 700	51 500	2.4	−45.3
4	PEG2000	25	86.3	22	18	4100	8600	14 400	1.7	−46.2
5	PEG2000	45	81.2	37	32	5700	13 200	27 000	2.0	−44.4
6	PEG2000	80	70.9	57	54	8300	20 500	43 300	2.1	−40.6

[a] Monomer-to-initiator molar feed ratio. [b] Determined by ^{1}H NMR spectroscopy of the crude reaction mixture. [c] DP calculated by feed composition and monomer conversion using the formula. DP = (M/In) × (conversion/100). [d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer. [e] Data obtained by SEC with polystyrene standards. [f] Data obtained by DSC.

and can be metabolized in the human body. On the other hand the fact that PCL is hydrophobic in nature means that it has a lower rate of degradation, which can be compensated by the PDXO segments of the triblock copolymer. PDXO is an amorphous, hydrophilic, and biocompatible polymer that can degrade rapidly.

PCL-diol acted as a chain extender when it was used as an initiator to polymerize CL in the presence of lipase-CA, and PCL homopolymer was therefore finally generated. Six polymers having different DP values were synthesized as reported in Table 4. The monomer conversion decreased with increasing monomer-to-initiator ratio following the same trend as observed for DXO. The ratio of oxymethylene protons at 4.0 ppm and terminal methylene protons at 3.6 ppm was used to determine the DP of each PCL sample. The DP values obtained were decreased by 10 (the repeat units counted for the PCL1250 macro-initiator from ^{1}H NMR spectroscopy) or by 16 (the repeat unit counted for the PCL2000 macro-initiator from ^{1}H NMR spectroscopy) to obtain the final values. The M_n values calculated from these DP results are presented in Table 4. The DP values calculated using eq 2 were similar to those obtained by ^{1}H NMR spectroscopy (proton peak ratio method), indicating similar polymerization behavior as observed before.

An increase in M_n with increasing monomer-to-PCL-diol ratio was observed from SEC chromatograms, and this confirms the role of PCL-diol as an initiator. The PDI values for all of the PCL samples were close to 1.3, indicating that the initiator had no effect on the molecular weight distribution or the transesterification reactions. A decrease in relative crytallinity of PCL homopolymers was observed with an increase in the polymer chain length, for reasons similar to those discussed before.

Use of PEG as a Macro-initiator. Dihydroxyl-terminated PEG of different molecular weights (1000 and 2000 g/mol) were used to initiate the polymerization of DXO or CL with lipase-CA. In this way, triblock copolymers of DXO or CL with PEG, viz. poly(DXO-*b*-EG-*b*-DXO) or poly(CL-*b*-EG-*b*-CL), were synthesized. Six different polymers of DXO were obtained by

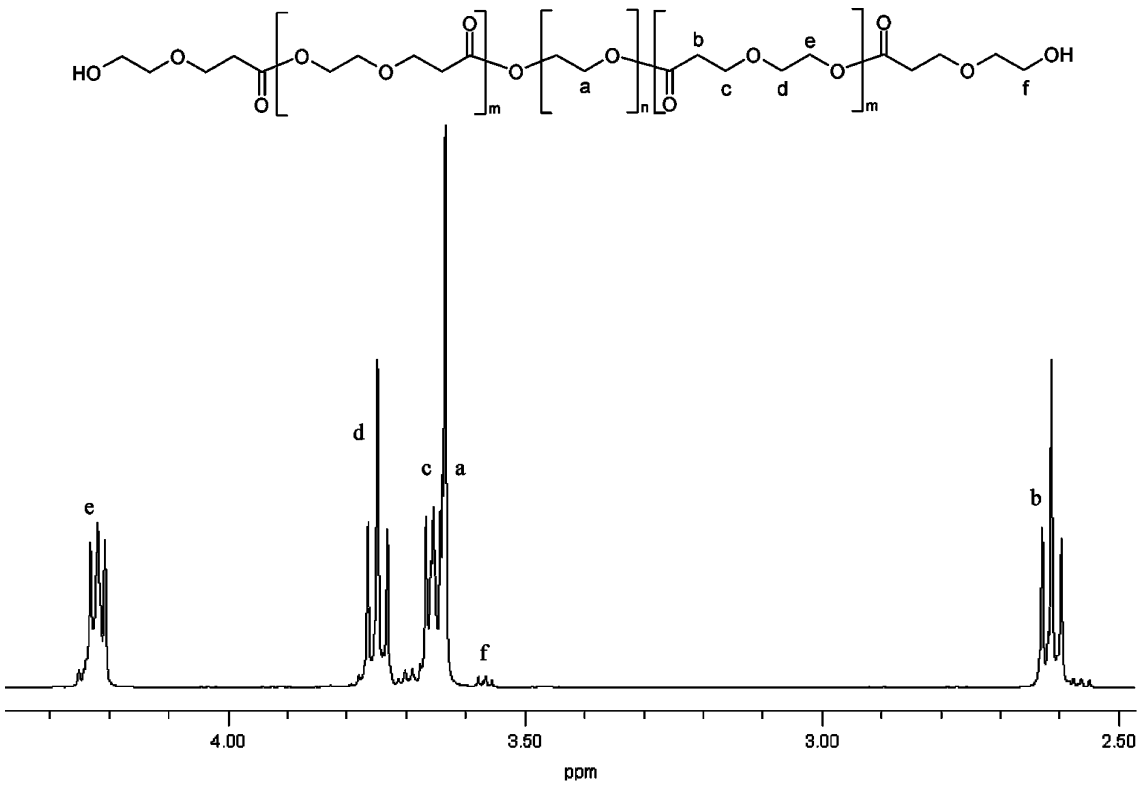


Figure 7. ^{1}H NMR spectrum of poly(DXO-*b*-EG-*b*-DXO) synthesized by the Laipse-CA-catalyzed ROP of DXO in the presence of PEG as an initiator (entry 1, Table 5).

changing the monomer-to-initiator molar feed ratio as indicated in Table 5. The percentage monomer conversion and DP were calculated from ^{1}H NMR spectra of the crude reaction mixture and the precipitated polymer sample, respectively. As can be seen in Table 5, the monomer conversion decreased with increasing monomer-to-initiator ratio. This observation follows the same trend as noticed before using 4-pentene-2-ol or PCL-diols as initiators, and therefore, it can be explained by similar reasons. The DP for each PDXO sample was calculated by taking the ratio of the peak intensity of the oxymethylene protons at 4.2 ppm to that of the terminal methylene protons at 3.6 ppm. A representative ^{1}H NMR spectrum of poly(DXO-*b*-EG-*b*-DXO) is shown in Figure 7. M_n was calculated from these DP values, and the results are presented in Table 5. The DP was also calculated using eq 2, and the living character of the polymerization was indicated when these DP values were compared with the values obtained by taking the ratio of proton peaks from ^{1}H NMR spectroscopy as described above. The values are presented in columns 5 and 6 of Table 5.

The expected increase in polymer molecular weight with increasing monomer-to-initiator ratio was observed in the SEC results. An increase in dispersity was observed with increasing DP of different poly(DXO-*b*-EG-*b*-DXO) samples because of the occurrence of possible transesterification reactions. Figure 8 shows the SEC chromatograms of different poly(DXO-*b*-EG-*b*-DXO) samples (entries 4−6 in Table 5) together with that of PEG2000. The complete consumption of PEG2000 as the initiator is revealed by the absence of any peak at higher retention times. T_g of the PDXO blocks in poly(DXO-*b*-EG-*b*-DXO) increased with increasing PDXO block length, as can be seen in Table 5.

Poly(CL-*b*-EG-*b*-CL) triblock copolymers were synthesized when PEG was used as initiator for CL polymerization in the presence of lipase-CA. Six different polymers with different DP values were generated, as reported in Table 6. The

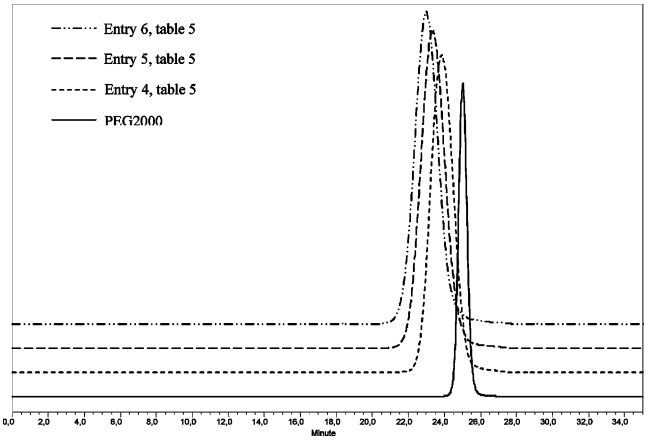


Figure 8. SEC chromatograms depicting the increase in molecular weight of poly(DXO-*b*-EG-*b*-DXO) (entries 4−6 in Table 5) copolymers with increasing monomer-to-initiator ratio in comparison with the initiator PEG2000.

percentage monomer conversion decreased with increasing monomer-to-initiator ratio, as observed before. The ratio of methylene protons due to PCL at 4.0 ppm to methylene protons due to PEG at ∼3.7 ppm was used to determine the DP of each PCL triblock copolymer. These DP results were used to calculate M_n values for the polymers, which are presented in Table 6. The DP values calculated using eq 2 were close to the values obtained by taking the ratio of proton peaks due to monomer and initiator.

The M_n values of the polymers, as obtained from SEC chromatograms, increased with increasing monomer-to-initiator molar ratio, confirming PEG's role as an initiator. The polydispersity of the poly(CL-*b*-EG-*b*-CL) polymers did not change with changing initiator or DP. The relative crystallinity of the PCL blocks in the different poly(CL-*b*-EG-*b*-CL) polymers

Table 6. Lipase-CA-catalyzed ROP of CL in the Presence of PEG as Macroinitiator

entry	initiator	M/In[a]	conversion[b] (%)	DP[c]	DP[d]	M_n[d]	M_n[e]	M_w[e]	PDI[e]	W_c[f] (%)
1	PEG1000	25	80.0	20	19	3200	9000	12 000	1.3	40.1
2	PEG1000	45	75.2	34	34	4900	15 200	19 300	1.3	39.0
3	PEG1000	90	63.0	57	54	7200	21 000	27 500	1.3	37.5
4	PEG2000	25	73.7	18	16	3800	8200	10 300	1.3	39.7
5	PEG2000	45	72.2	32	31	5500	12 100	16 000	1.3	36.8
6	PEG2000	80	64.9	52	52	7900	18 300	24 100	1.3	29.4

[a] Monomer-to-initiator molar feed ratio. [b] Determined by ^{1}H NMR spectroscopy of the crude reaction mixture. [c] DP calculated by feed composition and monomer conversion using the formula. DP = (M/In) × (conversion/100). [d] Determined by ^{1}H NMR spectroscopy of the precipitated polymer. [e] Data obtained by SEC with polystyrene standards. [f] Data obtained by DSC.

decreased with increasing PCL block length for reasons similar to those discussed before.

The triblock copolymers of DXO or CL with PEG generated in this way might find applications as biomedical tissue implants, sutures, or drug-delivery devices. The hydrophilic−hydrophobic character of the polymer can be controlled and tailored with a choice of blocks formed by PDXO or PEG (hydrophilic) and PCL (hydrophobic).

Conclusions

The lipase-CA-catalyzed ring-opening polymerization of seven-membered lactones has been extended to synthesize terminal-functionalized polyesters or triblock polyesters using an alcohol as the initiator. Macromers of PDXO or PCL containing terminal unsaturation, generated using 4-pentene-2-ol as the initiator, will be useful for synthesizing comblike polymers or for introducing hydrophilic or hydrophobic grafts into polyvinyls. Triblock copolymers of DXO or CL were synthesized using different dihydroxyl macro-initiators such as PCL-diol or PEG, where the hydrophilic or hydrophobic character of the copolymer was tailored by the choice of monomers and macro-initiators. This opens a novel pathway for the synthesis of polyesters having amphiphilic character. As indicated by the ^{1}H NMR results, all of the polymerizations were initiated by efficient introduction of the initiator group at the polymer chain terminal (monohydroxyl initiator) or inside polymer chain (dihydroxyl initiator), yielding polyester in a versatile single-step reaction. The degree of polymerization of the polyester chain segments could be easily controlled by varying the molar feed ratio of monomer to initiator. The role of alcohol as an initiator was confirmed by NMR, SEC, and FTIR analyses. The DP values calculated in different ways showed the living character of the lipase-CA-catalyzed polymerization of seven-membered ring lactones and also confirmed the role of the added alcohol as an initiator.

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