Enzyme-Catalyzed Ring-Opening Polymerization of ϵ-Caprolactone in Supercritical Carbon Dioxide

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ABSTRACT: We report the ring-opening polymerization reaction of ϵ-caprolactone in supercritical carbon dioxide ($scCO_2$) using an enzyme catalyst, Lipase B from *Candida antarctica* supported on macroporous beads (Novozym-435). Ring-opening polymerization of lactones is more commonly performed in organic solvents or in bulk using a Lewis acid catalyst. Recently there has been much interest in the replacement of such catalysts by enzymes. We demonstrate that the enzymatic route is viable in $scCO_2$, yielding poly(ϵ-caprolactone) (M_n = 12 000−37 000 g mol^{-1}) with molecular weights very similar to those obtained from the same enzyme catalysts in organic solvents, but with lower polydispersities (typical PDI = 1.4−1.6) and higher yields of polymer product (typically 95−98%). In the same process the unique "gaslike" mass transfer properties of $scCO_2$ can also be exploited to remove quantitatively any unconverted monomer and low molecular weight oligomers by $scCO_2$ extraction. It is also shown that the enzyme catalyst can be cleaned and recycled using $scCO_2$, while still producing high molecular weight polymer (M_n = 35 000−37 000 g mol^{-1}). Thus, a combination of enzyme catalyst and $scCO_2$ can be used repeatedly to prepare biodegradable poly(ϵ-caprolactone) (PCL) in the complete absence of potentially toxic organic solvents or metal catalysts.

Introduction

Supercritical fluids have been the focus of much research as potential replacements for conventional organic solvents. Much attention has been focused on supercritical carbon dioxide ($scCO_2$) primarily because it is cheap, environmentally friendly, nontoxic, and nonflammable, and its critical parameters are easily obtainable (T_c = 31.0 °C, p_c = 73.8 bar). However, the applications of $scCO_2$ have been limited by its solvent power, which lies somewhere between that of a nonpolar organic and perfluorinated solvent.[1] The attractive features of supercritical fluids are that they can easily be separated from the reaction products by lowering the pressure, and their solvent properties can be tuned by changing the density. Furthermore, supercritical fluids have solvent properties that are tunable between those of gases and liquids. Like gases they have a high diffusivity and low viscosity, whereas their densities are similar to those of liquids.[1−3]

Thus, there is tremendous scope for commercialization, and the unique solvent properties of $scCO_2$ are being exploited for the extraction of natural materials, for example, in decaffeination, ca. 100 000 tons of green coffee beans per year.[4] More recently, DuPont has built a substantial facility for the production of fluorinated polymers in $scCO_2$,[5] and Thomas Swan Ltd. and the Clean Technology Research Group at the University of Nottingham have developed a commercial plant for hydrogenation and a number of other reactions in $scCO_2$.[6,7]

Polymerization reactions have been studied extensively in $scCO_2$. The bulk of the work has been centered upon free radical dispersion polymerization[8,9] where the challenge has been to develop suitable stabilizers for use in $scCO_2$.[10,11] In all of these processes $scCO_2$ provides an advantage because the inherent plasticization of the polymer[8,9,12−14] enables monomer to diffuse rapidly to the active (radical) sites in the growing polymer particles. Other types of polymerization have also been explored.[8,9] Of particular relevance here is the ring-opening polymerization (ROP) of ϵ-caprolactone (ϵ-CL) in $scCO_2$ using dibutyltin dimethoxide as the initiator[15] and other polymers that have been prepared by ROP in $scCO_2$.[9,16−18] Thus, $scCO_2$ is now well established as a solvent in polymer chemistry.

Enzymes are highly active, selective, and environmentally friendly catalysts for polymer synthesis both in conventional organic solvents and in bulk.[19,20] In particular, the synthesis of polyesters by in vitro lipase-catalyzed ROP of lactones has been well documented.[21−26] The focus of such studies has been to eliminate the use of potentially toxic Lewis acid type catalysts that are used traditionally. A wide range of enzymes have been screened for the ROP of lactones, but the Lipase B from *Candida antarctica*, supported on macroporous beads (Novozym-435), has proved to be most active.

The polymerization of ϵ-CL, a seven-membered lactone, is a good model system to test the activity of an enzyme for lactone ROP.[19−25] Many studies reported that the ROP of ϵ-CL using Novozym-435 in bulk gave PCL of relatively low molecular weight and polydispersities greater than 2 (typically M_n = 3400−8500 g mol^{-1}, PDI = 2.1−2.5).[21−24] More recently, a considerable improvement was obtained with Novozym-435 catalysis by selecting toluene as the organic medium and using a ratio of ϵ-CL to toluene of 1:2 (w/v). This resulted in PCL in 86% yield (after solvent precipitation) with M_n and M_w/M_n of 44 800 g mol^{-1} and 1.7, respectively.[25]

The key question addressed in this paper is whether lipase-catalyzed lactone polymerizations can function effectively in $scCO_2$ and whether by using $scCO_2$ any

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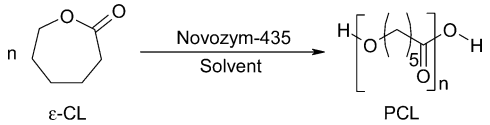

Figure 1. ROP of ϵ-CL using Novozym-435.

advantage is gained over working in conventional solvents. Enzymes have been utilized extensively in $scCO_2$ for processes ranging from acidolysis to chiral synthesis of esters, and there is extensive literature describing recent progress.[1,27] However, there is very little published work on enzyme-catalyzed polymerizations in $scCO_2$.[28−30]

Results and Discussion

Ring-Opening Polymerization. The reaction parameters for Novozym-435-catalyzed ϵ-CL ROP in traditional organic solvents have been systematically investigated (Figure 1).[25] The best results thus far were obtained in toluene with a ratio of ϵ-CL to toluene of 1:2 (w/v) using 10 mL of ϵ-CL and a Novozym-435 concentration of 10 wt % relative to the monomer. After a reaction time of 4 h at 70 °C, high molecular weight PCL (M_n 48 400 g mol^{-1}; PDI 1.7) was formed in 86% yield.

Our approach is to use $scCO_2$ as the solvent for the Novozym-435-catalyzed ROP of ϵ-CL. The monomer is soluble in $scCO_2$,[31] and the polymer product is insoluble but is efficiently plasticized.[12−14] Indeed, this led to a significant safety hazard (see Experimental Section). The reactions were performed in a reactor with a volume of 12.5 mL, and one-third of the autoclave was filled with ϵ-CL, giving conditions similar to those applied in conventional solvents, e.g., ϵ-CL to toluene ratio 1:2 (33 vol % ϵ-CL).[25] The results of a series of polymerization experiments are presented in Table 1, where an example of the reproducibility is demonstrated by a reaction repeated under identical conditions at a later date (entries 9 and 10). Our first priority was to establish the effect of reaction time under otherwise identical reaction conditions (entries 1−8). The highest molecular weight PCL was formed after 24 h (entries 2, 5, and 7). At shorter times monomer conversion to polymer appears incomplete, and extending the reaction to 72 h (entry 1) led to partial degradation of the polymer. Reaction temperature was also seen as an important variable. The temperature was reduced from 65 °C (entries 1−4) to 50 °C (entries 5 and 6) and to 35 °C (entries 7 and 8). In all cases, the density of the $scCO_2$ was kept constant. A small decrease in the molecular weight of the PCL product was observed that was most apparent at 6 h and 35 °C (entry 8) vs the 6 h reactions at 50 and 65 °C (entries 6 and 3, respectively). There also appeared to be a decrease in the yield as the temperature was reduced. From the ROP in organic solvents it is known that the concentration of the monomer has a strong influence on the polymer molecular weight and polydispersity.[25,26] In entries 9−11 the density of $scCO_2$ was decreased from 0.72 to 0.50 g cm^{-3} and thereby raised the effective concentration of ϵ-CL. This resulted in an increase in the molecular weight and yield of the PCL produced at 35 °C, while at 65 °C the increase in M_n was only small and there was no change in the yield. The effective concentration of ϵ-CL was reduced by increasing the density to 0.90 g cm^{-3} at 35 °C (entry 12). These results show that as the concentration of ϵ-CL is decreased, the molecular weight and yield both decrease. In general, these polymerizations in $scCO_2$ produce similar molecular weights and yields to those carried out in toluene.[25] The optimized results presented here are also significantly better than those published by Kobayashi for Novozym-435-catalyzed ROP of ϵ-CL using $scCO_2$ as the solvent, where the highest M_n was 17 000, but with a very high polydispersity of 4.0.[30] The loading of enzyme in the reaction was found to be crucially important. When Novozym-435 was reduced from 10 wt % through 5 wt % to 2.5 wt % (entries 9, 13, and 14, respectively), the molecular weight and yields were substantially reduced, which correlates well with results from polymerizations carried out in bulk.[22,24] Summarizing Table 1, the best results with respect to molecular weight and yield were obtained after 24 h at a low density of $scCO_2$ (entries 10 and 11), while temperature for 24 h reaction times did not appear to have a significant effect.

Table 1. Reaction Conditions and Results for the Novozym-435-Catalyzed ROP of ϵ-CL in $scCO_2$[a]

	T	p	$\rho(CO_2)$	t	GPC results[b]			yield[c]
entry	[°C]	[psi]	[g cm^{-3}]	[h]	M_n	M_w	PDI	[%]
1	65	3200	0.72	72	26 000	43 000	1.6	95
2	65	3200	0.72	24	30 000	51 000	1.7	97
3	65	3200	0.72	6	24 000	40 000	1.6	91
4	65	3200	0.72	2	17 000	30 000	1.8	93
5	50	2300	0.72	24	22 000	40 000	1.8	98
6	50	2300	0.72	6	18 000	29 000	1.6	69
7	35	1500	0.72	24	22 000	40 000	1.8	84
8	35	1500	0.72	6	12 000	18 000	1.5	70
9	35	1180	0.50	24	30 000	47 000	1.5	98
10[d]	35	1180	0.50	24	35 000	50 000	1.4	95
11	65	2010	0.50	24	35 000	54 000	1.5	98
12	35	3500	0.90	24	18 000	37 000	1.9	75
13[e]	35	1180	0.50	24	15 000	23 000	1.5	87
14[f]	35	1180	0.50	24	14 000	23 000	1.4	38
Recycling								
15[g]	50	2300	0.72	24	34 000	57 000	1.7	62[h]
16[g]	50	2300	0.72	24	35 000	67 000	1.9	42[h]
17[g]	50	2300	0.72	24	37 000	74 000	2.0	43[h]

[a] 10 wt % of Novozym-435 relative to ϵ-CL. [b] Relative to polystyrene. [c] Yield = [(weight of purified PCL)/(weight of ϵ-CL)] × 100. [d] Repeat of entry 9. [e] 5 wt % of Novozym-435 relative to ϵ-CL. [f] 2.5 wt % of Novozym-435 relative to ϵ-CL. [g] Novozym-435 contained in a filter pot during the reaction. [h] Yield = {(weight of extracted PCL)/[(weight of ϵ-CL) − (weight of PCL trapped in the filter pot)]} × 100.

Purification of the Polymer. In all of the published studies in conventional solvents or bulk the polymer product is purified by dissolution in an organic solvent and reprecipitation via an antisolvent, to remove residual monomer and low molecular weight oligomers. Our aim was to avoid the use of organic solvent by using the extractive properties of $scCO_2$. To achieve this, we first collected a portion of the raw material from the autoclave. The remainder was placed back into an autoclave and purged with $scCO_2$ at 4000 psi, 35 °C, for 4 h with a flow rate of approximately 5 mL of CO_2 min^{-1}. Any extracted materials were collected in a vial at the outlet tap and weighed and identified as monomer and oligomers by NMR and GPC. To demonstrate the efficiency of this method, the polymer was analyzed by GPC before and after extraction. Figure 2 shows typical GPC chromatograms before (a) and after (b) the extraction process.

In Figure 2a, signals caused by oligomeric reaction products occur after 17−18 min of elution time. After the extraction process with $scCO_2$ these signals disappeared (Figure 2b). The extract consisted of oligomers with a molecular weight of up to approximately 1500 g

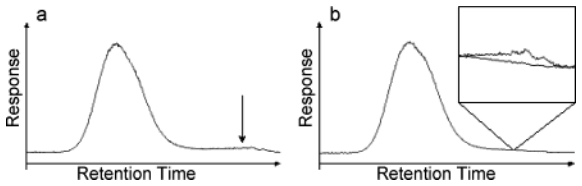

Figure 2. GPC chromatograms of PCL (a) crude PCL product (b) after extraction with $scCO_2$. Note that the oligomeric peaks (arrowed) completely disappear after extraction. The inset shows the expanded region of the GPC trace before (top trace) and after (bottom trace) extraction of the oligomers with $scCO_2$.

mol^{-1}, including the residual monomer. ^1H NMR analysis revealed that no detectable monomer was left in the purified PCL samples.

Accordingly, the purification of PCL with $scCO_2$ is efficient and environmentally benign since no organic solvents were used, and the polymer did not require drying as in the case of purification by precipitation.

In subsequent experiments (Table 1, entries 10−12) the purification of the PCL by extraction with $scCO_2$ was performed in a single step in the same autoclave directly after the polymerization. Careful control of the $scCO_2$ flow rate to maintain a constant autoclave pressure of 4000 psi during the extraction ensured that there was no extrusion of the polymer through the pipes or valves and that no blockages occurred. After the extraction for 4 h at 35 °C and 4000 psi, the autoclave was cooled with acetone/dry ice before opening and subliming away the CO_2. Analysis of the polymer by a combination of GPC and NMR demonstrated that there were no monomer residues remaining in the products after in-situ $scCO_2$ extraction.

Another important area is that of enzyme recycling. In conventional solvents, the enzyme is separated from the product PCL by dissolving and filtering to recover the supported enzyme beads. In our experiments (Table 1, entries 1−14) the supported enzyme beads are free inside the autoclave and are trapped within the resultant PCL product, and must be separated in the same way, by use of dissolution and filtering. Our aim was to see whether the recycling might be achieved *without* use of conventional solvents. To do this, the enzyme beads were placed in a small wire mesh filter pot, which was put in the bottom of the autoclave. Initial results demonstrated that containing the enzyme during the reaction leads to a modest increase in the molecular weight; however, the yield is reduced (compare entries 5 and 15). This may be due to a reduction in the total surface area of the enzyme or the PCL formed around the enzyme in the filter pot inhibiting the mass transfer to and from the enzyme. After removing the bulk of the product PCL from the autoclave and from the outside of the filter pot, gravimetric analysis showed that the weight of the enzyme beads (0.4 g) and filter pot had increased by 0.8 g—equivalent to twice the weight of Novozym-435. Clearly some of the PCL was trapped in the filter pot and on/within the enzyme beads. Various flow experiments were conducted in $scCO_2$ to try to remove this residual polymer. The principle here was to use $scCO_2$ to plasticize (effectively liquefy) the PCL such that it could easily be separated from the cross-linked polymer enzyme supporting beads. This did not occur under a simple flow of $scCO_2$. After some optimization, the best method for removing the polymer was found to be "spin-cleaning" in $scCO_2$. The separation was achieved by transferring the filter pot to a larger, 60 mL autoclave and attaching it directly to the shaft of the motor-driven stirrer blade. This autoclave was pressurized to 4000 psi at 35 °C, and the pot was spun at 4500 rpm for 1 h before the pressure was released. This spin cleaning was repeated a further three times. Under these conditions 90% of the adhered PCL was removed from the enzyme beads. Note that PCL is not soluble in $scCO_2$ under the conditions of our experiment.

The filter pot containing enzyme was then placed back in the 12.5 mL autoclave. On addition of fresh monomer, reusing the cleaned enzyme in a subsequent polymerization again led to high molecular weight polymer but with a decrease in yield (entry 16). The enzyme cleaning and recycling steps were repeated and demonstrated that there was no further change in molecular weight or yield—even after exhaustive $scCO_2$ cleaning and reuse (entry 17). It may be that the yield decreased after the first recycling step due to the leaching of the Lipase B from the immobilization matrix. The enzyme remaining in the beads after the first polymerization reaction and extraction process may be more tightly bound and, therefore, retained during the subsequent polymerization and reuse. Alternatively, a portion of the enzyme within the beads may be denatured, leading to a partial loss in overall catalyst activity. The retention of enzyme within the beads and activity of the enzyme retained within the recycled catalyst are currently being studied by IR microspectroscopy.[32]

Conclusions

$scCO_2$ is an environmentally benign replacement for organic solvents in the enzyme-catalyzed ROP of ϵ-CL. The monomer ϵ-CL is soluble in the supercritical fluid containing the dispersed enzyme beads, and PCL precipitates out during the polymerization reaction. The quality and yield of the polymer obtained can be optimized with a reaction time of 24 h using 33 vol % of ϵ-CL and a low density of CO_2 of 0.50 g cm^{-3}. The molecular weights are very similar to the results obtained in toluene, but in $scCO_2$ the PDI of the PCL was lower and the yields were higher.

Furthermore, the whole process can be carried out using only $scCO_2$, i.e., synthesis and recycling to clean the polymer by extracting any residual monomer and low molecular weight oligomers. By this strategy the need to use organic solvents to purify the polymer is eliminated. Such a step would be important in the manufacture of bioresorbable polymers such as PCL for biomedical applications. In addition, the enzyme can be recycled and reused without the use of any organic solvents. The enzyme retains activity despite prolonged exposure to $scCO_2$ and repeated pressurization and depressurization cycles. These results clearly demonstrate that in principle a one-pot, semicontinuous batch synthesis of PCL could be carried out using only $scCO_2$ as the processing solvent. This combination of enzyme catalyst and $scCO_2$ could be described as a truly "green" process for the formation of high molecular weight, biodegradable PCL.

Experimental Section

Materials. ϵ-Caprolactone (99%) was purchased from Aldrich, stirred with CaH_2 for 24 h under argon, distilled under reduced pressure, and kept in a Schlenk under argon until used. Novozym-435 (10 wt % lipase B from *Candida antarctica* on a macroporous acrylic resin, specified activity 7000 PLU g^{-1}) was a gift from Novozymes. Prior to its use in the experiments, the enzyme was dried under an oil pump vacuum for 30 min. Carbon dioxide (99.99%, H_2O <2 ppm) was obtained from BOC Gases.

Methods. *General Procedure (Entry 9).* Novozym-435 (0.4 g) was dried under an oil pump vacuum. After 30 min, the dried enzyme beads were transferred to the body of the 12.5 mL autoclave containing a magnetic stirrer bar, to which ϵ-CL (4.2 mL) was added. The autoclave was immediately closed and placed into an aluminum heating block, preheated to 35 °C. The autoclave was briefly purged with CO_2 and then filled to 1180 psi at 35 °C, at which point the stirring was started. After 24 h the heating and stirring were both turned off, and the autoclave was placed into an acetone/dry ice bath. Once the pressure inside the autoclave had fallen to approximately atmospheric pressure, the autoclave was opened, covered, and left in a freezer until the dry ice had sublimed, leaving 4.73 g of PCL and enzyme. At this point a small sample of the product was taken for analysis. The remainder of the product was put into a 60 mL volume autoclave and pressurized to 4000 psi at 35 °C without stirring. The outlet tap was opened slightly with the inlet tap fully open, producing a flow of CO_2 through the autoclave of approximately 5 mL min^{-1}. After 4 h the heating was turned off, and both the outlet and inlet taps were closed. Acetone/dry ice was used to freeze the contents of the autoclave, after which the autoclave was opened as before. Product (4.66 g) was recovered from the autoclave, corresponding to a yield of 98%.

For the Recycling Studies (e.g., Entry 15). Novozym-435 (0.4 g) was placed into the wire mesh filter pot (Swagelok TF SS-4F-K4-230, 230 μm filter strainer, volume approximately 5 mL) and dried under oil pump vacuum for 30 min. The wire mesh filter pot containing the enzyme (4.31 g) was placed into the body of a 12.5 mL autoclave containing a magnetic stir bar, to which ϵ-CL (4.2 mL) was added. The bulk of the PCL (3.41 g) was collected and extracted with $scCO_2$ as before, leading to 2.18 g of PCL recovered. Additionally, the filter pot containing the enzyme and some PCL (5.10 g) was removed and was attached to the shaft of a motor driven stirrer blade inside a larger, 60 mL volume autoclave, which was pressurized to 4000 psi at 35 °C. The wire mesh filter pot was spun at 4500 rpm for 1 h, causing some of the plasticized PCL to be forced out, after which the pressure was released. This process was repeated a further three times, reducing the weight of the filter pot to 4.38 g, corresponding to a removal of over 90% of the occluded PCL.

Important Safety Note. Poly(caprolactone) is very efficiently plasticized by $scCO_2$,[12−14] a phenomenon that we and others have sought to exploit.[33,34] Thus, when venting, the softened and swollen polymer was found to extrude very effectively through high-pressure pipework and valves, producing an extruded "sausage" of poly(caprolactone). However, under atmospheric conditions the plasticization effect is lost, and the polymer hardens within the valve, preventing further release of pressure. When attempting to vent through the second valve on the autoclave, the same problem resulted. Thus, we were faced with the difficult problem of releasing the pressure through careful opening of the main threaded seal. A far safer methodology, adopted in all of our subsequent experiments, was to place the autoclave, after the appropriate reaction, into an acetone/dry ice bath (approximately −78 °C) to freeze the CO_2/polymer mixture, whereby the pressure dropped to approximately atmospheric pressure. To extract the monomer and low molecular weight oligomers, the autoclave was purged with $scCO_2$ at 4000 psi and 35 °C for 4 h (flow rate: approximately 5 mL of CO_2 min^{-1}). Since PCL is insoluble in $scCO_2$ under these conditions, only the monomer and low molecular weight oligomer are removed. The autoclave was then depressurized and opened as described above, and the reaction mixture could be removed and stored in a freezer at −20 °C, during which time all dry ice sublimed.

Polymer product (both before and after extraction) and the collected extracts were analyzed by GPC using an LC 1120 HPLC pump (Polymer Laboratories), chloroform as the solvent at 30 °C, two PLgel 5 μm Mixed-D columns (Polymer Laboratories), and an evaporative light scattering detector (Polymer Laboratories PL-ELS 1000). Calibration was carried out using polystyrene standards. Both the sample analysis and the calibration were conducted at a flow rate of 1 mL min^{-1}.

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