

Candida antarctica Lipase B Catalyzed Polycaprolactone Synthesis: Effects of Organic Media and Temperature

Ajay Kumar and Richard A. Gross*

NSF Center for Biocatalysis and Bioprocessing of Macromolecules, Polytechnic University, Department of Chemistry and Chemical Engineering, Six Metrotech Center, Brooklyn, New York 11201

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Engineering of the reaction medium and study of an expanded range of reaction temperatures were carried out in an effort to positively influence the outcome of Novozyme-435 (immobilized Lipase B from *Candida antarctica*) catalyzed ϵ -CL polymerizations. A series of solvents including acetonitrile, dioxane, tetrahydrofuran, chloroform, butyl ether, isopropyl ether, isooctane, and toluene ($\log P$ from -1.1 to 4.5) were evaluated at 70°C . Statistically (ANOVA), two significant regions were observed. Solvents having $\log P$ values from -1.1 to 0.49 showed low propagation rates ($\leq 30\%$ ϵ -CL conversion in 4 h) and gave products of short chain length ($M_n \leq 5200$ g/mol). In contrast, solvents with $\log P$ values from 1.9 to 4.5 showed enhanced propagation rates and afforded polymers of higher molecular weight ($M_n = 11500$ – 17000 g/mol). Toluene, a preferred solvent for this work, was studied at ϵ -CL to toluene (wt/vol) ratios from 1:1 to 10:1. The ratio 1:2 was selected since, for polymerizations at 70°C , 0.3 mL of ϵ -CL and 4 h, gave high monomer conversions and M_n values ($\sim 85\%$ and $\sim 17\,000$ g/mol, respectively). Increasing the scale of the reaction from 0.3 to 10 mL of CL resulted in a similar isolated product yield, but the M_n increased from 17 200 to 44 800 g/mol. Toluene appeared to help stabilize Novozyme-435 so that lipase-catalyzed polymerizations could be conducted effectively at 90°C . For example, within only 2 h at 90°C (toluene- d_8 to ϵ -CL, 5:1, $\sim 1\%$ protein), the % monomer conversion reached $\sim 90\%$. Also, the controlled character of these polymerizations as a function of reaction temperature was evaluated.

Introduction

Recently, research on enzymatic polymerizations in non-aqueous or nontraditional media has been receiving increased attention as a new tool for building polymer chains.¹ Polymers with well-defined structures can now be prepared by in vitro enzyme catalysis.^{2–8} In contrast, attempts to attain similar levels of polymer structural control by conventional methods might not be possible, or may not be practical, due to requirements of multiple protection–deprotection steps. Earlier, investigations of enzymatic polyester syntheses were mainly by bacterial fermentation.⁹

Enzyme-catalyzed transesterification reactions between diesters and diols have been used to prepare polyesters via condensation polymerization.^{10–13} These generally require long reaction times and give low molecular weight products. Ring-opening chemistry offers an important alternative route since leaving groups that can limit monomer conversion or degree of polymerization are not generated during polymerizations.^{3b}

A rapidly increasing number of publications have appeared that document studies on various aspects of in vitro enzyme-catalyzed lactone ring opening polymerizations.^{2,3,14–16} Our group^{2f–h} and others^{3f} has been investigating the mechanistic and kinetic features of lipase-catalyzed ring-opening polymerization of lactones. Recently, we investigated the affects

of lipase (*Candida antarctica* Lipase B) concentration on ring-opening bulk polymerization of ϵ -caprolactone.^{2c} We found that the monomer consumption followed a first-order rate law and that no chain termination occurred.

Despite significant advancements in recent years, a number of problems still exist in transferring the protocols of in vitro enzyme-catalyzed polyester synthesis from the laboratory to an industrial scale. Of particular concern is the relatively low catalytic activities displayed by enzymes when used in nonaqueous media.¹⁷ The problem is well illustrated in studies by Kobayashi et al.¹⁹ and Dong et al.¹⁸ that describe low reaction rates and molecular weight values for lipase-catalyzed ϵ -CL polymerizations. This necessitates the use of comparatively large amounts of enzymes to ensure that the desired extent of polymerization occurs within a reasonable time scale. The productivity of enzymatic catalysis, in terms of units of polymer obtained per units of enzyme used, will to a large extent determine the feasibility of employing an enzymatic process in commercial processes. Surprisingly, limited effort has been directed at enhancing the kinetics of in vitro enzyme-catalyzed ring opening lactone polymerization. Such work would transform these new polymerization routes from academic curiosities to methods with reaction kinetics that are worthy of attention by industrial scientists. In that spirit, this paper describes work carried out in our laboratory on Novozyme-435 (immobilized *Candida antarctica* lipase B) catalyzed ring-opening polymerizations of ϵ -CL. We explored how engineering of the reaction media,

* To whom correspondence may be addressed. E-mail: Kumar@poly.edu, rgross@poly.edu.

by manipulating the solvent structure and concentration, can be used to expand the range of temperatures at which lipase-catalyzed polymerizations can be conducted. Furthermore, systematic regulation of these variables led to new knowledge on how to control the propagation kinetics, molecular weight, and "living" character of these polymerizations.

Material and Methods

Polymerization grade ϵ -caprolactone, a gift from Union Carbide, was first dried over calcium hydride and then distilled under reduced pressure in nitrogen atmosphere. Anhydrous acetonitrile, dioxane, tetrahydrofuran, chloroform, butyl ether, isopropyl ether, isooctane, toluene, and toluene- d_8 were purchased from Aldrich Chemical Co. Toluene was dried over calcium hydride, and distilled under nitrogen atmosphere. Coulomat A and Coulomat C were purchased from EMscience. Novozyme-435 (specified activity 7000 PLU/g) was a gift from Novo Nordisk Co. All liquid chemical transfers were performed by syringe through rubber septum caps under nitrogen atmosphere. Novozyme-435 (specified activity 7000 PLU/g) was a gift from Novo Nordisk Co.

Novozyme-435 Catalyzed in Vitro Polymerization of ϵ -Caprolactone. Novozyme-435 (12 mg) dried in a vacuum desiccator (0.1 mmHg, 25 °C, 24 h) was transferred under nitrogen atmosphere into oven dried 7 in. long premium (60–360 MHz) NMR tube. The tubes were stoppered with rubber septa, sealed with Teflon tape, and ϵ -CL (0.12 mL) and toluene- d_8 (0.6 mL) were added by syringe under nitrogen. The NMR tubes were placed in the NMR spectrometer with the temperature probe set at the desired temperature. NMR data were recorded, and the tube was taken out, shaken well, and put in the probe for the next recording. All of these procedures were performed as rapidly as possible to avoid fluctuations in the reaction temperature. After a desired monomer conversion, the reactions were terminated by adding an excess of cold chloroform and removing the enzymes by filtration (glass-fritted filter, medium pore porosity). The chloroform in the filtrate was in large part removed by rotary evaporation and the polymer in the concentrated solution was precipitated in methanol. The precipitate was isolated by filtration and dried (0.1 mmHg, 50 °C, 24 h). The molecular weight and dispersity of the samples were determined by gel permeation chromatography (GPC). Experiments such as the effect of solvent and monomer concentration on ϵ -CL polymerization were performed in 10 mL Pyrex culture tubes instead of NMR tubes with stirring (220 rpm) with a batch size of 0.3 mL of CL. The workup and isolation of the products formed were as described above.

Instrumentation Methods. ϵ -CL polymerization was monitored in situ by ^1H NMR to determine: (i) monomer conversion, (ii) number average molecular weight, and (iii) total number of polymer chains. ^1H NMR spectra were recorded on a Bruker NMR spectrometer (model DPX300) at 300 MHz. The chemical shifts in parts-per-million (ppm) for ^1H NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference. ^1H NMR

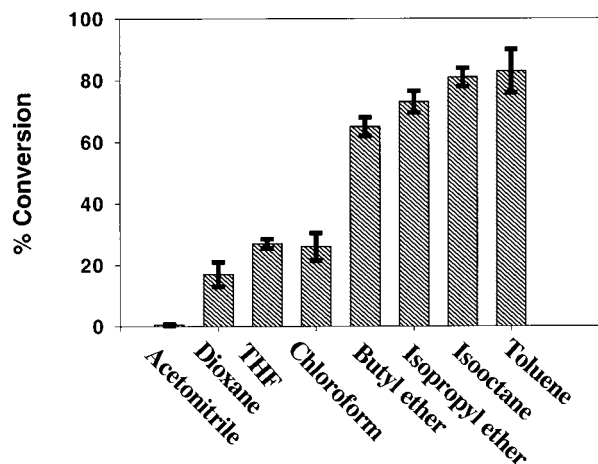


Figure 1. Effect of the solvent on the percent monomer conversion for Novozyme-435 catalyzed polymerizations of ϵ -CL at a monomer/solvent ratio 1:3 for 4 h. The Novozyme-435 concentration is 10 wt % relative to the monomer, and the catalytic protein is 10% (w/w) of Novozym-435. The enzyme concentration remained unchanged for all other experiments described in this paper.

spectra of poly-(O=C-CH₂-(CH₂)₂-CH₂-CH₂-O)- were as follows: ^1H NMR (CDCl₃) δ 4.07 (t, J 6.5 Hz, OCH₂), 3.61 (t, J 6.5 Hz, HOCH₂), 2.31 (t, J 7.5 Hz, COCH₂), 1.65 and 1.30 [m, (CH₂)₃] ppm. The parameters used for the NMR experiments were as follows: 4.0 wt %/wt polymer in chloroform- d , temperature 28–105 °C, pulse width 60°, 18 000 data points, relaxation delay 1.0 s, and 32 transients.

Molecular weights were determined by GPC using a Waters HPLC system equipped with model 510 pump, Waters model 717 autosampler, model 410 refractive index detector, and model T-50/T-60 detector of Viscotek Corp. with 500, 10³, 10⁴, and 10⁵ Å Ultrastaygel columns in series. Trisec GPC software version 3 was used for calculations. Chloroform was used as the eluent at a flow rate of 1.0 mL min⁻¹. Sample concentrations of 0.2% wt/vol and injection volumes of 0.1 mL were used. Molecular weights were determined based on conventional calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich Chemical Co.

Reaction initial water contents (wt % water) were measured by using an Aqua star C 3000 titrator with Coulomat A and Coulomat C from EMscience. Enzyme water content was determined by stirring 53 mg of Novozyme-435 in Coulomat A within the Aqua star closed septum container and titrating it against Coulomat C and was found to be less than 0.8%. Similarly the water content of solvent was determined by stirring 10 mL of each solvent and was found to be less than 0.01%.

Result and Discussion

Effect of Solvent Engineering on Novozyme-435 Catalyzed ϵ -CL Polymerization. Polymerizations at 70 °C were conducted while using different organic media (see Figures 1 and 2). The concentration of the catalytic protein relative to monomer was maintained at about 1% (wt/wt) throughout this work. Reactions were performed by transferring ϵ -CL (0.3 mL) and Novozyme-435 (30 mg) into Pyrex tubes stoppered with rubber septa under a nitrogen atmosphere (see

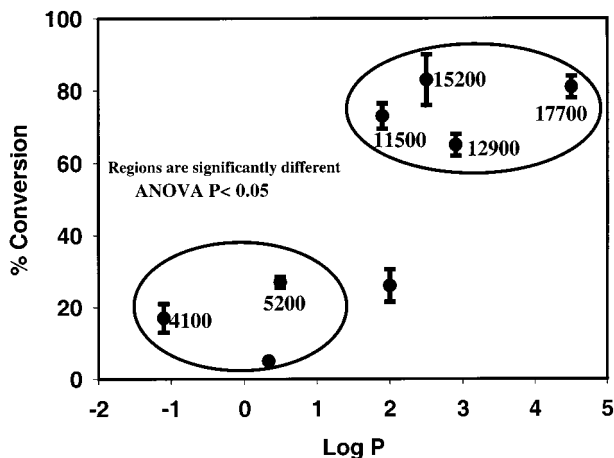


Figure 2. Effect of dielectric constant on the percent monomer conversion for Novozyme-435 catalyzed polymerizations of ϵ -CL at a monomer/solvent ratio 1:3 for 4 h.

Experimental Section). Solvents (0.6 mL) including acetonitrile, dioxane, tetrahydrofuran, chloroform, butyl ether, isopropyl ether, isooctane, and toluene were transferred by syringe into reactions that were stirred for 4 h and maintained at 70 °C. Investigation of the relationship between $\log P$ and polymer isolated yields showed that two $\log P$ regions exist that give differences in polymer isolated yields that are statistically significant (ANOVA). Use of solvents in the first group (dioxane, -1.1; acetonitrile, 0.33; tetrahydrofuran, 0.49) resulted in slower polymerization kinetics and lower chain molecular weights. Acetonitrile, despite having a slightly higher $\log P$ value than dioxane (17% yield), showed almost no polymerization. A partial explanation for this may be a deactivation of the enzyme in polar media due to enzyme conformational changes.¹⁸ Also, polar media can negatively influence the distribution and interactions of water in the outer layer of the protein molecules. In contrast, the second group of solvents (isopropyl ether, 1.9; toluene 2.5; butyl ether, 2.9; isooctane, 4.5) resulted in relatively higher monomer conversion and polymer molecular weights. Polymerization of ϵ -CL in isooctane and toluene gave the highest percent monomer conversion (about 80% within 4 h) and moderate molecular weights (15 000). It is noteworthy that butyl ether, which had a greater $\log P$ value than toluene (2.9 versus 2.5), gave a relatively lower percent monomer conversion and M_n (about 60% and 12 900 g/mol, respectively) (Figures 1 and 2). Thus, the observed differences cannot be explained by simply considering $\log P$ values. A better understanding of how solvent geometry, dipole moments, solubilization of substrates, and other factors influence the physicochemical and catalytic properties of enzymes is needed.²⁰ With respect to solubility of the reaction substrates, formed PCL readily dissolved in acetonitrile, dioxane, tetrahydrofuran, chloroform, and toluene but was sparingly soluble in isopropyl and butyl ethers. Polymerization of ϵ -CL using isooctane had two immiscible liquids (ϵ -CL and isooctane) and a solid-phase enzyme catalyst. In contrast, polymerizations using toluene gave a solution in which ϵ -CL and polymer were soluble and the catalyst remained insoluble. We concluded that for planned studies of reaction kinetics and mechanism, polymerizations conducted in toluene were advantageous to those in isooctane.

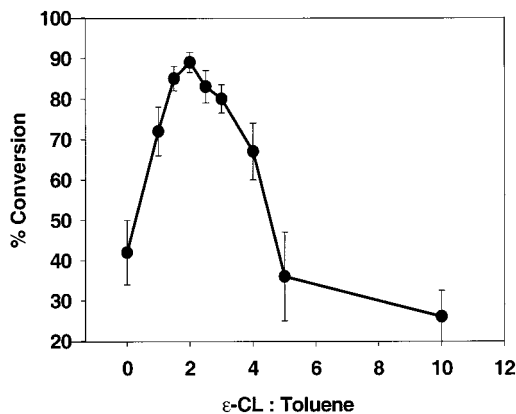


Figure 3. Plot of percent monomer conversion versus the ratio of toluene to ϵ -CL used for polymerizations at 70 °C.

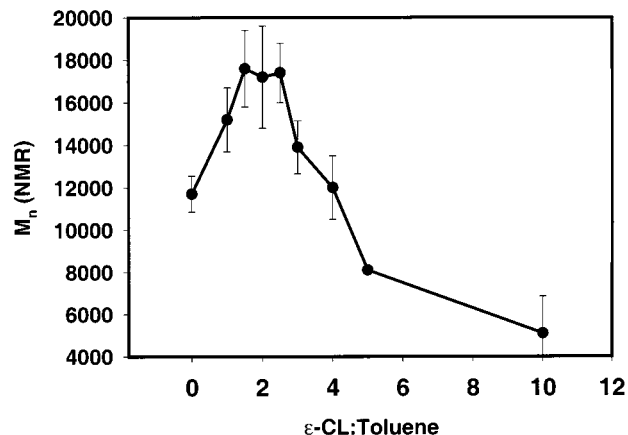


Figure 4. Plot of M_n versus the ratio of toluene to ϵ -CL used for polymerizations at 70 °C.

Effect of Monomer Concentration on Reaction Rate and M_n . Ratios of toluene to ϵ -CL of 0:1 (solventless), 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 4:1, 5:1, and 10:1 (wt/vol) were investigated for polymerizations at 70 °C for 4 h (see Figures 3 and 4). To bulk or solventless ϵ -CL reaction mixtures, the addition of toluene (e.g., 2 parts relative to ϵ -CL, wt/vol) resulted in increased percent monomer conversion, product M_n , and reduced polydispersity (M_w/M_n) values. For example, solventless polymerizations gave % ϵ -CL conversion, M_n , and polydispersity values of 41%, 10 800 g/mol, and 2.1, respectively. In contrast, when the polymerization consisted of toluene/ ϵ -CL 2:1 vol/vol, % ϵ -CL conversion, M_n , and polydispersity were 85%, 17 200 g/mol, and 1.8, respectively. Also, a region exists between toluene/ ϵ -CL ratios of 1.5 to 3 where Novozyme-435 catalyzed ϵ -CL polymerizations occur with substantially increased reaction rates. In addition a region between toluene/ ϵ -CL 1.5 and 2.5 gave products of higher molecular weights (see Figures 3 and 4). The experiments described above were on a scale of 0.3 mL of ϵ -CL. A reaction was carried out to scale-up PCL synthesis to the multigram level. Therefore, the method used followed that described above except that CL (10 g), toluene (20 mL), and Novozyme-435 (1 g) were added to a round-bottom flask (250 mL) and the reaction was maintained at 70 °C, for 4 h, with magnetic stirring. The isolated product was obtained in 86% yield with a M_n and polydispersity index of 44 800 g/mol and 1.7, respectively. Therefore, increasing the scale of the reaction from 0.3 to 10 mL of CL resulted in a similar

isolated product yield, but the M_n increased from 17 200 to 44 800 g/mol. Possible explanations may lie in differences of heat transfer rates and other factors such as the susceptibility to water uptake during reactions. Systematic studies where mixing and other physical variables are changed in a controlled manner are planned to understand this result.

The polydispersity of the polymers formed was also influenced by the ratio of toluene to monomer used. Toluene-to-monomer ratios of 0:1, 5:1, and 10:1 gave products with polydispersity values of 2.09, 1.52, and 1.38. A short discussion of factors controlling the molecular weight distribution in lipase-catalyzed polymerizations such as relative rates of initiation to propagation, the occurrence of chain transfer, termination, and transesterification was published by us previously.^{2f} More recently, studies were completed that compare lipase-catalyzed polymerizations based on different polymerization mechanism models (manuscript in preparation).

Temperature Effects on Novozyme-435 Catalyzed ϵ -CL Polymerization. The temperature effects were monitored by in situ NMR analyses. In other words, the NMR probe temperature was adjusted to different reaction temperatures (60, 70, 80, 85, 90, 100, and 105 °C), and the reactions were performed in NMR tubes using toluene- d_8 as the solvent (see Materials and Methods). NMR experiments at a ratio 1:2 (vol/vol) toluene- d_8 to ϵ -CL created difficulty in locking signals due to increased viscosity; thus, a ratio 5:1 (vol/vol) toluene- d_8 to ϵ -CL was selected. At this concentration, the results in the NMR tube were similar to those of reactions carried out in vials outside the NMR. The NMR instrument was locked and was maintained at fine shim that avoided broadening of signals at high conversion due to increased viscosity. The signals at δ 3.99 (t, J 6.5 Hz, OCH_2^a), 2.48 (t, J 7.5 Hz, COCH_2^a), and 1.71 and 1.52 (m, $(\text{CH}_2)_3^a$) were observed at zero time and were assigned to the protons of ϵ -CL monomer. Signals at δ 4.18 (t, 6.5 Hz, OCH_2^b), 3.62 (t, 6.5 Hz, HOCH_2^b), and 2.42 (t, J 7.5 Hz, COCH_2^b) appeared after the onset of polymerization reactions and were assigned to PCL protons. The ratio of the signals at δ 4.18 to 3.99 and δ 4.18 to 3.62 were used to calculate the monomer conversion and M_n , respectively. Control reactions were conducted by using a thermally deactivated Novozyme-435, at 100 and 105 °C, and by using identical conditions as those employed for polymerizations with active Novozyme-435. This experiment showed that after 3 h, at 105 °C, the monomer conversion was <5 mol %.

The results of in situ NMR analyses of percent monomer conversion as a function of reaction time and temperature are shown in Figure 5. The initial slope of the percent monomer conversion versus time plots for each reaction temperature (Figure 5) was used to calculate the apparent rate constants (k_{app}) of the polymerizations (Figure 6). Figure 5 shows that the result of increasing the reaction temperature from 60 to 70, 80, 85, and 90 °C was more rapid conversion of monomer. However, further increases in the reaction temperature from 90 to 100 and 105 °C gave decreased rates of monomer conversion. An explanation for decreased monomer conversion rates for reaction temperatures >90 °C is the occurrence of protein denaturation and deactivation at

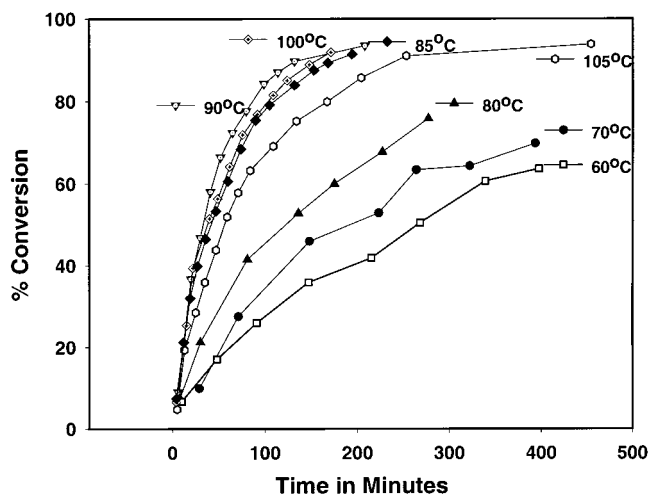


Figure 5. Plots of percent monomer conversion as a function of reaction time for a series of polymerizations carried out between 60 and 105 °C. The ratio of toluene- d_8 to CL was 5:1.

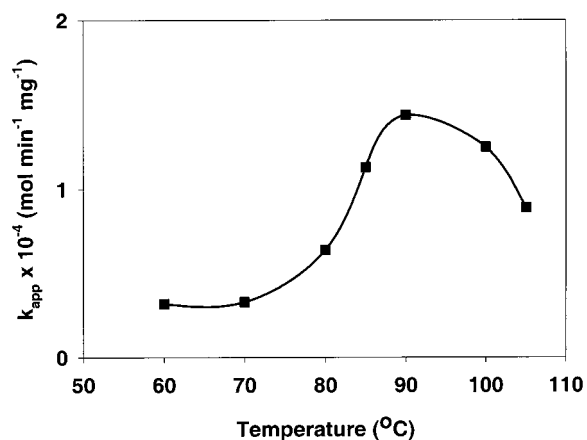


Figure 6. Influence of the reaction temperature on the apparent propagation rate constant $k_{\text{app}} \times 10^{-4}$ (mol min⁻¹ mg⁻¹) of Novozyme-435. The ratio of toluene- d_8 to CL was 5:1.

these elevated temperatures. Analysis of Figure 6 led to the same conclusions with the exception of the 60–70 °C temperature interval, which has almost similar k_{app} that can be explained by a short lag period at 70 °C (Figure 5). Hence, for Novozyme-435 catalyzed ϵ -CL polymerizations in toluene, 90 °C is the preferred reaction temperature for optimal rates of ϵ -CL conversion to polymer. These conditions resulted in >90% monomer conversion within 2 h.

The results in Figures 5 were used to construct plots of M_n versus monomer conversion for different reaction temperatures (Figure 7). Plots of M_n versus reaction temperature shown in Figure 8 were constructed by abstracting M_n values from Figure 7 at three representative monomer conversions (25, 45, 63.5%). Observation of all three curves in Figure 8 shows that the maximum and minimum M_n values occurred at 60 and 90 °C, respectively. At monomer conversions of 45 and 63.5%, M_n increased with the following order of reaction temperatures: 90 < 100 < 105 = 85 < 70 = 80 < 60 °C. Thus, although 90 °C gave the most rapid monomer conversion, this temperature resulted in products of the lowest M_n . Conversely, reactions at 60 °C that gave the slowest monomer conversion produced polymers with the highest M_n .

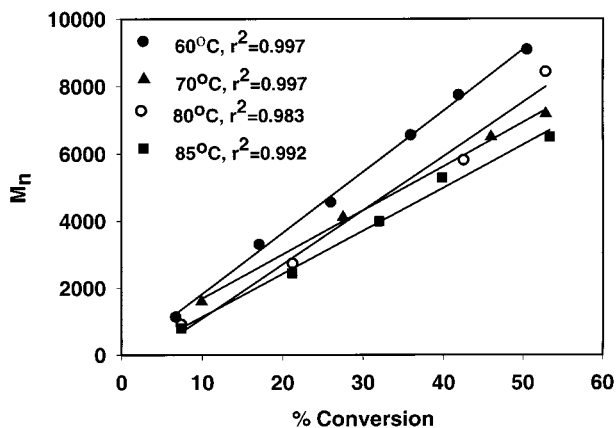


Figure 7. Plots of M_n versus conversion for reactions carried out at temperatures between 60 and 85 °C. The ratio of toluene- d_8 to CL was 5:1.

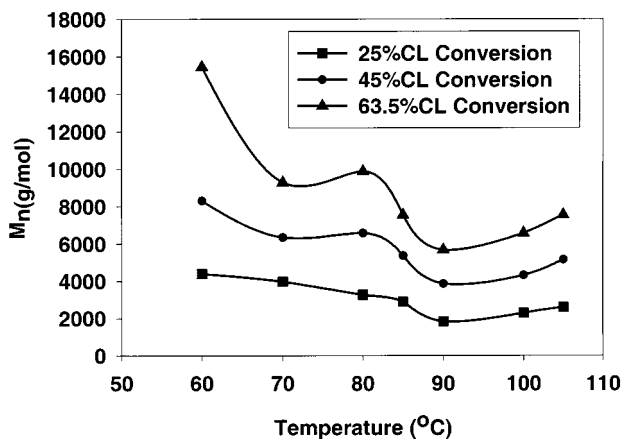


Figure 8. Plots of M_n versus the reaction temperature that was generated by selecting results from Figure 6 at 25, 45, and 65.3% ϵ -CL conversions. The ratio of toluene- d_8 to CL was 5:1.

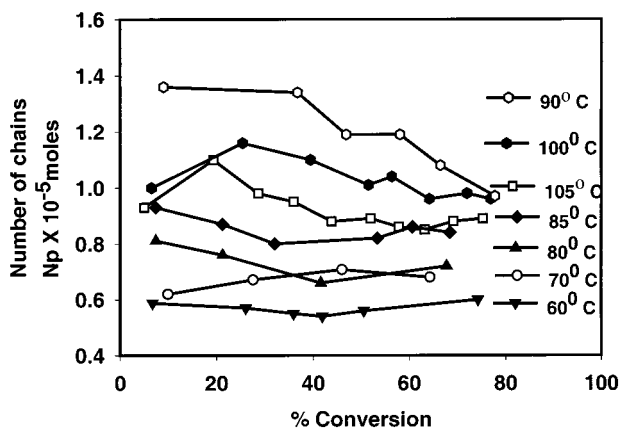


Figure 9. Plots of N_p versus conversion for polymerizations carried out 60, 70, 80, 85, 90, 100, and 105 °C. The ratio of toluene- d_8 to CL was 5:1.

Plots of the total number of chains ($[N_p]$, per millimole of monomer) versus percent conversion for reactions conducted at different temperatures are shown in Figure 9. General trends observed in Figure 9 are as follows: (i) N_p increased with an increase in reaction temperature from 60 to 90 °C, (ii) N_p decreased as the reaction temperature was increased from 90 to 105 °C, (iii) N_p remained relatively constant to high conversion for reactions conducted at 60, 70, 80, and 85 °C, (iv) N_p tended to decrease with an increase

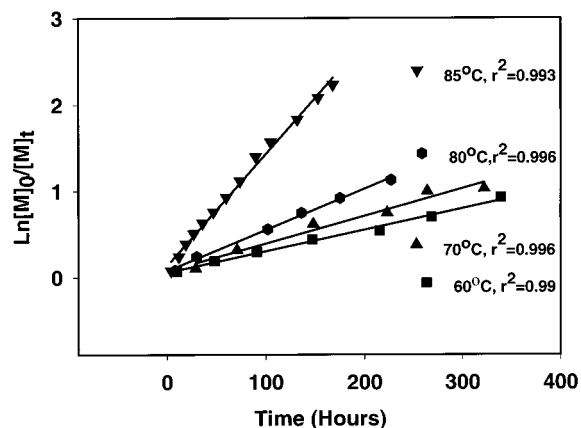


Figure 10. Plots of $\ln[M_0/M_t]$ versus percent conversion for polymerizations carried out 60, 70, 80, and 85 °C. The ratio of toluene- d to CL was 5:1.

in conversion above about 30% for reactions at 90, 100, and 105 °C.

Previous description of the polymerization mechanism^{2f} implicates water as the initiator when no other nucleophiles such as alcohols or amines are added to the reactions. The presence of water in reactions and its ability to initiate chains will influence N_p . Precautions were taken to ensure that water remaining in reactions did not vary as a function of the reaction conditions. In other words, consistency was maintained in the methods used to dry the reaction components and exclude moisture (see Materials and Methods). An explanation for increased molecular weights at the lowest reaction temperature (60 °C) is that fewer chains are formed. This may result from insufficient thermal energy to break interactions between certain water molecules bound to the protein and/or catalyst matrix. Indeed, inspection of Figure 9 shows that the number of chains is the lowest for the 60 °C polymerizations. Furthermore, the increase in thermal energy supplied by raising the temperature from 60 to 90 °C and corresponding changes in N_p results in a decrease in M_n from 60 to 70 and 80 to 90 °C (Figure 8). The fact that N_p does not significantly vary between 70 and 80 °C is consistent with the similarity of product M_n values at these temperatures (see Figures 8 and 9). A decrease in the number of chains at low conversion as the reaction temperature increased from 90 to 100 and 105 °C may be due to protein denaturation that leads to the initiation of a lower number of total chains. Also, the observed decrease in N_p under these conditions may also occur by condensation reactions between chains. A lower number of propagating chains in combination with protein denaturation is also consistent with the decrease in polymerization rates observed as the reaction temperature was increased from 90 to 100 and 105 °C.

Reactions conducted at 60, 70, 80, and 85 °C show values of N_p that changes little for monomer conversions between about 5 and 75%. This suggests that the number of propagating chains remains unchanged during the polymerization. To further analyze this type of behavior, plots of $\ln[M_0/M_t]$ versus reaction time (Figure 10) were constructed for the 60–85 °C reactions and monomer conversions up to about 55%. Correlation coefficients (r^2) from linear regression analysis of the data were between 0.990 and 0.996, indicating linearity. Thus, to 55% conversion, these results

indicate that there is no chain termination, and monomer consumption followed a first-order rate law. Furthermore, inspection of M_n versus conversion plots in Figure 7 shows, for reactions between 60 and 85 °C, to 55% conversion, the data appear to fit the linear regression line. The r^2 values for M_n versus conversion at 60, 70, 80, and 85 °C are 0.998, 0.998, 0.983, and 0.992, respectively. This indicates that chain transfer reactions do not occur. In previous studies, we described similar behavior for porcine pancreatic lipase as well as Novozyme-435 catalyzed polymerizations of ϵ -CL.^{2c,2f} The latter were for solventless polymerizations at 70 °C. Additional studies will be needed to provide a better understanding of why these polymerizations show "living" or "immortal" characteristics and the boundaries of such behavior.

Summary and Conclusions

The reaction solvent, monomer concentration, and temperature had profound effects on the polymerization rate, M_n , and polydispersity for Novozyme-435 catalyzed ϵ -CL polymerizations. Studies of polymerizations carried out in different solvents showed that the most rapid rates of polymerization and highest product molecular weights resulted by using toluene and isooctane. For example, the Novozyme-435 catalyzed polymerization of ϵ -CL (24 h, toluene- d_8 to ϵ -CL 5:1 (vol/vol), 60 °C) gave PCL with an M_n and polydispersity of 21 500 g/mol and 1.9, respectively. Since toluene solubilized both ϵ -CL and PCL while isooctane dissolved only the former, toluene was selected to facilitate subsequent solution kinetics and other studies. Variation in the ratio of toluene to ϵ -CL in reactions at 70 °C showed that percent ϵ -CL conversion and PCL molecular weight were largest for ratios about 2:1. Using these conditions and increasing the scale of the reaction from 0.3 mL to 10 g of ϵ -CL, PCL with M_n 44 800 g/mol and polydispersity 1.7 was prepared. This represents a significant improvement in the efficiency and molecular weights achieved for lipase-catalyzed polymerizations reported by us^{2f} and others^{3g} for lactones with ring sizes of seven or lower. The effect of reaction temperature on propagation kinetics and product molecular weight was studied by direct measurements of the reactions by an in situ NMR method. This method exploited the fact that both the monomer and polymer were soluble during the course of the polymerization in toluene- d_8 . Novozyme-435 catalysis at 90 °C was found to give the most rapid polymerization rate. For example, within only 2 h at 90 °C (toluene- d_8 to ϵ -CL, 5:1, ~1% protein), the percent monomer conversion reached ~90%. The propagation kinetics was also studied using diagnostic tools of living polymerizations. The following was observed for reactions carried out to about 55% monomer conversion at temperatures between 60 and 85 °C: (i) N_p did not vary, (ii) linear relationship for plots of $\ln[M_0/M_t]$ versus time, and (iii) linear relationship for plots of M_n versus percent monomer conversion. These results indicate that, under these reaction conditions, termination and chain transfer did not occur. Therefore, it appears that these polymerizations share many features of immortal polymerizations.

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