Solvent-Free Adipic Acid/1,8-Octanediol Condensation Polymerizations Catalyzed by *Candida antartica* Lipase B

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ABSTRACT: Bulk condensation polymerizations of adipic acid and octanediol, catalyzed by *Candida antartica* Lipase B (CALB), were investigated. The polymers formed by 8 and 24 h polymerizations using CALB immobilized on Accurel and Lewatit had similar molecular weights (e.g., M_n at 24 h ≈15 000). CALB "free" of the immobilization resin was also active for the polymerization but, relative to its immobilized forms, gave slower chain growth (M_n ≈ 10 000 by 48 h). For all three catalyst systems at degree of polymerization (DP) ≥ 20, dispersity (M_w/M_n) was ≤1.5. Since random processes of step-growth condensation polymerizations give dispersity values ≥ 2, the dispersity of products obtained using CALB as the catalyst is believed to result from the unique chain length or mass selectivity of the lipase. Gel permeation chromatograms showed that between 15 min and 4 h chain growth occurred rapidly so that the fraction of product with M_p values > 2910 increased from 28 to 78%. At 70 °C the catalyst activity at 4 h remained unchanged but decreased by 15 and 21% at 24 and 48 h. Unexpectedly, an increase in the concentration of CALB on Lewatit from 0.1 to 1 wt % protein resulted in only a small increase in M_n (e.g., at 24 h, 14 500 vs 17 800). However, decrease in the percent protein to 0.5% had a large detrimental effect. Between 65 and 90 °C the polymerizations occurred with little dependence on the reaction temperature.

Introduction

Biotransformations represent an effective and sometimes preferable alternative to conventional chemical synthesis for the production of fine chemicals and optically active compounds.[1] Recently, the use of enzymes in polymer science has been discussed within comprehensive reviews.[2]

Research on the synthesis and properties of aliphatic polyesters continues to be of great importance due to the needs of the biomedical community,[3] the potential to build polymers from annually renewable building blocks[4] (e.g., lactic acid and fatty acid derived materials), and to prepare polymers that, when disposed in the environment, are biodegradable.[5] Normally, polyester synthesis is performed by ester interchange reactions or by direct esterification of hydroxyacids or diacid/diol combinations.[6] The use of chemical catalysts for these reactions requires harsh reaction conditions (e.g., temperatures > 200 °C) as well as metal catalysts that may be problematic for certain product end uses.[7] These reaction conditions can limit product molecular weight and eliminate the possibility of using building blocks that are not stable at such temperature−catalyst conditions. For example, the condensation polymerization of 2-allylpropane-1,3-diol with adipic acid catalyzed by Ti(O_iPr)$_4$ (220 °C under nitrogen) gave a yellow gel, suggesting that side reactions and decomposition occurred.[8] Lipase-catalyzed condensation polymerizations are metal-free and can be performed at reduced temperatures (see below).

Previous studies on lipase-catalyzed condensation polyesterifications focused primarily on reactions between diols and activated diacids.[9] Prominent examples of activating groups used by these researchers include bis(2,2,2-trichloroethyl) and vinyl esters.[9b,c] For example, Russell and co-workers[10] showed that, by using Novozyme-435, the solvent-less copolymerization of divinyl adipate and 1,4-butanediol gave the corresponding polyester with M_w 23 200 g/mol. However, activation of diacids with such groups is expensive and limits the potential technological impact of these methods.

Important progress has been made in lipase-catalyzed copolymerizations of acid and alcohol building blocks using nonactivated free acids. Linko et al.[11] reported the copolymerization of 1,4-butanediol with sebacic acid in diphenyl ether under reduced pressure using the lipase from *Mucor miehei* (36.5 wt %). In 7 days and 37 °C, poly(1,4-butylsebacate) was formed with M_w 42 000. Similarly, *M. miehei* (36.5%) catalyzed the condensation polymerization of adipic acid and hexanediol in diphenyl ether (37 °C, 7 days, 0.15 mmHg) to give poly(1,6-hexyladipate) with M_n 16 000 and M_w/M_n 4.4.[12] In addition, the polymerization of aliphatic diols with isopthalic acid was described[13] using Novozyme-435 (*Candida antartica* Lipase B on Lewatit) as the catalyst. For example, the copolymerization of isophthalic acid with 1,6-hexanediol at 70 °C (7 days, 8.1% catalyst) yielded a polymer with M_w 55 000 (dispersity and solvent not reported). Taylor[14] reported the formation of low-M_n polyesters by the direct condensation of diacids and diols in solventless conditions at 40−100 °C. Uyama et al.[15] reported that *Candida antarctica* lipase (immobilized without disclosure of matrix, 17 wt % relative to monomers) catalyzed condensation reactions between sebacic acid with 1,5-pentanediol in bulk (70 °C, 48 h) to give aliphatic polyesters with M_n and M_w/M_n (fractionated by precipitation in nonsolvent) of 14 000 and 2.3, respectively. Binns et al.[16] studied copolymerizations of adipic acid and 1,4-butanediol using Novozyme-435. The reactions were performed under solvent-free conditions for 4 and 10 h at 40 and 60 °C, respectively. The GPC of the reaction mixture after 4 and 14 h had different product distributions, the former showing a discrete array of predominantly

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hydroxy-terminated oligomers and the latter displaying the polyesters with M_w about 2200.

The above-cited literature demonstrates the feasibility of performing lipase-catalyzed condensation polymerizations with nonactivated diacids and diols. Problems that will need to be overcome for the practical use of these methods include the use of long reaction times[11,15] and excessive quantities of lipase[11,13,15] (see above). Also, uncertainty exists as to the molecular weights attained for lipase-catalyzed diol/diacid polymerizations due to fractionation of products prior to molecular weight analysis.[11,13,15] Furthermore, previous studies on diol/diacid polycondensations have not considered the extent that the enzyme is deactivated which will ultimately dictate the possibility of its reuse. Moreover, the practice of fixing the reaction time while varying the reaction parameters could obscure differences in product end-group structure, molecular weight, and dispersity values that would otherwise be seen during time course studies.[11,15]

Motivated by the important benefits lipase-catalyzed polymerizations can provide (e.g., selectivity, mild reaction conditions) and the aforementioned progress in this area, our laboratory embarked on a program to gain further insights into lipase-catalyzed condensation polymerizations. Adipic acid and 1,8-octanediol were selected as model substrates to study the effect of selected reaction parameters on the course of its polymerization. The polymerizations were performed in bulk without activation of the diacid. For reasons elaborated below, the catalyst used was Novozyme-435 which consists of *Candida antartica* Lipase B (CALB) physiadsorbed on Lewatit. The potential of using CALB physiadsorbed on an alternative matrix as well as without immobilization was investigated. The effect of CALB concentration and reaction temperature over the ranges from 1 to 0.01 wt % and 65 to 90 °C, respectively, were studied. The polymers formed were characterized to determine their molecular weight averages and polydispersity. Furthermore, the retention of activity by Novozyme-435 as a function of reaction time for bulk polymerizations performed at 70 °C is reported.

Experimental Section

Materials. Adipic acid and 1,8-octanediol were purchased from Aldrich Chemical Co. and used as received. SP 525 is an aqueous solution of *Candida antartica* Lipase B (CALB) (26.5 mg of enzyme/mL) which was a gift from Novozymes (Denmark). Novozyme-435 was also provided by Novozymes (Denmark) and consists of CALB physically adsorbed within the macroporous resin Lewatit VPOC 1600 (supplied by Bayer). CALB immobilized on Accurell was also provided by Novozymes (Denmark).

General Procedure for Lipase-Catalyzed Condensation Polymerizations between Adipic Acid and 1,8-Octanediol. Novozyme-435 (1 wt % relative to the total weight of monomer), dried in a vacuum desiccator (0.1 mmHg, 25 °C, 24 h), was transferred into a round-bottom flask (100 mL flask, 20 mmol diacid/diol) containing the monomers, adipic acid, and 1,8-octanediol. The reactions were performed in bulk, and the flasks were capped with a rubber septum. The reaction flask was then placed into a constant preset temperature oil bath on a magnetic stirrer (IKA Werke: Rct Basic) at 220 rpm for a predetermined time. Vacuum was applied (10 mmHg) to facilitate removal of water. Aliquots of about 20 mg were removed at selected time intervals and analyzed directly by GPC. In other words, the product formed was analyzed by GPC without any step such as precipitation that could result in fractionation of the product. At the final time point studied (e.g., the predetermined time to terminate the reaction), an excess of cold chloroform (2−3 washings) was added, the mixture was stirred for 15 min, and the enzyme beads were removed by filtration (glass-fritted filter, medium porosity). Washing of the beads with cold chloroform was repeated two to three times. The samples were characterized by ^{1}H NMR and GPC.

The protein contents of nonimmobilized CALB, CALB on Accurel (0.22 w/w), and Lewatit (0.1 w/w) differ; the amount of the catalyst used was normalized so that, in all experiments, the wt % of CALB relative to substrate was 0.1%. The protein content of the nonimmobilized CALB was determined using the microwell plate protocol of the BCA protein assay kit (see below).

Protein Content Determination of Nonimmobilized CALB. SP 525, the aqueous solution of *Candida antartica* Lipase B (10 mL, 26.5 mg of enzyme/mL), was lyophilized (Labcon Freeze Zone 6 series) to remove excess water (−40 °C, 0.131 bar, 8 h). The protein content of the final enzyme solution, determined using the standard microwell plate protocol of the BCA protein assay kit,[17] was 64.4 mg of enzyme/mL.

Assay Protocol for Lipase in Organic Media. The lipase activity in organic media was determined by the lipase-catalyzed esterification of lauric acid with propanol. To 1 mL of toluene were added lauric acid (200 mg), propanol (75 μL), recovered catalyst (20 mg), and dried molecular sieves (for water removal). After agitation (200 rpm) at 70 °C for 3 h the reaction was terminated by filtering off the enzyme. The filtrate was assayed for propyl laurate by gas chromatography (GC) (Perkin-Elmer gas chromatograph, 8500) using the following conditions: column, DB 5 (30 m × 0.32 mm × 1 μm); detector, flame ionization (FID); carrier gas, helium at a flow rate of 15 mL/min; temperature program, 45 °C (hold 1 min) to 100 °C at 7 °C/min (hold 10 min) to 280 °C at 10 °C/min (hold 4 min); injector temperature, 350 °C; and detector temperature, 350 °C. From the GC data, the recovered enzyme activity was calculated as residual activity = [(peak area (propyl laurate) of the recovered catalyst)/(peak area (propyl laurate) of the unused catalyst)] × 100.

Other Instrumental Methods. *Nuclear Magnetic Resonance (NMR).* The polyesters formed were characterized using proton ^{1}H NMR spectrometry. ^{1}H NMR spectra were recorded on a Bruker NMR spectrometer (model DPX-300) at 300 MHz. The chemical shifts in parts per million (ppm) for ^{1}H NMR spectra were referenced relative to tetramethylsilane (TMS, 0.00 ppm) as the internal reference.

Gel Permeation Chromatography (GPC). Molecular weights were determined by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 510 pump, a Waters model 717 autosampler, a model 410 refractive index detector, and a model T-50/T-60 detector of Viscotek Corp. with 500, 10^3, 10^4, and 10^5 Å Ultrastyragel 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. Sample concentrations of 0.2% w/v and injection volumes of 100 μL were used. Molecular weights were determined on the basis of a conventional calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich Chemical Co.

Reaction Water Content. The water (wt %) in reactions was measured by using an Aqua star C 3000 titrator with Coulomat A and Coulomat C from EM-Science. The water (w/w) in reaction mixtures was determined by adding the sample (20 mg) in Coulomat A, stirring in a closed septum container, and titrating against Coulomat C. The total water content (w/w) in the reactions was ∼1.5%.

Results and Discussion

Enzyme Selection, Immobilization, and Polymerization Activity. Previous work on the synthesis of polyesters using enzymes of different origin showed that Lipase B from *Candida antartica* (CALB) physiadsorbed onto Lewatit (Novozyme 435) is the preferred lipase−

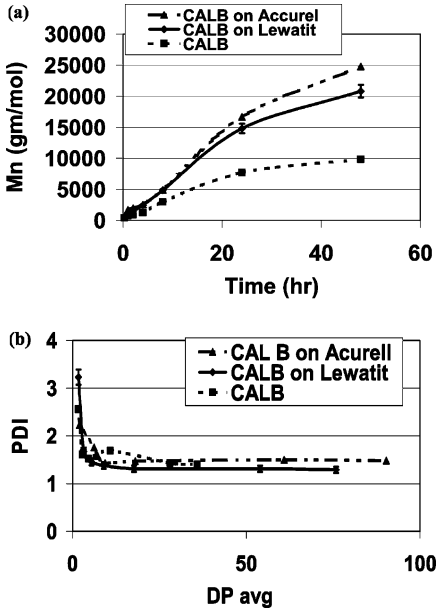

Figure 1. Condensation polymerization of adipic acid with 1,8-octanediol in bulk [70 °C, 1% w/w catalyst:monomer]: (a) effect of enzyme origin and matrices on M_n; (b) effect of enzyme origin and matrices on PDI vs DP_{avg}. The error bars were calculated from triplicate runs for the CALB on Lewatit experiments.

catalyst for polymerizations of activated acids with alcohols[10,18] and by ring-opening of lactones.[19,20] Furthermore, our laboratory showed that, of the different immobilization supports studied for the physiadsorption of CALB, Accurel was preferred relative to Lewatit for polymerizations of different sized lactones.[20] These results prompted us to compare the bulk lipase-catalyzed polymerization of adipic acid with 1,8-octanediol (70 °C, 1% w/w catalyst:monomers) using CALB as well as CALB immobilized on Lewatit (Novozyme-435) and Accurel (Figure 1). Lewatit and Accurel consist of poly(methyl methacrylate-*co*-butyl methacrylate) and polypropylene, have protein contents of 0.1 and 0.22% w/w, surface areas of 110−150 and 80−100 $m^2\ g^{-1}$, and average pore diameters of 140−170 and 50−500 Å, respectively.[21] Since the protein contents of CALB on Accurel and Lewatit differ, the amount of the catalyst used was normalized so that, in all experiments, the wt % of CALB relative to substrate was 0.1%. During the first 8 h of the polymerizations, the M_n values of products from CALB immobilized on Accurel and Lewatit gave similar results (M_n at 24 h ≥15 000). Small but significant differences in M_n (24 800 vs 20 800) were found when these reactions were extended to 48 h. CALB was active for the polymerization but gave slower chain growth ($M_n \approx$ 10 000 by 48 h). Higher activity of CALB in immobilized vs free form is consistent with the established ability of lipases adsorbed onto solid supports to have increased stability and activity relative to that of these enzymes in free form.[22] Fundamental studies are in progress to determine whether improvements in the performance of CALB and other related enzymes can be realized by better understanding interactions between polymeric surfaces and enzymes.

Figure 1b displays plots for PDI (M_w/M_n) vs DP_{avg} for free CALB as well as CALB immobilized on Accurel and Lewatit. At about DP_{avg} 20 and above, PDI is ≤1.5 for all three catalyst systems. Furthermore, immobilization of CALB on Lewatit allows us to achieve PDI values as low as 1.2. In contrast, by chemically catalyzed condensation polymerization of simple diol/diacid systems such as this, PDI values ≥ 2 are predicted.[6] For the latter, chain buildup is assumed to occur by random processes so that the kinetics of reactions between oligomer building blocks is independent of their chain lengths. In contrast, the results herein on lipase-catalyzed condensation polymerizations suggest that chain growth occurs with selectivity. In other words, the lipase reacts at different rates as a function of substrate chain length, resulting in products that are relatively more uniform in length. Studies are in progress to carefully follow the progress of chain growth to understand how enzyme selectivity ultimately provides chains of such uniformity. Many benefits can be derived by the formation of chain segments of more uniform chain length. For example, chain uniformity can be used to attain polymers that crystallize more rapidly than their analogues that have less uniform polyester chains.[14,16] In contrast to the above, Linko et al.[13] using the lipase from *M. miehei* for hexanedioic/1,6-hexanediol copolymerization reported the formation of a product with M_n and M_w/M_n 16 000 and 4.4, respectively. This indicates that selectivity during the buildup of chain segments to give narrow polydispersity products is not a general characteristic of all lipases. Low dispersities were also reported by Uyama et al.[15] using CALB catalysis for condensation polymerizations. However, since these workers fractionated the products by precipitation prior to molecular weight analysis, it could not be concluded that the origin of the narrow dispersity products was lipase selectivity.

GPC Monitoring of Chain Growth. The evolution of molecular weight as a function of time was studied for CALB on Lewatit-catalyzed adipic acid/1,8-octanediol polycondensation in bulk at 70 °C. Figure 2 displays GPC traces of products for reaction times of 15 min, 2 h, 4 h, 24 h, and 48 h as well as the chromatogram for a control reaction with deactivated catalyst (70 °C, 48 h). The peak positions of oligomers based on peak molecular weights (M_p) are designated by letters (Figure 2). Peak areas were determined by deconvolution of peaks assuming a Bernoulli distribution. The areas were measured by cutting and weighing. The data from this analysis allowed the following semiquantitative discussion of chain growth as a function of time. Comparison of the area under peaks for 15 min and 4 h time points shows that chain buildup is rapid. At 15 and 4 h, the percent of products with M_p 0 to 1090 and >2910 were 58 vs 6 and 28 vs 78, respectively. By 48 h, the percent of chains with oligomers > 2910 was 94%. Thus, an uninterrupted increase with time of higher molecular weight chains with concurrent depletion of oligomers with $M_p \leq 2910$ was observed. This is consistent with the retention of a substantial fraction of the original enzymatic activity throughout the 48 h polymerization (see below). Observation of the GPC trace for the control reaction verifies that chain growth occurs due to enzyme catalysis. Binns et al.[8,16] described a solventless polycondensation between 1,4-butanediol and adipic acid at 40 °C catalyzed by immobilized CALB. They concluded that the lipase-catalyzed polycondensation polymerization occurred by a step-growth mechanism. Our results are also consistent with a contribution of step-growth kinetics to propagation. However, a previous study by our laboratory suggests that polymer formation occurs with concurrent transesterification reactions.[23] Furthermore,

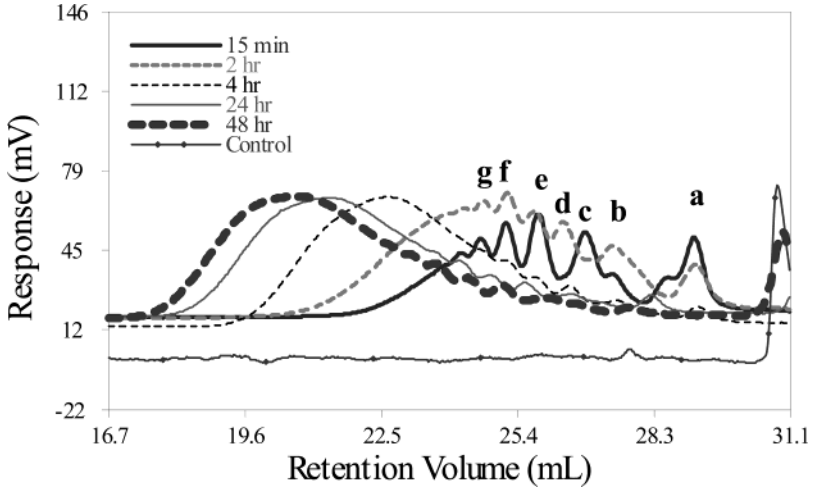

Figure 2. GPC traces vs time for the condensation polymerization of adipic acid and 1,8-octanediol in bulk (70 °C, 1% w/w catalyst:monomer substrates). The peak molecular weights are (a) 310, (b) 450, (c) 670, (d) 1090, (e) 1600, (f) 2240, and (g) 2910 g/mol.

both condensation and hydrolysis reactions must occur although the equilibrium position is shifted to polymer formation by the removal of water under vacuum. Hence, the details of the polymerization mechanism along with kinetic constants for competing reactions remain to be studied.

Catalyst Activity vs Reaction Time. The retention of catalyst activity at 4, 24, and 48 h for the bulk polycondensation between adipic acid and 1,8-octanediol was measured. The polymerization was catalyzed by CALB on Lewatit at 70 °C with 1 wt % catalyst. The assay for enzyme activity was by GC where the extent of propyl laurate formed in toluene was determined (see Experimental Section). The enzyme activity remains unchanged during the first 4 h of the reaction. By 24 and 48 h, the residual activity of the catalyst beads dropped to 85 and 79%, respectively. Thus, even after a 48 h reaction at 70 °C, the catalyst retains almost 80% of its original activity and, therefore, could be reused for at least another polymerization cycle. In contrast, Binns et al.[8] reported that recovered Novozyme-435 beads had 35% of their original activity for an in-bulk 1,4-butanediol/adipic acid copolymerization conducted in vacuo for 17 h at 60 °C. Although details of how mixing of reactants was performed was not described by Binns et al.,[8] it may be that larger shear applied to the catalyst particles resulted in larger extents of catalyst loss in activity. The detailed factors that influence changes in enzyme activity as a function of reactor design, and operation are currently under study in our laboratory.

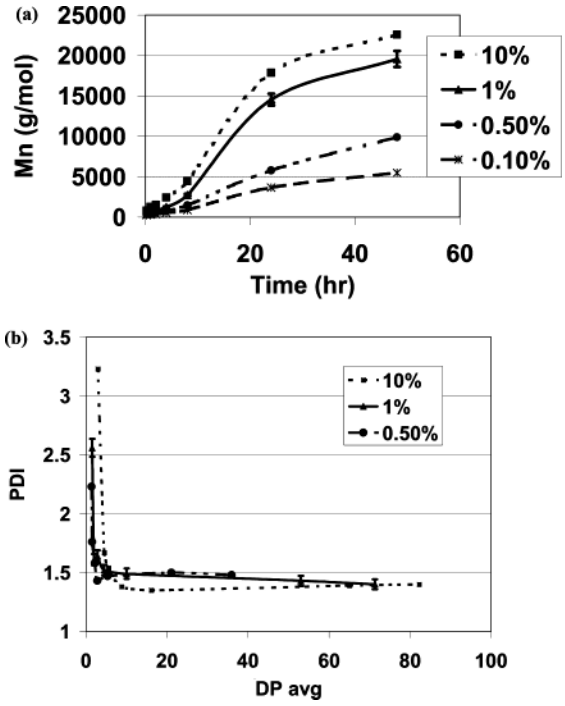

Figure 3. Effect of catalyst concentration for the polymerization of adipic acid and 1,8-octanediol (bulk, 70 °C) on (a) the extent of chain growth as a function of time and (b) the polydispersity index (PDI) of products formed. The error bars were calculated from triplicate runs for the 1% CALB on Lewatit experiments.

Effect of Catalyst Concentration. The catalyst (CALB on Lewatit) concentrations studied were 10, 1, 0.50, and 0.10 wt % relative to monomer (Figure 3). Since the concentration of CALB physiadsorbed within the Lewatit resin is 10 wt %, the corresponding percent protein in these reactions was 1, 0.1, 0.05, and 0.01 wt %, respectively. Reactions run for 24 and 48 h clearly show that decreasing the catalyst concentration results in lower molecular weight products. By 48 h, the M_n of the products obtained at 10, 1, 0.50, and 0.10 wt % catalyst were 22 600, 19 570, 9870, and 5480, respectively. Although the M_n values were smaller when the catalyst was reduced from 10 to 1%, the difference in M_n was much less than the order of magnitude decrease in the catalyst used (see Figure 3). In contrast, a further decrease in the catalyst concentration from 1 to 0.5% had a much larger impact on the extent of chain growth vs time. Therefore, when both high catalyst efficiency and chain molecular weights are the goals, the preferred catalyst concentration is 1% or 0.1% CALB. In comparison, an increase in the concentration of the *M. miehei* lipase by 7-fold (10−73%) gave an increase in product M_w from 20 000 to 42 000.[12] These workers did not report catalyst concentrations below 10%. Furthermore, studies by Uyama et al.[15] of CALB-catalyzed 1,4-butanediol/sebacic acid polymerizations did not report effects of catalyst concentration. The efficiency of CALB for the catalysis of diol/diacid polymerization is extraordinary considering that the role of CALB in nature is presumed to be fatty acid acylation and hydrolysis of

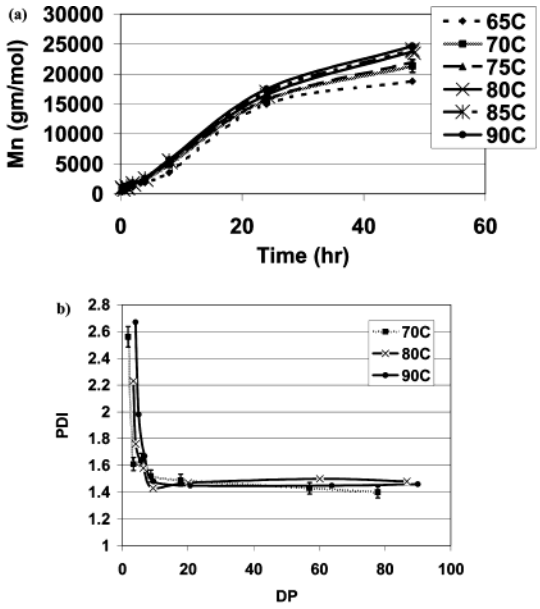

Figure 4. Effect of reaction temperature for the polymerization of adipic acid and 1,8-octanediol in-bulk (1% w/w catalyst: monomer) on (a) the extent of chain growth as a function of time and (b) the polydispersity index (PDI) of products formed. The error bars were calculated from triplicate runs for the 70 °C CALB on Lewatit experiment.

low molar mass substrates (e.g., glycerol fatty acid esters). Inspection of Figure 3b shows that, within the range of catalyst concentrations between 0.5 and 10%, polyesters with narrow dispersity (PDI ≤ 1.5) were formed. Furthermore, within this range of enzyme concentrations, PDI was invariable.

Effect of Reaction Temperature. Bulk polycondensations between adipic acid and 1,8-octanediol, catalyzed by 1% CALB on Lewatit, were performed at 65, 70, 75, 80, 85, and 90 °C. Figure 4a shows for reaction times up to 24 h there is no significant affect of temperature on M_n. However, at the extremes of 65 and 90 °C at 48 h, a small but significant difference between the M_n values (18 800 and 24 700, respectively) was found. Overall, small or no change in the extent of chain buildup was found between reactions conducted at temperatures that differed over this 25 °C range. This result is in good agreement with a previous study by our laboratory where monomer conversion for CALB catalyzed ring-opening polymerization showed little variation over an even broader temperature range (20−108 °C).[23] For bulk condensation polymerizations between sebacic acid and 1,4-butanediol, Uyama et al.[15] reported an increase in yield and polymer M_n when the temperature was increased from 50 to 60 °C (e.g., M_n from 2300 to 3800) but little further change at 70 °C. Our study did not include 50 °C, but the reported invariability of yield and M_n between 60 and 70 °C is consistent with the results herein. Linko et al.[13] reported that, for Novozyme 435-catalyzed polymerization of 1,6-hexanediol with isophthalic acid in diphenyl ether, M_w increased by increasing the reaction temperature from 45 to 60 to 70 °C (2000, 38 000, and 55 000, respectively). However, these workers did not report product PDI or yield after solvent fractionation. Other reports on lipase-catalyzed aliphatic polyester synthesis from diols and diacids did not study the influence of temperature on the polymerizations.[16,11] Inspection of Figure 4b shows that, within the range of temperatures from 70 to 90 °C and product DPs from 20 to 90, the PDI values stayed between 1.4 and 1.5. Thus, within this range of polymerization temperatures, PDI was invariable.

Summary and Conclusions

The condensation polymerization of adipic acid and 1,8-octanediol catalyzed by CALB was selected as the model system for this study. The polymerizations were conducted in the absence of solvent and without the use of groups that activate the monomer carboxylic acids. The influence of the macroporous support on the activity of CALB was studied for the adipic acid/1,8-octanediol polymerization. Without optimization of the CALB loading on Accurel, the polymerization activity of CALB on Accurel was similar to that of CALB on Lewatit (Novozyme 435). CALB without immobilization also catalyzed the polymerization, but at a slower rate than was observed using immobilized CALB. Under a wide range of reaction conditions and without fractionation the products formed had M_w/M_n values of ≤1.5. Such low polydispersity values demonstrate that CALB catalyzes chain growth with chain-length selectivity. This ability of CALB to form products of substantially lower polydispersity than would have been attained using conventional chemical catalysts is an important attribute of the use of CALB. The growth of polymer chains was found to be nearly invariable for reaction temperatures from 65 to 90 °C. A similar lack of sensitivity over an even broader range of polymerization temperatures (20−108 °C) was reported earlier by us for CALB-catalyzed lactone polymerizations. Generally, increases in temperature result in increased reaction rate in the absence of catalyst decomposition. However, if CALB sufficiently reduces the activation energy of the rate-determining step, increases in temperature will have less effect on the rate. Indeed, we believe this to be the reason that CALB-catalyzed polymerizations performed at 65, 70, 75, 80, 85, and 90 °C occurred at nearly identical propagation rates.

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