

Lipase-Catalyzed Polycondensations: Effect of Substrates and Solvent on Chain Formation, Dispersity, and End-Group Structure

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The effects of substrates and solvent on polymer formation, number-average molecular weight (M_n), polydispersity, and end-group structure for lipase-catalyzed polycondensations were investigated. Diphenyl ether was found to be the preferred solvent for the polyesterification of adipic acid and 1,8-octanediol giving a M_n of 28 500 (48 h, 70 °C). The effect of varying the alkylene chain length of diols and diacids on the molecular weight distribution and the polymer end-group structure was assessed. A series of diacids (succinic, glutaric, adipic, and sebacic acid) and diols (1,4-butanediol, 1,6-hexanediol, and 1,8-octanediol) were polymerized in solution and in bulk. It was found that reactions involving monomers having longer alkylene chain lengths of diacids (sebacic and adipic acid) and diols (1,8-octanediol and 1,6-hexanediol) give a higher reactivity than reactions of shorter chain-length diacids (succinic and glutaric acid) and 1,4-butanediol. The bulk lipase-catalyzed condensation reactions were feasible, but the use of diphenyl ether gave higher M_n values (42 400 g/mol in 3 days at 70 °C). The polydispersity varied little over the conditions studied giving values ≤ 2 . No specific trend with respect to end-group structure as a function of time was observed. At 70 °C, the retention of catalyst activity in the bulk was independent of substrate structure but was higher when reactions were conducted in diphenyl ether than in bulk.

Introduction

The past 16 years have seen tremendous progress in the use of enzymes in organic media to catalyze a wide variety of small-molecule transformations.¹ The rationale for using enzymes as catalysts in polymer synthesis and polymer modification are discussed at length elsewhere in comprehensive reviews.² Specific attributes of enzymes that have motivated our laboratory to study enzyme-catalyzed routes to polymer synthesis and modification are discussed below.

Aliphatic polyesters continue to be of interest for biodegradable medical materials,³ for the potential to build polymers from annually renewable building blocks⁴ (e.g., lactic acid- and fatty acid-derived materials), as well as 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 often require harsh reaction conditions (e.g., temperatures > 200 °C) and metal catalysts, which 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, in the condensation polymerization of 2-allylpropane-1,3-diol with adipic acid catalyzed by $\text{Ti}(\text{O}^i\text{Pr})_4$ (220 °C under nitrogen), a yellow-colored gel was obtained

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 polyesterification reactions focused primarily on reactions between diols and activated diacids.⁹ Prominent examples of activated ester groups used by these researchers include bis-(2,2,2-trichloroethyl) and vinyl esters.^{9b,c} For example, Russell and co-workers¹⁰ showed that by using Novozyme-435, the solventless copolymerization of divinyl adipate and 1,4-butanediol gave the corresponding polyester with a weight-average molecular weight (M_w) of 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 monomers 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 at 37 °C, poly(1,4-butyl sebacate) was formed with a M_w of 42 000. Similarly, the lipase from *M. miehei* (36.5%) catalyzed the condensation polymerization of adipic acid and hexanediol in diphenyl ether (37 °C, 7 days, 0.15 mm Hg) to give poly(1,6-hexyl adipate) with a M_n of 16 000 and a M_w/M_n of 4.4.¹² In addition, the polymerization of aliphatic diols with isophthalic acid was described¹³ using Novozyme-435 (*Candida antartica* lipase B on Lewatit) as the catalyst. Furthermore, the copolymer-

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ization of isophthalic acid with 1,6-hexanediol at 70 °C (7 days, 8.1% catalyst) yielded a polymer with a M_w of 55 000 (dispersity and solvent not reported). Taylor et al.¹⁴ reported the formation of polyesters with low M_n values 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% by weight relative to monomers) catalyzed condensation reactions between sebacic acid and 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) values of 14 000 and 2.3, respectively. Binns et al.¹⁶ studied reactions 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 analysis of the reaction mixture after 4 and 14 h gave different product distributions, the former reaction time showing a discrete array of predominantly hydroxyl-terminated oligomers and the latter displaying the polyesters with M_w of about 2200.

The above references firmly established the feasibility of performing polymerization reactions between diacids and diols in the presence and absence of solvents. Recently, our laboratory selected adipic acid/1,8-octanediol as a model system for lipase-catalyzed polycondensations.¹⁷ This paper seeks to further extend our understanding of lipase-catalyzed polycondensations by focusing on the effects of reaction medium and the monomers used. The progress of reactions as a function of time is reported, and products were characterized to determine molecular weight averages, polydispersity, and end-group structure. The diols studied include 1,4-butanediol, 1,6-hexanediol, and 1,8-octanediol ($\text{HO}(\text{CH}_2)_n\text{OH}$, where $n = 4, 6, \text{ and } 8$, respectively). The diacids studied include succinic, glutaric, adipic, and sebacic acids ($\text{HOOC}(\text{CH}_2)_m\text{COOH}$, where $m = 2, 3, 4, \text{ and } 8$, respectively). Furthermore, the retention of activity by Novozyme-435 as a function of substrates was determined for both solution and solvent-free media.

Experimental Section

Materials. Diacids (succinic, glutaric, adipic, and sebacic acid) and diols (1,4-butanediol, 1,6-hexanediol, and 1,8-octanediol) were purchased from Aldrich Chemical Co and used as received. Anhydrous diphenyl ether, xylene, tetraethylene glycol, dimethyl ether, and 2-methoxyethyl ether were purchased from Aldrich Chemical Co and used as received. Novozyme-435 (specific activity 7000 PLU/g) was provided by Novozymes (Denmark) and consists of *Candida antarctica* lipase B (CALB) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (supplied by Bayer). Lewatit consists of poly(methyl methacrylate-*co*-butyl methacrylate) and has a protein content of 0.1 w/w, surface area of 110–150 m² g⁻¹, and average pore diameter of 140–170 Å.¹⁸

General Procedure for Lipase-Catalyzed Condensation Polymerizations between Diacids and Diols. Novozyme-435 (1% by weight 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) contain-

ing the monomers (20 mmol diacid/diol). The reactions were performed in bulk, as well as in different solvents. The flasks were capped with a rubber septum, and as applicable, the solvent (2:1 v/v of total monomer) was added via syringe. 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 mm of Hg) to facilitate removal of water. Aliquots of about 20 mg were removed at selected time intervals. The reactions were terminated by adding excess cold chloroform (2–3 washings), stirring for 15 min, and removing the enzyme by filtration (glass-fritted filter, medium porosity). The samples were characterized by ¹H NMR and gel permeation chromatography (GPC) to determine the molecular weight distribution and to analyze the formation of different species generated.

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 follows: 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 (¹H) NMR. 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.

The end-group structure analysis was determined using oxalyl chloride treatment. ¹H NMR of nonfractionated products were used to analyze the polymer end-group structure. The signals were observed at δ 4.08 (O=COCH₂), 3.64 (CH₂OH), 2.34 (O=CCH₂), 1.66, and 1.24 (all other methylenes). The methylene next to free acid was not resolved and is often concealed within the methylene signal of its ester (2.34). Therefore, the product was derivatized with oxalyl chloride, and the signal at 3.64 shifted to 4.21 and a new signal at 2.9 appeared. These signals are due to the methylene carbons next to the oxalyl chloride derivatized chain-end hydroxyl and carboxyl groups, respectively. The ratio of the two signals was used to determine the relative amount of hydroxyl to carboxyl chain-ends.

Gel Permeation Chromatography (GPC). Molecular weights were determined by gel permeation chromatography (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 Corporation with 500, 10^3 , 10^4 , and 10^5 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. Sample concentrations of 0.2% (w/v) and injection volumes of 100 μ L were used. Molecular weights were determined on the basis of conventional calibration curve generated by narrow molecular weight polystyrene standards obtained from Aldrich Chemical Company.

The relative formation of different molecular weight species in Figure 2 was determined by assuming a Bernoulli distribution for overlapping peaks and by cutting and weighing curve areas. Peak molecular weights in Figure 2 were determined on the basis of polystyrene standards. These peak molecular weights were used to identify discrete regions of GPC traces.

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 \sim 1.5%.

Results and Discussion

The rationale for the choice of Novozyme-435 as the catalyst for this work is described elsewhere.¹⁷ Novozyme-435 consists of *Candida antarctica* lipase B (CALB) physisorbed on "Lewatit" resin, which is a macroporous polyacrylate (see Experimental Section). The Novozyme-435-catalyzed condensation polymerization reaction carried out in bulk on 1,8-octanediol with adipic acid showed little or no change for reaction temperatures varying from 65 to 90 $^{\circ}$ C,¹⁷ so 70 $^{\circ}$ C was used throughout this study.

Effect of Solvent. The effects of organic solvents on lipase reactivity are complex, and it is difficult to predict the behavior of different lipases in the same solvent system or of different solvents with the same lipase. Previous work was carried out in our laboratory on solvent properties, such as $\log P$ (logarithm of partition coefficient) and its correlation to Novozyme-435 activity for ϵ -caprolactone ring-opening polymerization reactions.¹⁹ Of the solvents studied, those with $\log P$ values between 1.9 and 4.5 gave more efficient polymerizations (e.g., toluene and isooctane, $\log P = 2.5$ and 4.5, respectively).¹⁹ Furthermore, when solvents with $\log P$ values between -1.1 and 0.49 were used, the polymerization reactions were relatively slow.¹⁹ This result generally agrees with results found by others that have conducted similar experiments with low molar mass substrates and products. For example, Laane et al.²⁰ found that lipase (yeast lipase, mold lipase)-catalyzed transesterification reactions between tributyrin and heptanol were slow for $\log P < 2$, moderate in solvents having $\log P$ between 2 and 4, and fast in apolar solvents with $\log P > 4$.

The boiling point of the solvent is an additional consideration for condensation polymerizations. The solvent should

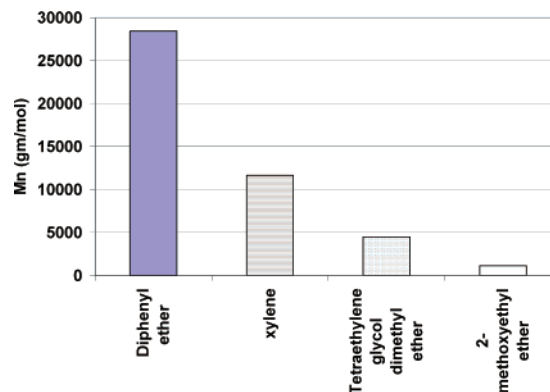


Figure 1. Effect of solvent on M_n for polymerization of adipic acid and 1,8-octanediol [70 $^{\circ}$ C, 24 h, 1% w/w catalyst/monomer, solvent/substrates 2:1 (v/w)].

have a sufficiently high boiling point ($bp \geq 130$ $^{\circ}$ C) to remain in the reactor during the removal of the byproduct (e.g., water). Linko et al.¹² evaluated solvents with relatively high boiling points for the polyesterification of bis(2,2,2-trifluoroethyl sebacate) and 1,4-butanediol. Of the six solvents studied (diphenyl ether, dodecane, hexyl ether, isoamyl ether, triethylene glycol dimethyl ether, and veratrole), diphenyl ether was found to be preferred giving a M_w of 27 700 (37 $^{\circ}$ C, 72 h).

In the present study, four solvents (diphenyl ether, xylene, tetraethylene glycol dimethyl ether, and 2-methoxyethyl ether) were selected for study of the adipic acid/1,8-octanediol polymerization reaction (70 $^{\circ}$ C, 48 h) as a model system. Figure 1 shows that diphenyl ether gave the product having the highest molar mass. The M_n values for polymerizations conducted in diphenyl ether ($\log P = 4.05$), xylene ($\log P = 3.09$), tetraethylene glycol dimethyl ether ($\log P = -1.03$), and 2-methoxyethyl ether ($\log P = -0.48$) were 28 500, 11 670, 4500, and 1130, respectively.²¹ Thus, as was found by Linko et al.¹² with a different lipase (from *M. miehei*) using an activated ester, diphenyl ether was again the preferred solvent. Also, the general trends discussed above with respect to $\log P$ were found to apply to the present system.

Molecular Weight Distribution. In another study by our laboratory,¹⁷ the change of the molecular weight distribution as a function of time for adipic acid/1,8-octanediol polycondensations performed in bulk at 70 $^{\circ}$ C was reported. To determine the differences between solvent-free and solvent-based reaction media on molecular weight, adipic acid/1,8-octanediol polycondensations were performed as above but in diphenyl ether. Figure 2a displays GPC traces of the products obtained for reaction times of 15 min and 2, 4, 24, and 48 h, as well as the chromatogram for a control reaction with a deactivated catalyst (70 $^{\circ}$ C, 48 h). Oligomer peak positions are designated by letters, and their peak molecular weights (M_p) are given in the Figure 2a legend. Areas under the peaks were determined by cutting and weighing to approximate the relative amounts of oligomers found of differing M_p . At 15 min and 4 h (Figure 2b), the percent of products with M_p values ranging from 0 to 450, 670 to 1090, 1600 to 2240, 2910, and >2910 was 55 vs 11, 40 vs 15, 5 vs 18, 0 vs 8, and 0 vs 38, respectively. By 48 h, the percent of product with a $M_p > 2910$ was 89%. Therefore, similar

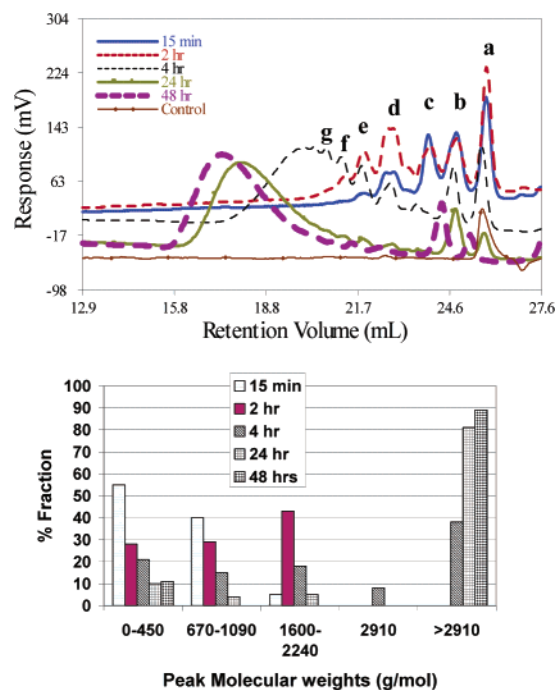


Figure 2. (a) GPC traces vs time for condensation polymerization of adipic acid and 1,8-octanediol in diphenyl ether (70 °C, 1% w/w catalyst/monomer substrates, 2:1 v/w solvent). The peak molecular weights are represented as follows: a = 310, b = 450, c = 670, d = 1090, e = 1600, f = 2240, and g = 2910 g/mol. Panel b shows the wt % relative to total sample that selected molecular species are found in the product. Results are from analysis of GPC traces displayed in Figure 3a.

to identical reactions performed in bulk,¹⁷ an uninterrupted increase with time of higher molecular weight chains with the concurrent depletion of oligomers with $M_p \leq 2910$ was observed. However, comparison of GPC traces for bulk and solution reactions shows that for the latter, oligomers with $M_p \leq 2910$ persisted for longer reaction times. In other words, discrete oligomer fractions were found in the polymerization reaction mixture at longer reaction times for polymerization reactions run in diphenyl ether relative to those run in bulk, giving higher polydispersities for the former. This result will be further elaborated below when polydispersity for solution and bulk reactions are compared. Observation of the GPC trace for the control reaction verifies that polymer formation occurs because of enzyme catalysis.

Effect of the Diol and Diacid Chain Length on Average Degree of Polymerization. The effect of the diol chain length was studied by performing Novozyme-435-catalyzed condensation polymerizations of adipic acid with 1,4-butanediol, 1,6-hexanediol, and 1,8-octanediol (Figure 3). In Figure 3a, the reactions were conducted in diphenyl ether at 70 °C under vacuum (10 mm of Hg) with 1 wt % catalyst (e.g., 0.1% CALB) relative to monomer. To study the progress of the reactions as a function of time, aliquots were withdrawn from reactions after 24, 48, and 72 h. Except for the results at 24 h using 1,6-hexanediol and 1,8-octanediol, the other data show increased molecular weight as the diol length was increased. The average degree of polymerization (DP_{avg}) of the product from adipic acid/1,8-octanediol increased with reaction time (Figure 3). However, changes in molecular weight were relatively small between 24 and

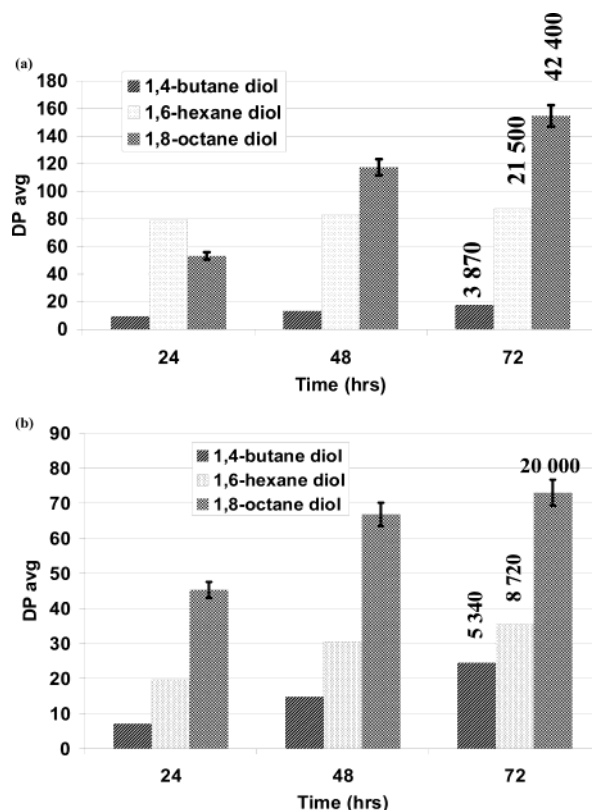


Figure 3. Degree of polymerization (DP_{avg}) as a function of time and substrates (diol chain length) [70 °C, 1% w/w catalyst/monomer] (a) in diphenyl ether (2:1 v/w solvent) and (b) in bulk. The error bars were determined on the basis of triplicate runs.

72 h when 1,4-butanediol and 1,6-hexanediol were used. The highest M_n achieved in this study was that for the 72 h adipic acid/1,8-octanediol polymerization in diphenyl ether ($M_n = 42\,400$, $PDI = 1.5$). The observation of a continuous increase in DP_{avg} over the 72 h reaction time suggests that at least some fraction of the original enzyme activity remains (see below).

Figure 3b shows the results with the identical substrates and reaction conditions but in a solvent-free system. As in solution, bulk reactions also showed more rapid build-up of chains with increase in the diol length. However, from 48 to 72 h, polymerizations in bulk showed little or no further increase in DP_{avg} . This contrasts markedly to the solution polymerization of 1,8-octanediol in which the DP_{avg} increased from 120 to 155 over the same time interval. The most significant difference between 1,6-hexanediol and 1,8-octanediol polymerizations conducted in solution and bulk is that, without solvent, the increase in the DP_{avg} with time occurs more slowly. This result is likely due to greater constraints on chain diffusion for bulk polymerizations. However, it may also be that the enzyme has enhanced activity for substrate polymerization when the reaction is conducted in diphenyl ether rather than in bulk.

Studies are currently looking more closely at the effects of diphenyl ether concentration in reactions, as well as process parameters (e.g., agitation rate, shear, viscosity) to improve the kinetics of chain build-up in bulk reactions. Study of the progress of adipic acid/1,8-octanediol polymerizations in diphenyl ether for reaction times ≤ 10 h was also studied (See Figure S1, Supplementary Information). The

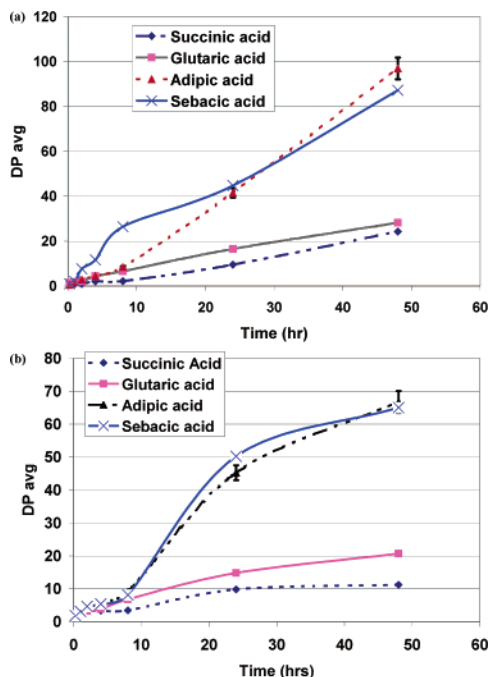


Figure 4. Degree of polymerization (DP_{avg}) as a function of time and substrates (diacid chain length) [70 °C, 1% w/w catalyst/monomer] (a) in diphenyl ether (2:1 v/w solvent/substrates) and (b) in bulk. The error bars were calculated on the basis of triplicate runs.

DP_{avg} values were 1.7, 2.8, 4.6, and 8.4 for reactions at 1, 2, 4, and 8 h, respectively.

Figure 4 shows the results of CALB-catalyzed polymerizations as a function of diacid chain length in solvent-based and bulk systems (parts a and b, respectively). The more rapid polymerization in diphenyl ether of sebacic (C_{10}) acid relative to shorter diacid substrates was evident by 4 and 8 h. However, by 24 and 48 h, the DP_{avg} for sebacic and adipic diacid (C_6) polymerizations was statistically equivalent. Similarly, polymerizations of succinic (C_4) and glutaric (C_5) diacids proceeded at similar rates. Thus, by 24 h, the relative extent of chain build-up for polymerizations with octane-1,8-diol in diphenyl ether was sebacic \approx adipic $>$ glutaric \approx succinic acids. It may be that the rate of sebacic/1,8-octanediol polymerization is more rapid than for the other diacids studied; however, it appears that as the reaction progresses the rate of hydrolysis relative to propagation increases.

Figure 4b shows results with the identical substrates and reaction conditions in the solvent-free system. The general trends for the solvent-free and solvent-based systems are almost identical. Significant differences for the former are as follows: (i) the early stage of sebacic acid copolymerizations does not exhibit the notably more rapid increase in DP_{avg} , and (ii) by 48 h, the DP_{avg} with glutaric acid is greater than that with succinic acid. As was found above with different diols (Figure 3), the relative increase in DP_{avg} between 24 and 48 h was larger for solution than bulk polymerizations.

Of relevance to this study is the work by Linko et al.¹³ who described how altering the substrate chain length affects the outcome of lipase-catalyzed diol/diacid polymerizations. They used the lipase from *M. miehei* (36.5 wt % catalyst) in diphenyl ether (37 °C, 7 days). For adipic acid copolymer-

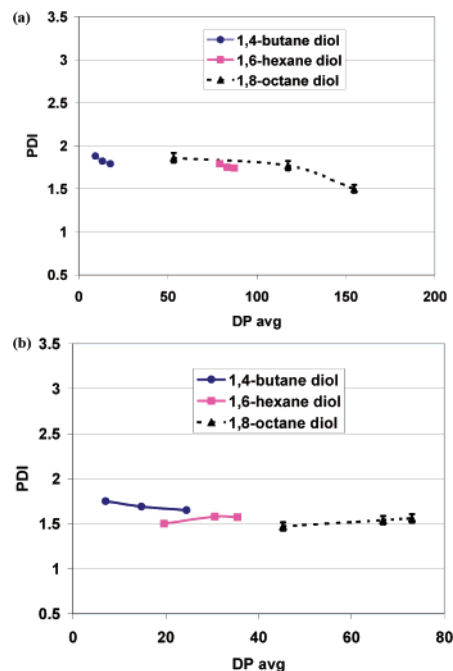


Figure 5. Effect of DP_{avg} and diol used on PDI for condensation polymerization of adipic acid [70 °C, 1% w/w catalyst/monomer] (a) in diphenyl ether (2:1 v/w solvent/substrates) and (b) in bulk. The error bars were calculated on the basis of triplicate runs.

izations, the product DP_{avg} increased from 12 to 18 as the diol length increased from 1,4-butanediol to 1,6-hexanediol. Figure 3a shows that the corresponding increase in diol chain length with Novozyme-435 in diphenyl ether (70 °C, 48 h) gave a much larger increase in DP_{avg} (15 to 81). Uyama et al.,¹⁵ using immobilized CALB (matrix not disclosed, 60 °C, 48 h), reported that copolymerizations of sebacic acid with 1,4-butanediol, 1,5-pentanediol, and 1,8-octanediol have DP_{avg} (M_w/M_n , yield) values of 27 (1.6, 80%), 51 (2.3, 86%), and 12 (1.4, 83%). Similar to the results by Uyama et al.¹⁵ with C_4 and C_5 diols, Figure 3b shows that an increase in diol chain length from C_4 to C_6 also resulted in a large increase in DP_{avg} (15 to 31). However, Uyama et al.¹⁵ also reported a large decrease in DP_{avg} from 51 to 12 as the diol chain length was increased from C_5 to C_8 . This result is unexpected and inconsistent with the results herein.

Effects of Substrates and Solvent on Polydispersity (PDI, M_w/M_n). PDI values as a function of the diol chain length from reactions performed in diphenyl ether and in bulk are shown in Figure 5, parts a and b, respectively. Both in solution and solvent-free conditions, PDI is independent of the diol chain length. However, the PDI values were generally larger for polymerizations in solution. PDI values for DP_{avg} from 50 to 120 were between 1.75 and 1.85 (Figure 5a). The standard deviation for dispersity values was ± 0.1 .²² In contrast, for bulk polymerizations of different diols with DP_{avg} from 20 to 75, the PDI values were between 1.48 and 1.65. A narrower polydispersity for bulk polycondensations was similarly found for reactions between 1,8-octanediol and different chain length diacids. In the range of DP_{avg} 10–30, PDI values in diphenyl ether were generally between 2 and 3 (Figure 6a). In comparison, for DP_{avg} 10 to 30, bulk reactions had PDI values between 1.29 and 1.40. This trend of higher PDI values in solution than in bulk continues at

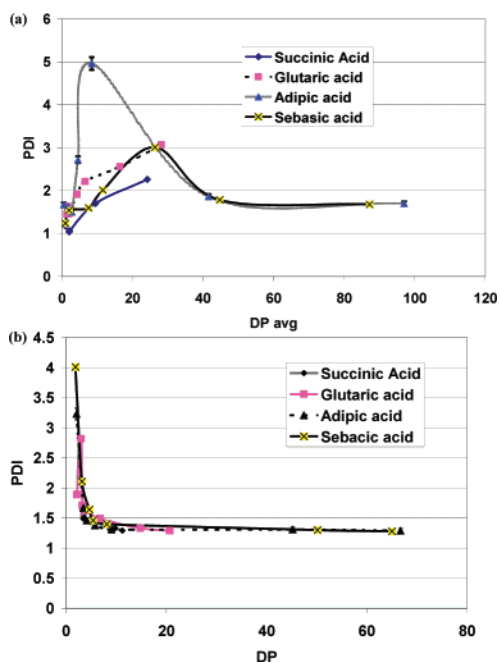


Figure 6. Effect of DP_{avg} and diacid used on PDI for condensation polymerizations of adipic acid [70 °C, 1% w/w catalyst/monomer] (a) in diphenyl ether (2:1 v/w solvent) and (b) in bulk. The error bars were calculated on the basis of triplicate runs.

higher DP_{avg}. At DP_{avg} values from 40 to 100 for 1,8-octanediol/adipic or sebacic acid solution polymerizations, the PDI values are between 1.65 and 1.85. In contrast, for the same polymerizations in bulk, for DP_{avg} between ~40 and 70, the PDIs are between 1.28 and 1.34. The differences in PDI as a function of the diacid substrate in diphenyl ether at low DP_{avg} (e.g., about DP_{avg} 10) are nonsystematic (Figure 6a). In contrast, PDI values at DP_{avg} < 20 for different diacids polymerized in bulk show much less variation than those of their solution polymerization counterparts.

There are few reports that compared solvent-free and solvent-based systems for lipase-catalyzed condensation polymerizations. To our knowledge, this is the first report to explore polydispersity as a function of reaction time, substrate structure, and the reaction medium (e.g., solution versus bulk). Opportunities by others to explore PDI for lipase-catalyzed diol/diacid polycondensations have been missed because of product fractionation prior to molecular weight analysis.^{11,13,15} Furthermore, polydispersities reported when activated diesters were used are not relevant to the current system because of competing hydrolysis reactions that deactivate a fraction of diester monomers, as well as ester groups at chain termini.²³

Binns et al.¹⁶ described Novozyme-435-catalyzed polymerizations of adipic acid (A) with 1,4-butanediol (B) in bulk and toluene. They also studied this polymerization by using AB and BAB monomer adducts. In all cases, polymerizations in toluene relative to solvent-free gave products of broader dispersity. For example, under analogous reaction conditions, the M_w (M_w/M_n) values for bulk and toluene polymerizations were 2227 (1.5) and 1341 (2.3). Thus, although the polymerization conditions and solvents used differ, both this report and that by Binns et al.¹⁶ agree that for solvent-based systems product dispersity is higher. The

Table 1. Effect of Substrates and Reaction Time on End-Group Structure (OH/COOH Ratio) for Condensation Polymerizations of Nonactivated Diacids and Diols [70 °C, 1% w/w Catalyst/Monomer]

Effect of Diacid			
diacid	time (h)	bulk	diphenyl ether
succinic acid	24	3.6:1	
	48	3.3:1	3.4:1
glutaric acid	8	2.5:1	
	24	2.9:1	
adipic acid	48	2.6:1	5:1
	8	3.7:1	
	24	2.4:1	
	48	6.7:1	3.2:1
sebacic acid	8	3.2:1	
	24	5.5:1	
	48	1.5:1	3.3:1
Effect of Diol			
diol	time (h)	bulk (vacuum)	diphenyl ether
1,4-butanediol	24	2.8:1	
	48	2.2:1	
	72	3.3:1	3.7:1
1,6-hexanediol	24	4.6:1	
	48	3.8:1	
	72	3.3:1	5.6:1
1,8-octanediol	24	2.4:1	
	48	6.7:1	
	72	5.4:1	10:1

broader molecular weight distributions found for polymerizations in solution can be explained by interchain transesterification reactions. That is, interchain transesterification reactions may occur at a greater rate when Novozyme-435-catalyzed polymerizations are conducted in solution instead of using a solvent-free system. Thus, chain growth occurs by esterification reactions between building blocks with acid and alcohol terminal groups. The lipase selectivity during chain growth gives rise to M_w/M_n values < 1.5. However, when the reaction conditions are altered such that the probability of interchain transesterification is increased, then M_w/M_n will increase above 1.5 to values reminiscent of those found for conventional chemically catalyzed polyesterifications conducted at elevated temperatures in the presence of acidic or organometallic reagents.

End-Group Structure. Selected samples withdrawn as aliquots from polymerizations were analyzed by ¹H NMR to determine their end-group structure. This required modification of the end groups with oxalyl chloride prior to NMR analyses (see Experimental Section).²⁴ The results of this work (Table 1) showed no systematic variations in end-group structure as a function of the reaction time or chain length of the diacid and diol substrates. Furthermore, no significant differences in end-group structure were observed for solution versus bulk polymerizations. In all cases, products had chain ends with hydroxyl-to-carboxy ratios of > 1. Most often, these values were in the range from 3:1 to 5:1. Deviation from hydroxyl-to-carboxyl ratios of 1:1, despite the use of equimolar diol and diacid substrates, may be due to low-level impurities.

Binns et al.²⁵ reported the formation of low-molecular-weight poly(butyl adipate) by Lipozyme IM-20 catalysis in

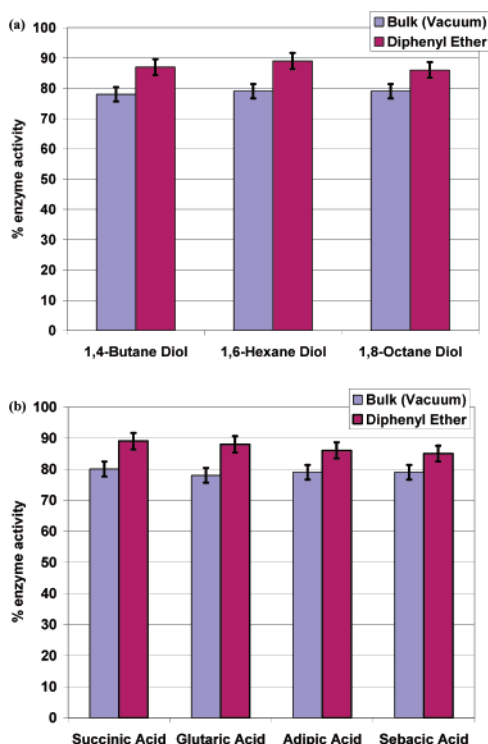


Figure 7. Percent retention of enzyme activity as a function of monomer structure and reaction medium for condensation polymerization of diacids and diols at 70 °C after 48 h: (a) effect of diol; (b) effect of diacid. The error bars were calculated on the basis of triplicate runs.

diisopropyl ether. For example, a 140 h reaction at about 40–44 °C gave the polyester with $M_n = 4172$. They found that the polyester chains had a preponderance of hydroxyl-terminated moieties. This was explained by the poor solubility of adipic acid in the low-polarity solvent. They also reported that during much of the reaction, a part of the acid remained undissolved. If found to be true for reactions herein, then this would explain diol/diacid ratios > 1 and up to 10:1 for poly(octyl adipate) formed at 72 h in diphenyl ether (Table 1).

Effects of Substrates and Solvent on Catalyst Activity versus Reaction Time. Recently our laboratory reported that, after 48 h, the percent retention of enzyme activity for bulk adipic acid/1,8-octanediol polycondensation (70 °C, 1 wt % catalyst) was 79%.¹⁷ The assay for enzyme activity was by GC where the extent of propyl laurate formed in toluene was determined (See Experimental Section). The percent activity remaining after 48 h bulk polymerizations of adipic acid with either 1,4-butanediol or 1,6-hexanediol was 78% and 79%, respectively (see Figure 7a). Similarly, the percent activity remaining after 48 h bulk polymerizations in which octanediol was reacted with either succinic, glutaric, or sebacic acid was 80%, 78%, and 79%, respectively (see Figure 7b). Thus, the large differences in reaction rate as a function of the diol and diacid chain length (see Figures 3 and 4) were not due to differences in retained lipase activity during the reaction.

Similar studies of enzyme activity were carried out with recovered enzyme samples after filtration from the reactions carried out in diphenyl ether. The percent activity remaining after 48 h polymerizations in diphenyl ether of adipic acid

with 1,4-butanediol, 1,6-hexanediol, or 1,8-octanediol was 87%, 89%, and 86%, respectively (see Figure 7a). Similarly, the percent activity remaining after 48 h solution polymerizations in which octanediol was reacted with either succinic, glutaric, adipic, or sebacic acid was 89%, 88%, 86%, and 85%, respectively (see Figure 7b). The standard deviation for activity values was $\pm 3\%$. The increased percent retention of catalyst activity in diphenyl ether may be in part explained by increased shear on the enzyme for the bulk polymerizations. Also, the increase in the reaction rate observed in diphenyl ether as compared to bulk polymerizations could partly be due to increased percent retention of enzyme activity in diphenyl ether. The detailed factors that influence changes in enzyme activity as a function of reactor design and operation are currently under study in our laboratory.

Summary and Conclusions

The ability to conduct the above reactions by using Novozyme-435 at 1 wt % (relative to the total monomer concentration) is noteworthy. Normally, polymerizations with Novozyme-435 are conducted with concentrations of catalyst $\geq 10\%$.^{6–8} Furthermore, the concentration of the active protein (CALB) in Novozyme-435 is about 10% of the total catalyst weight. Because to the best of our knowledge the CALB used has not been evolved for polyester synthesis, the good activity of CALB for the polymerizations performed herein is very promising. Future development of this catalyst by genetic methods will lead to requirements of substantially reduced enzyme concentrations and, possibly, commercially viable processes.

The effects of lipase and organic solvents are very complicated, and it is difficult to predict the behavior of different lipases in the same solvent system or of different solvents with the same lipase. Diphenyl ether was found to be the preferred solvent that gave the highest molecular weight product ($M_n = 28\,500$ in 48 h).

Studies on the effect of monomer chain length for polyesterifications with unactivated diacids and diols showed that systems with longer chain length diacids (sebacic and adipic acid) and diols (1,8-octanediol and 1,6-hexanediol) give higher reactivity than systems with shorter chain length of diacids (succinic and glutaric acid) and 1,4-butanediol. Running bulk lipase-catalyzed condensation reactions is feasible, although using diphenyl ether gave higher M_n values. Both in solution and solvent-free conditions, PDI is independent of the diol chain length. However, the PDI values were generally larger for polymerizations in solution.

Studies on end-group analysis of the polymer formed showed no systematic variations in end-group structure as a function of the reaction time or chain length of the diacid and diol substrates. Furthermore, no significant differences in end-group structure were observed for solution versus bulk polymerizations.

There was no substantial change in the percent retention of enzyme activity as a function of the monomer structures used. Comparison of polymerizations performed in solvent-free media and in diphenyl ether showed that the percent retention of enzyme activity was slightly greater when using diphenyl ether ($\sim 87\%$ versus 80%).

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Supporting Information Available. Figure S1 showing early times of chain growth versus time and substrate. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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