

***Candida antarctica* Lipase B-Catalyzed Synthesis of Poly(butylene succinate): Shorter Chain Building Blocks Also Work**

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Lipase catalysis was successfully employed to synthesize high molecular weight poly(butylene succinate) (PBS). Attempts to copolymerize succinic acid with 1,4-butanediol were unsuccessful due to phase separation of the reactants. To circumvent this problem, monophasic reaction mixtures were prepared from diethyl succinate and 1,4-butanediol. The reactions were studied in bulk as well as in solution. Of the organic solvents evaluated, diphenyl ether was preferred, giving higher molecular weight products. After 24 h in diphenyl ether, polymerizations at 60, 70, 80, and 90 °C yielded PBS with M_n of 2000, 4000, 8000, and 7000, respectively. Further increase in reaction time to 72 h resulted in little or no further increase in M_n . However, increasing the reaction time produced PBS with extraordinarily low M_w/M_n due to the diffusion and reaction between low-molecular weight oligomers and chains that occurs at a greater frequency than interchain transesterification. Time-course studies and visual observation of polymerizations at 80 °C revealed PBS precipitates at 5 to 10 h, limiting the growth of chains. To maintain a monophasic reaction mixture, the polymerization temperature was increased from 80 to 95 °C after 21 h. The result was an increase in the PBS molecular weight to $M_w = 38\,000$ ($M_w/M_n = 1.39$). This work paves the way for the synthesis of PBS macromers and polymers that contain variable quantities of monomers with chemically sensitive moieties (e.g., silicone, epoxy, vinyl). Furthermore, this study established the feasibility of using lipase catalysis to prepare polyesters from α,ω -linear aliphatic diethyl ester/diol monomers with less than six carbons.

Introduction

Biodegradable polymers have been extensively used in biomedical applications, for example, as biomaterials for constructing absorbable bone plates, other surgical fixation devices, surgical sutures, and controlled release drug carriers.¹ The biocompatibility, toxicity, and immunogenicity of polymers play important roles in these applications. Other influential factors include a predictable rate of biodegradation and desirable mechanical properties.

Aliphatic polyesters are a group of remarkable biodegradable polymers that can be processed into various forms, such as fibers, films, and injection-molded devices.² They are suitable to replace many conventional thermoplastics because of their good mechanical strength and variable melting temperatures. Commercial aliphatic polyesters are produced mainly through condensation polymerization of aliphatic dicarboxylic acids with diols, transesterification reaction of diesters with diols, polymerization of hydroxy acids, and ring-opening polymerization of lactones.³

Poly(butylene succinate) (PBS) is an important member of the aliphatic polyester family. PBS and related copolymers have shown considerable promise for uses as environmentally biodegradable thermoplastics, as well as bioabsorbable/biocompatible medical materials. In both cases, practical applications require that the polymer possess a high molecular weight ($M_n > 20\,000$) so that it has useful mechanical properties.

High molecular weight PBS materials, including commercial products sold by Showa Highpower Co., Ltd., have been synthesized by using organometallic catalysts at high reaction

temperatures (≥ 190 °C).^{4,5} Residual metals in these materials are difficult to remove because of strong metal-ester interactions. These metals can cause undesirable effects on the environment upon material disposal, limit polymer use in applications such as electronics, and may be unacceptable components of medical materials. Furthermore, the combination of high reaction temperatures and organometallic catalysts can lead to monomer/polymer decomposition reactions and, hence, discoloration and decreased product molecular weight. Also, if one wishes to elaborate further on the structure of PBS by including chemical entities that are unstable under traditional chemical condensation conditions (e.g., silicone, epoxy, vinyl), new low-temperature polymerization methods are needed.

Lipase-catalyzed polycondensation reactions have been explored by us and others at temperatures of 90 °C and below.^{6–11} Previous studies on lipase-catalyzed condensation polymerizations primarily focused on reactions between diols and activated diesters, such as 2,2,2-trichloroethyl and vinyl esters.¹² For example, Russell and co-workers showed that, by using physically immobilized *Candida antarctica* Lipase B (Novozym 435, abbreviated as N435), the copolymerization of divinyl adipate and 1,4-butanediol in bulk yielded the corresponding polyester with a weight average molecular weight (M_w) of 23 200.¹³ However, activated monomers are expensive and therefore limit the potential impact of the polymerization method.

Review of literature in recent years shows important progress in lipase-catalyzed condensation polymerization of conventional (unactivated) diacids and diols. Linko, et al. reported the copolymerization of sebacic acid with 1,4-butanediol in diphenyl ether under vacuum using the lipase catalyst from *Mucor miehei* (36.5 wt %).^{14,15} After 7 days of reaction at 37 °C, poly(1,4-butylene sebacate) with M_w of 42 000 was isolated through

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reprecipitation of the polymer in a chloroform/methanol mixture (no yield reported). Taylor, et al. performed copolymerizations between adipic acid and 1,4-butanediol (bulk, 40–60 °C, 27–39 h) under vacuum to prepare polyesters of low molecular weight (i.e., $M_n \leq 5000$).¹⁶ Uyama, et al. reported that *Candida antarctica* lipase immobilized on an undisclosed matrix (17 wt % vs total monomer) catalyzed copolymerization (bulk, 70 °C, 48 h) between sebacic acid and 1,5-pentanediol to yield, after reprecipitation, poly(pentamethylene sebacate) with M_n and M_w/M_n of 14 000 and 2.3, respectively.¹⁷ Important to the subject of this paper is that all the above examples used nonactivated α,ω -linear aliphatic diacid and/or diol monomers with six or more carbons.

Our laboratory studied lipase-catalyzed polycondensation reactions using adipic acid/1,8-octanediol as model substrates.⁶ The effects of solvents on the polymerization rate, product molecular weight, polydispersity, and polymer end group structures were investigated. Diphenyl ether was found to be a preferred solvent for polymerization of adipic acid with 1,8-octanediol. The authors also investigated effects of diacid and diol monomer chain length on polymer molecular weight and end-group structures.⁶ Results showed that lipase-catalyzed polymerization of α,ω -linear aliphatic diacid and diol substrates was strongly influenced by monomer chain length. α,ω -Linear aliphatic diacids and diols with six or more carbons were more rapidly copolymerized by N435 to form polyesters. For example, N435-catalyzed copolymerization of adipic acid and 1,8-octanediol at 70 °C for 72 h resulted in poly(octamethylene adipate) with M_n and M_w/M_n of 42 400 and 1.5, respectively.

Since shorter-chain substrates lead to important aliphatic polyesters with unique physical properties, a serious gap in lipase-catalyzed polymerization methodology remains to be solved. Access to polymer structures from 4-carbon building blocks by lipase catalysis can provide semicrystalline materials, with useful properties, that also contain chemically sensitive, third-component monomers that enhance polymer performance. Herein we report the first successful enzyme-catalyzed synthesis of high molecular weight PBS from practical monomer precursors.

Experimental Section

Materials. Diethyl succinate, 1,4-butanediol, diphenyl ether, dodecane, and diglyme were purchased from Aldrich Chemical Co. in the highest available purity and were used as received. N435 (specific activity 7000 PLU/g) was a gift from Novozymes (Bagsvaerd, Denmark) and consists of *Candida antarctica* Lipase B (CAL-B) physically adsorbed within the macroporous resin Lewatit VPOC 1600 (poly[methyl methacrylate-*co*-butyl methacrylate], supplied by Bayer). Lewatit VPOC 1600 has a surface area of 110–150 m²/g and an average pore diameter of 100 nm. N435 contains 10 wt % CAL-B, which is located on the outer 100 μ m of the 600 μ m average diameter Lewatit beads.⁹

Instrumental Methods. ¹H- and ¹³C NMR spectra were recorded on a Bruker DPX 300 NMR spectrometer. The chemical shifts reported were referenced to internal tetramethylsilane (0.00 ppm) or to the solvent resonance at the appropriate frequency. The number and weight average molecular weights (M_n and M_w , respectively) of the polymers were measured by gel permeation chromatography (GPC) using a Waters HPLC system equipped with a model 510 pump, a Waters model 717 autosampler, and a Wyatt Optilab DSP interferometric refractometer with 500, 10³, 10⁴, and 10⁵ Å Ultrastayragel 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 2 mg/mL and injection volumes of 100 μ L were used. Molecular

weights were determined on the basis of a conventional calibration curve generated by narrow polydispersity polystyrene standards from Aldrich Chemical Co. All polymer molecular weights reported in this paper correspond to nonprecipitated reaction products.

General Procedure for N435-Catalyzed Condensation Polymerization of Diethyl Succinate with 1,4-Butanediol. N435 (10 wt % vs total monomer), dried under 0.1 mmHg vacuum at 25 °C for 24 h, was transferred into a 50 mL round-bottom flask containing diethyl succinate (4.00 g, 22.96 mmol) and 1,4-butanediol (2.07 g, 22.96 mmol), with or without an organic solvent (200 wt % vs total monomer). The reaction mixture was magnetically stirred and heated at a temperature between 60 and 90 °C for a predetermined time. The reaction was carried out under atmospheric pressure for the first 2 h to convert the monomers to oligomers. The reaction pressure was then reduced to 40 mmHg. A J-KEM vacuum regulator was employed to control the reaction pressure. At pre-selected time intervals during the polymerization reactions, aliquots of about 20 mg were withdrawn, added to HPLC grade chloroform to dissolve the products, and then filtered to remove the lipase catalyst. Catalyst particles were washed with chloroform twice, and the combined filtrates (polymer solutions in chloroform) were directly analyzed by GPC.

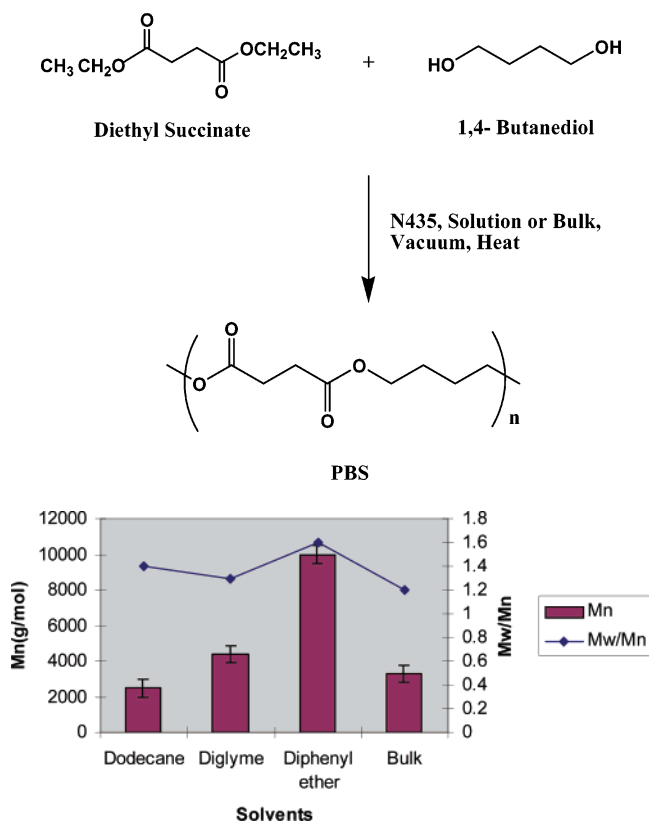
Two-Stage Enzymatic Reaction to Prepare High Molecular Weight PBS. N435 (0.61 g, dried under 0.1 mmHg vacuum at 25 °C for 24 h) was added to a solution of diethyl succinate (4.00 g, 22.96 mmol) and 1,4-butanediol (2.07 g, 22.96 mmol) in diphenyl ether (12.14 g). Mixing in reactions was by magnetic stirring. The following sequence of reaction conditions was performed: (i) 80 °C for 2 h under atmospheric pressure, (ii) pressure reduced to 2 mmHg using a J-KEM vacuum regulator while maintaining the reaction at 80 °C for 5 to 24 h, (iii) reaction temperature increased to 95 °C for 20 to 30 h. During polymerization reactions, aliquots of about 20 mg were withdrawn and treated as described in the previous section. The filtrates from these aliquots were analyzed by GPC.

The product mixture at the last time point was dissolved in chloroform and then filtered to remove the catalyst. The resulting chloroform solution was slowly added with stirring to methanol to precipitate a white crystalline polymeric product. The precipitated polymer was washed with methanol three times and then dried in vacuo at 50 °C for 16 h. The results of ¹H- and ¹³C NMR spectral analysis were as follows: ¹H NMR (CDCl₃) (ppm): 1.71 (4H, m, -CH₂-), 2.62 (4H, s, -COCH₂-), 4.12 (4H, m, -CH₂O-), low intensity signals due to end groups were observed at 1.26 (t, CH₃CH₂O-), 3.67 (t, HOCH₂CH₂CH₂CH₂O-); ¹³C NMR (CDCl₃) (ppm): 25.2 (-CH₂-), 29.0 (-CH₂CO-), 64.2 (-OCH₂-), 172.3 (-CO-), low intensity signals due to end-groups at 14.2, 60.7 assigned to CH₃CH₂O-CO- end groups, and resonances at 25.1, 29.1, 62.2, 64.6 that correspond to HOCH₂CH₂CH₂CH₂O- end groups.

Results and Discussion

Substrate Selection. The most convenient substrates for PBS synthesis are succinic acid and 1,4-butanediol. However, succinic acid was found to have low solubility in 1,4-butanediol under the reaction conditions, thus leading to two separate liquid phases in the reaction mixture. As a result, the copolymerization between the two monomers was slow, and only low molecular weight oligomers were formed during the reaction. This problematic phase separation was avoided by replacing succinic acid with diethyl succinate as the acyl donor. The reaction mixture consisting of diethyl succinate and 1,4-butanediol, either in the presence or absence of an organic solvent, formed a single liquid phase which facilitated efficient diffusion between reactants during copolymerizations.

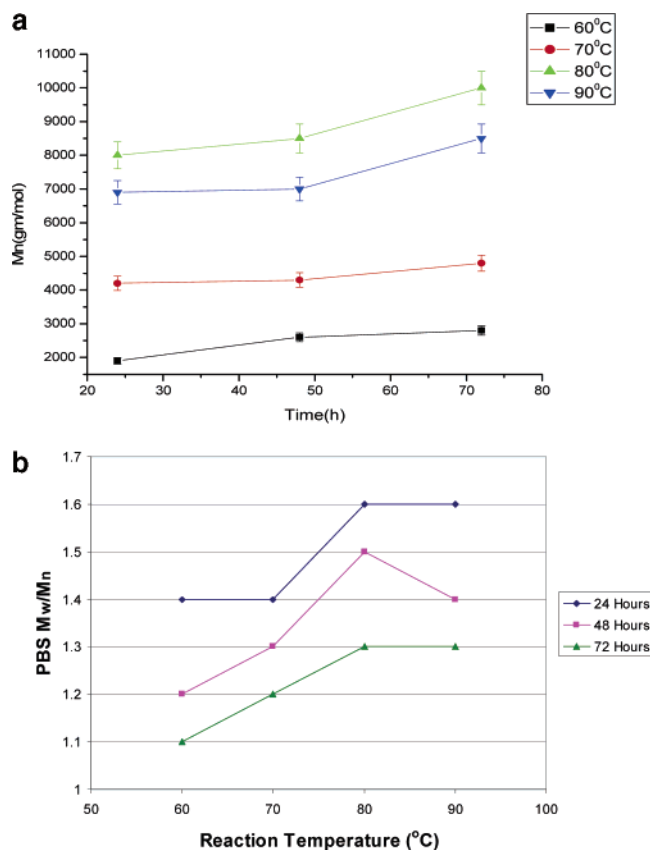
Effects of Reaction Conditions on Lipase-Catalyzed Copolymerization of Diethyl Succinate with 1,4-Butanediol. Scheme 1 illustrates the general synthetic strategy for N435-

Scheme 1. Condensation Copolymerization of Diethyl Succinate with 1,4-Butanediol to Form PBS**Figure 1.** Effects of solvent on PBS molecular weight and polydispersity. The synthesized polymers were not fractionated. Error bars were calculated on the basis of triplicate experiments.

catalyzed PBS synthesis. The copolymerization reactions were performed at mild temperatures ($<100\text{ }^\circ\text{C}$), under vacuum, either in solution or in bulk. The molar ratio of diethyl succinate to 1,4-butanediol was 1:1, and the catalyst was physically immobilized *Candida antarctica* Lipase B (N435, 10 wt % catalyst or 1 wt % protein vs total monomer).

Diethyl succinate/1,4-butanediol copolymerization reactions were studied in bulk as well as in solution. The organic solvents evaluated include diphenyl ether, dodecane, and diglyme. They were selected since they have high boiling points (i.e., low volatility under vacuum), are chemically inert under the selected reaction conditions, include a diverse range of structures (alkane, aliphatic and aromatic ethers), and have log P values greater than or equal to 1.9. It's generally accepted that lipase activity is improved at higher log P values where the water structure around enzymes remains intact.¹⁰

Polymerizations in diphenyl ether, dodecane, and diglyme, as well as a solvent-free reaction, were performed at $80\text{ }^\circ\text{C}$ under vacuum (40 mmHg) for 72 h. The number average molecular weight (M_n) and polydispersity (M_w/M_n) values of PBS were analyzed by GPC, and the results are shown in Figure 1. The polymer of highest molecular weight was obtained in diphenyl ether. Values of product M_n (M_w/M_n) obtained from the reactions in dodecane, diglyme, diphenyl ether, and in bulk were 2500 (1.4), 4400 (1.3), 10 000 (1.6), and 3300 (1.2), respectively. Previously, our laboratory studied N435-catalyzed condensation polymerizations between adipic acid and 1,8-octanediol in xylene, tetramethylene glycol dimethyl ether, 2-methoxyethyl ether, and diphenyl ether.⁶ As was found here, it was reported that, by using diphenyl ether as solvent, polyesters of higher M_n were obtained. Hence, even though the structures of the

**Figure 2.** (a) Effects of reaction temperature and time on PBS M_n for the N435-catalyzed copolymerization of diethyl succinate with 1,4-butanediol. (b) Effects of reaction temperature and time on PBS polydispersity (M_w/M_n) for the N435-catalyzed copolymerization of diethyl succinate with 1,4-butanediol.

monomers and polymers were different, the cumulative results from these studies show diphenyl ether is an exceptional solvent that enables N435 to form high molecular weight polyesters by step-condensation polymerization reactions. Figure 1 also shows that the M_w/M_n values range from 1.2 to 1.6. Polydispersity values less than or equal to 2 for nonfractionated products formed by N435-catalyzed condensation polymerizations were previously reported by our laboratory^{11,18} and by Binns et al.¹⁹ In general, by random processes of step-growth condensation polymerization, the polydispersity of resulting polymers will have values greater than or equal to 2. The comparatively lower polydispersity of polyesters formed by N435-catalyzed condensation polymerization reactions is explained by the unique chain length or mass selectivity of the lipase. Higher polydispersity values in diphenyl ether are likely due to interchain transesterification reactions. That is, when N435-catalyzed polymerizations are conducted in solution instead of using a solvent-free system, interchain transesterification reactions may occur at a greater rate.

Effects of reaction temperature on PBS molecular weight averages and polydispersity were studied for polymerizations in diphenyl ether. Figure 2a delineates product M_n as a function of reaction time at different temperatures. In 24 h, the polymerization reaction at 60, 70, 80, and $90\text{ }^\circ\text{C}$ yielded PBS with M_n values of 2000, 4000, 8000, and 7000, respectively. Further increase in reaction time resulted in little or no change in the product molecular weight. This led us to focus on the course of reactions over shorter time intervals, as is discussed below. Figure 2b shows M_w/M_n values for the above set of polymers described in Figure 2a. The general trends observed are

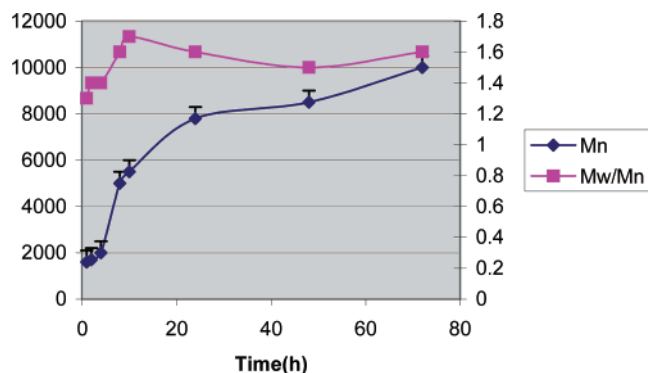


Figure 3. Effects of reaction time on PBS M_n and M_w/M_n for the N435-catalyzed copolymerization of diethyl succinate with 1,4-butanediol at 80 °C in diphenyl ether.

increased M_w/M_n for reactions conducted at higher temperatures up to 80 °C, and decreased M_w/M_n as the reaction time was increased from 24 to 48 and 72 h. These results are explained by an increased frequency of transesterification reactions when reactions are performed at higher reaction temperatures. Longer reaction times permit low molar mass products to diffuse to the catalyst and form products of higher molecular weight. This reduces the low molecular weight fraction, thereby decreasing M_w/M_n . Apparently, for 24–72 h reaction times, the addition of low molecular weight oligomers to polymer chains occurs at a greater frequency than transesterification reactions, allowing the attainment of products with extraordinarily low M_w/M_n values (1.1–1.3 at 72 h, Figure 2b). As above, M_w/M_n values are significantly below that for statistically random, stepwise polycondensation reactions.

Since M_n was nearly unchanged with increase in reaction time from 24 to 72 h, a study was performed to characterize chain growth from 0 to 24 h at 80 °C in diphenyl ether. Figure 3 shows that, during the first 10 h of the reaction, a rapid increase in M_n up to 5500 occurred. Thereafter, M_n increased slowly to 8000 and 10 000 by 24 and 72 h, respectively. Close inspection of physical changes in the reaction mixture showed that, at about 8 h, PBS precipitates. After 20 h, the reaction mixture formed a gel.

Phase separation of the product during the reaction causes infrequent collisions between substrate and catalyst molecules. This explains the slowed chain growth beyond 10 and 24 h observed in Figures 3 and 2a, respectively.

Consistent with the results reported previously on the N435-catalyzed polycondensation of aliphatic diacids with diols in diphenyl ether, the PBS polydispersity increased initially to about 1.7 then decreased after 10 h to about 1.5 at 48 h.⁶ The decrease in M_w/M_n at extended reaction times is explained above and was further exacerbated by precipitation of product in the reaction medium.

Temperature-Variied Two-Stage N435-Catalyzed Polymerizations between Diethyl Succinate and 1,4-Butanediol.

On the basis of observations discussed above, a temperature-varied two-stage process was designed and studied to circumvent the phase separation of PBS during the reaction. The copolymerization of diethyl succinate with 1,4-butanediol in diphenyl ether was carried out first at 80 °C for 5–21 h at 1.8–2.2 mmHg to form PBS with M_n between 5000 and 10 000. Subsequently, the reaction temperature was raised to 95 °C at 1.8–2.2 mmHg to re-form a monophasic reaction mixture. Figure 4 shows that, by this strategy, synthesis of PBS with $M_w > 35 000$ was achieved (details are summarized in Table A in Supporting Information). During polymerizations at 95 °C, precipitation of

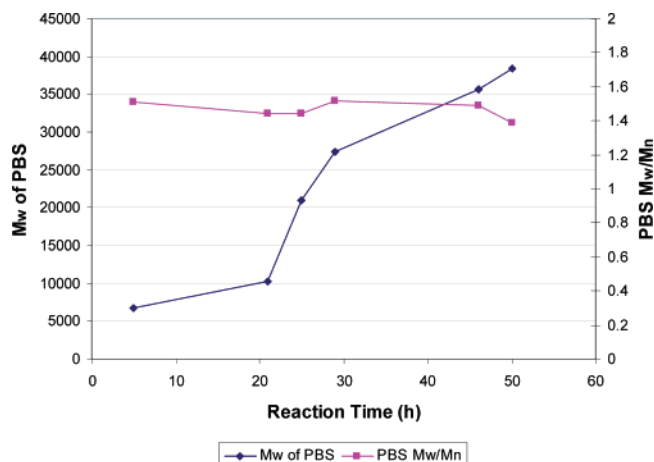


Figure 4. Temperature-varied two-stage N435-catalyzed polymerization: Effects of reaction time on PBS M_w and M_w/M_n . Reaction was performed in diphenyl ether at 80 °C during 0–21 h and at 95 °C during 21–50 h.

PBS was not observed. Instead of nearly invariant molecular weight values with increasing reaction time above 21 h (see Figures 2a and 3), Figure 4 shows an abrupt increase in PBS molecular weight after 21 h. In other words, by increasing the reaction temperature to 95 °C at 21 h, a monophasic reaction mixture was formed, and PBS molecular weight increases through 50 h. Furthermore, in contrast to the M_w/M_n values of ≥ 1.8 observed using chemical catalysts for aliphatic polyester synthesis by step-condensation reactions,^{4a} PBS at 50 h has an M_w/M_n value of 1.39.

A control experiment without using N435 catalyst was performed in parallel under reaction conditions identical to those described above. GPC analysis of the reaction products indicates that essentially no polymer ($M_n \geq 500$) was formed during the 50 h period of noncatalyzed reaction.

Finally, it needs to be noted that the polymer products formed during polycondensations between diethyl succinate and 1,4-butanediol were analyzed by GPC and NMR spectroscopy. No monomer was detected in the reaction mixture after 5 h at 60–90 °C under 2–40 mmHg pressure. Furthermore, under such mild reaction conditions, loss of the monomers and low molecular weight oligomers due to evaporation is minimal. Ethanol was found to be the only byproduct in the cold trap during the polymerization. Thus, the monomer conversion and PBS yield based on added substrates for all polymerization reactions described in the previous sections are essentially 100%.

Conclusions

We report, for the first time, an efficient metal-free enzymatic route to synthesize high molecular weight PBS. Slow diffusivity of the substrates and growing polymer chains resulting from polymer precipitation was a key factor limiting further molecular weight increase after 5–10 h of reaction time. This problem was circumvented by carrying out polymerization reactions in solution using a temperature-varied, two-stage process. In other words, by increasing the polymerization temperature to 95 °C during the second stage, the polymer remained soluble in the reaction medium so that the reaction proceeded in a single liquid phase that facilitated further molecular weight increase. The results of this work pave the way for the synthesis of PBS macromers and polymers that contain variable quantities of chemically sensitive moieties (e.g., silicone, epoxy, vinyl). Direct incorporation of such groups by traditional

chemical methods (e.g., ≥ 150 °C, organometallic or acid-catalyzed) would result in nondesired coproducts.

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Supporting Information Available. Table showing the effects of reaction time on PBS M_n and M_w/M_n during the temperature-varied two-stage N435-catalyzed copolymerization of diethyl succinate with 1,4-butanediol. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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