Rational Control of Polymer Molecular Weight and Dispersity during Enzyme-Catalyzed Polyester Synthesis in Supercritical Fluids

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Abstract: Enzyme-catalyzed polyester synthesis has been investigated with special emphasis on modulation of polymer molecular weight and dispersity. Supercritical fluids were also used for isolating low-dispersity polyester fractions by changing pressure. Enzymatic synthesis of low-dispersity polyesters was achieved in supercritical fluoroform. The synthesized polymer molecular weight can be controlled by changing the pressure.

Introduction

Supercritical fluids have attracted significant attention as powerful extraction solvents[1] and have been applied in various industrial extraction processes.[2] Enzymes are known for their ability to selectively catalyze reactions in both aqueous and nonaqueous media.[3] With supercritical fluids as the reaction medium, enzyme activity[4] and selectivity[5] can be manipulated by varying either the pressure or temperature of the system. Because of their pressure dependent physical properties, supercritical fluids have also been used in examining the effect of solvent physical properties on conventionally catalyzed reactions.[6]

There is a growing interest in the biocatalytic synthesis of specialty polymers since such an approach can generate additional properties such as chirality and biodegradability. Biologically synthesized polymers have been applied as absorbents, biodegradable materials, chiral adsorbents, liquid crystals, and perm-selective membranes.[7] The enzymatic syntheses of polyacrylates,[8] polyamides,[8] polyesters,[9,10] and polyphenols[11,12] in conventional organic solvents have been successfully accomplished.

Low molecular weight linear aliphatic oligoesters which are hydroxy-terminated at both ends have special commercial significance as intermediates for polyurethane synthesis[13,14] and as plasticizers.[15] The current commercial synthesis is based on the acid/base-catalyzed condensation polymerization reaction between a diacid/diester and a diol. However, the use of traditional chemical catalysts is limited because all catalysts tend to have an undesirable effect on the subsequent polyurethane reactions.[15] Indeed, the highly reactive nature of isocyanates poses special problems if contaminating acid/base exists.[16] Ether, aldehyde, and lactone formation by side reactions also complicates downstream purification processes after acid/base-catalyzed polymerization. The potential selectivity of an enzyme-based approach is made more attractive by the known tendency of enzyme-catalyzed polyester synthesis to result in relatively low molecular weight polymers.

We have investigated the lipase-catalyzed transesterification of a diester and diol (Figure 1). Synthesized polyesters were characterized using laser desorption mass spectrometry (LD-MS), gel permeation chromatography (GPC), and nuclear magnetic resonance (^{1}H and ^{13}C) and FT-IR spectroscopy. The fractionation of synthesized polymer in supercritical fluids indicated that the solubility of the synthesized polymer chains is a sensitive function of pressure. This further suggested that a single step polyester synthesis of low dispersity and molecular weight can be achieved under supercritical conditions. Further, one should be able to alter the molecular weight merely by controlling pressure. The results of such an approach are shown below.

Results and Discussion

Molecular Weight Determination. In the literature molecular weights of many enzyme-synthesized polyesters have been described without the use of polyester standards (monodisperse polyester standards are currently not commercially available). In the absence of the polyester standards of the type we

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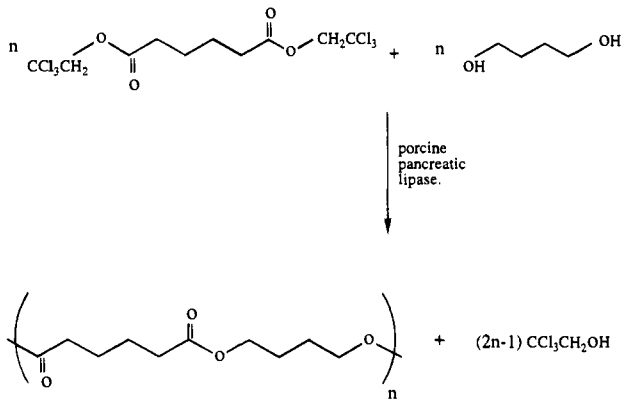

Figure 1. Lipase-catalyzed polytransesterification between bis(2,2,2-trichloroethyl) adipate and 1,4-butanediol.

synthesized, an alternative method of obtaining absolute molecular weights was required. Recently there has been considerable attention paid to a remarkable new technology for polymer molecular weight determination: laser desorption mass spectrometry (LD-MS). LD-MS enables structural and size characterization of intact low molecular weight polymers with 99.95% accuracy.[17,18] Mass spectroscopy techniques in which "soft" ionization is *not* performed (such as field desorption or fast atom bombardment) involve a danger of fragmentation of polymeric species. High-energy pulsed lasers as ionization sources do not have this limitation. LD-MS, however, can be applied only with great difficulty for molecular weights above 10 000 and for highly nonpolar species. Also, the distribution can be skewed to lower masses if there is preferential desorption of oligomers[19] or if there is inadequate postacceleration.[20]

We determined the absolute molecular weights for many of our synthesized polyesters using LD-MS and then used this information to correct the molecular weights obtained from GPC by differential refractometry. It should be noted that using polystyrene molecular weight standards on a GPC for molecular weight determination of polyesters, the molecular weight is overestimated (by about 25%). This may account for our difficulty in obtaining "high" molecular weight polyesters which have been reported previously.

It is interesting to note that for conventionally catalyzed polyester synthesis, the resulting polymers have a broad distribution in molecular weight, whereas the enzyme-synthesized material has a particularly large low molecular weight content. The reason for this is probably related to the changing specificity of the enzyme with chain extension. Since the enzyme is more active with shorter chain substrates, the resulting polymer mixture will have a relatively low concentration of high molecular weight material. Since LD-MS data offer accurate analysis of different masses of polymers and its relative distribution, these data are being used to develop a predictive model for lipase-catalyzed polyester synthesis which will be published elsewhere.

Time Course of Polymer Synthesis. Using the techniques described below we have studied the lipase-catalyzed synthesis of low molecular weight poly(1,4-butylene adipate). Given our interest in controlling the molecular weight of the polymer product, it is important to distinguish between possible mechanisms for molecular weight limitation. The achievable molecular weight in an enzyme-catalyzed polytransesterification could be limited by a combination of enzyme instability, the equilibrium position for the reaction, and the effect of molecular weight of the substrates on enzyme specificity. Study of the time course of the reaction gives important information about the governing forces for the enzyme-catalyzed synthesis, although most previous research in this field (with the notable exception of the work of Morrow) has only considered endpoint analysis of the reaction mixture.

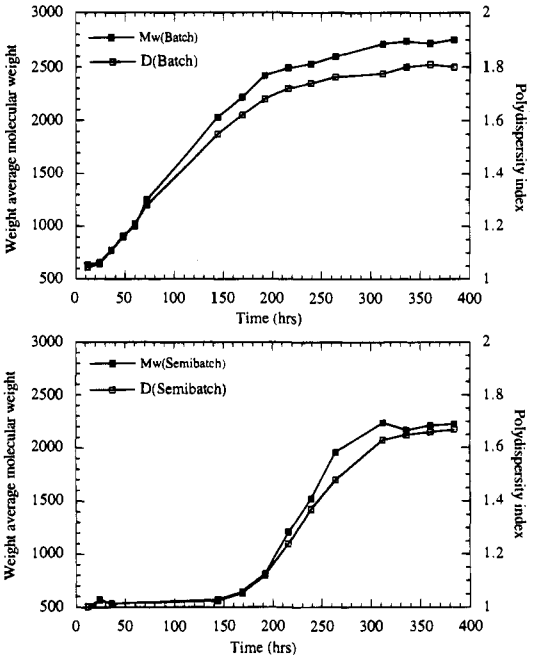

Figure 2. Progress and results of the lipase-catalyzed polytransesterification of bis(2,2,2-trichloroethyl) adipate by 1,4-butanediol in anhydrous ether under atmospheric conditions in a sealed vessel (top) and in semibatch mode (bottom). For experimental details, see text.

Figure 2 (top) describes the increase in molecular weight during the lipase-catalyzed polytransesterification between bis-(2,2,2-trichloroethyl) adipate and 1,4-butanediol in anhydrous ether under atmospheric conditions in a sealed vessel. The lipase was selected on the basis of a preliminary screen of lipases known to catalyze polytransesterifications. Figure 2 (bottom), however, describes the same synthesis in semibatch mode, where the enzyme is added stepwise during synthesis. The degree of polymerization only increases after a number of aliquots of enzyme have been added, and the molecular weight increase is faster than that in batch mode during the polymerization stage due to the availability of fresh enzyme.

Once the molecular weights observed in the batch system are obtained in the semibatch experiment, no further polymerization is observed (even after the addition of fresh enzyme). Since there is no possibility of the loss of reacting functional groups on the polymer chains,[21] further reaction must therefore be thermodynamically limited. Achievable polyester molecular weight during enzyme-catalyzed synthesis has been increased by shifting the equilibrium in the forward direction;[22] however, the high molecular weights reported (determined using ^1H NMR,

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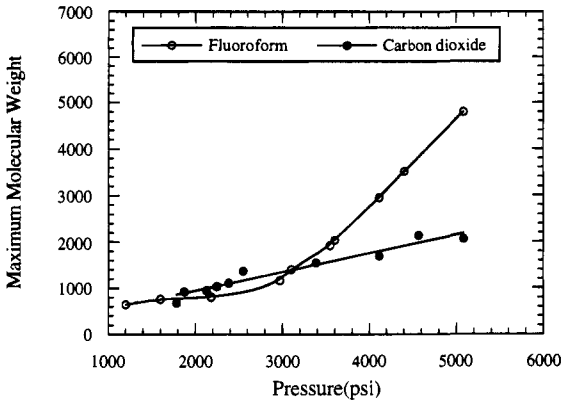

Figure 3. Extraction of commercial poly(1,4-butylene adipate) in carbon dioxide and fluoroform at 50 °C. The dispersity of the extracted fractions in carbon dioxide increased from 1.03 to 1.08 (the maximum molecular weight extracted represents the longest chain length solubilized at a given pressure).

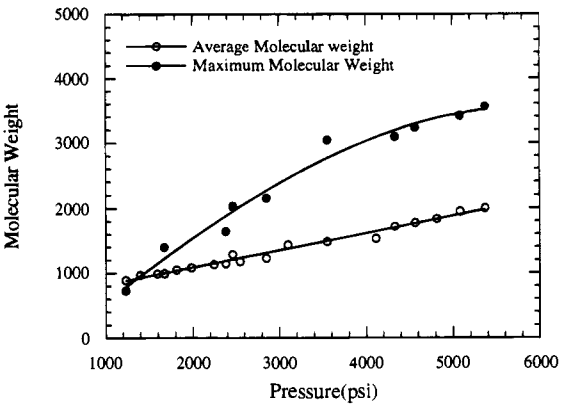

Figure 4. Extraction of enzyme-synthesized polyester in supercritical fluoroform at 50 °C. The polydispersity index of the extracted fractions increased from 1.02 to 1.1.

which can be insensitive at higher molecular weights,[23] or using GPC without correcting for the polymer type[24]) were not achieved in our studies.

Biocatalytic Polyester Synthesis Yield. After loss of low oligomeric species during the purification procedures, the final yield of the pure polyester (after precipitation) from the batch reaction was approximately 40% based on the starting materials. There is a loss of 60% of the low molecular weight oligomers, which does not precipitate under the conditions used for polymer recovery. Naturally, optimization of recovery could be performed if necessary.

Polymer Fractionation on the Basis of Molecular Weight. The commercial synthesis of poly(1,4-butylene adipate) usually generates a broad molecular weight distribution in the product polymer (as is typical of a step-condensation polymerization for varying extents of reaction).[25] In addition, the formation of ether and aldehydes due to side reactions[14] is a well-known problem. There have been attempts to control polymer molecular weight distribution during enzyme-catalyzed polymer syntheses. For example, polyphenol molecular weight can be controlled by precipitating the polymer product by adjusting the dioxane content in an aqueous/organic system.[10] Since, for an SCF, the solubility parameter is a strong function of pressure, possible application for fractionation of oligomers has been suggested.[1] Polymer chains can be dissolved in, or precipitated from, a solution by varying pressure. It is possible to exploit the pressure dependence of solubility parameter for polymer processing. A commercially available sample of poly(1,4-butylene adipate), with an average molecular weight of 8500 (determined using LD-MS) and a polydispersity index of 2.4 can be fractionated into low-dispersity polymer fractions by extracting with supercritical fluoroform or carbon dioxide (Figure 3). Extraction was also performed on a sample of the lipase-synthesized poly(1,4-butylene adipate) described above (with average molecular weight of 4100 and polydispersity index of 1.37) (Figure 4). As expected, due to a steady increase in the solvent solubility parameter with pressure the molecular weight of the polymer that can be solubilized and extracted from a polymer sample increases with pressure. Also, since fluoroform is a better solvent than carbon dioxide, it can solubilize higher molecular weight chains than carbon dioxide.

Table 1. Effect of Pressure on Molecular Weight and Dispersity during Lipase-Catalyzed Polymerization in Supercritical Fluoroform[a]

pressure (psi)	maximum molecular weight of the soluble polymer	average molecular weight and dispersity of the synthesized polymer	average molecular weight and dispersity of the precipitated polymer
900	739[b]	701 (1.07)[b]	764 (1.02)[b]
1600	1076	778 (1.11)	1272 (1.03)
2400	1982	1035 (1.18)	2130 (1.03)
3000	2189	1338 (1.23)	2590 (1.05)

[a] The polytransesterification of bis(2,2,2-trichloroethyl) adipate by 1,4-butanediol (100 mM) is catalyzed by porcine pancreatic lipase (0.4 g/mmol of diester) suspended in fluoroform at 50 °C. [b] The precipitated material for the biocatalysis experiment performed at 900 psi was extracted into a supercritical fluid extractor in which the minimum pressure achievable was 1200 psi because of minimum pressure limitations in our system.

Reactive Separation of Low-Dispersity Polyester during Biocatalytic Synthesis. The rate at which a solid enzyme catalyzes a reaction with a solid substrate will be much less than that for the reaction where either the catalyst or the substrate is in solution. Indeed, this well-recognized fact is the basis for the enzyme-catalyzed commercial synthesis of aspartame[26] by Dutch State Mines in which the precipitation of the product drives the equilibrium to product formation, and prevents further chain extension. This is also the basis of the fact that modification and/or polymerization reactions on sugar molecules are carried out in hydrophilic water-miscible solvents such as pyridine or dimethylformamide.[27] Therefore, one would expect an enzyme which catalyzes chain extension polymerizations in a supercritical fluid to the essentially unable to increase the molecular weight of a polymer which is also insoluble in the fluid. For a supercritical fluid, the size the polymer which can be solubilized will be sharply dependent on temperature and pressure, as shown by the extraction data presented above. One could predict, therefore, that the precipitated product of an enzyme-catalyzed polymer synthesis in supercritical fluids should have relatively low dispersity, and the polymer molecular weight will be density-dependent.

To test our hypothesis, we examined the potential of using pressure as an adjustable parameter to control the molecular weight and dispersity of the polyester product obtained during the lipase-catalyzed polytransesterification of bis(2,2,2-trichloroethyl) adipate by 1,4-butanediol (Table 1 and Figure 5 bottom). As the pressure is increased, there is, as expected, a steady increase in the average molecular weight of both the soluble

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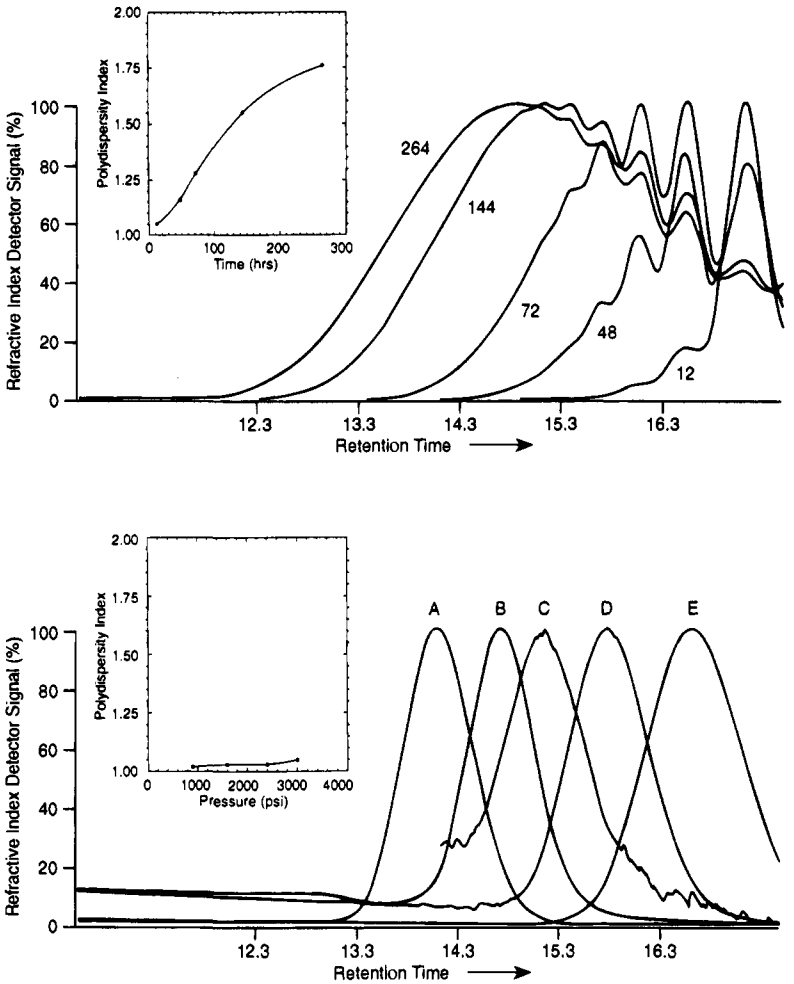

Figure 5. Lipase-catalyzed polytransesterification between bis(2,2,2-trichloroethyl) adipate and 1,4-butanediol in anhydrous ether (top) and in supercritical fluoroform at different pressures (bottom). Curve A is the chromatogram for a polystyrene standard with $M_w = 5050$ and polydispersity index of 1.05. Curves B, C, D, and E are the gel permeation chromatograms for the enzyme-synthesized polymers at 3000, 2400, 1600, and 900 psi, respectively.

and precipitated polymers. The increasing pressure, and thus solvent solubility parameter, also results in a steady rise in the maximum chain length for the soluble material. An example of the effect of pressure on the fluoroform solubility parameter has been published previously.[5] As in conventional solvents, the increase in the maximum molecular weight of the *soluble* polymer, at any given pressure, is matched by an increase in the polydispersity index of the material. The molecular weight of the *precipitated* polymer also increases with pressure, and its average molecular weight is equivalent to only one or two monomers more than the soluble material. However, since the precipitated polymer will have a very low rate for enzyme-catalyzed chain extension, it retains low dispersity after precipitation. In addition, the precipitation of the materials drives the equilibrium reaction in solution toward synthesis. For a typical reaction carried out at 1400 psi in fluoroform, the overall yield of monodisperse polyester based on total starting monomers is around 6−8%. We are now optimizing the system for efficient recovery of the precipitated polymer in a continuous system. At high pressures, the change of solubility parameter with pressure becomes less pronounced, and the fine control of dispersity which is achievable at low pressures will be less precise. Nevertheless, the polydispersity index for the precipitated material (although relatively poor yield) is still far lower than what can be achieved using a conventional approach (Figure 5). Finally, the molecular weight of the precipitated polymer can also be predicted from extraction data on the enzyme-synthesized polyester. It is apparent that the maximum molecular weight of the material extracted from traditionally-synthesized polymer, at any given pressure, is approximately equivalent to the average molecular weight of the precipitated polymer which has been synthesized, in situ, at that particular pressure (Figure 6).

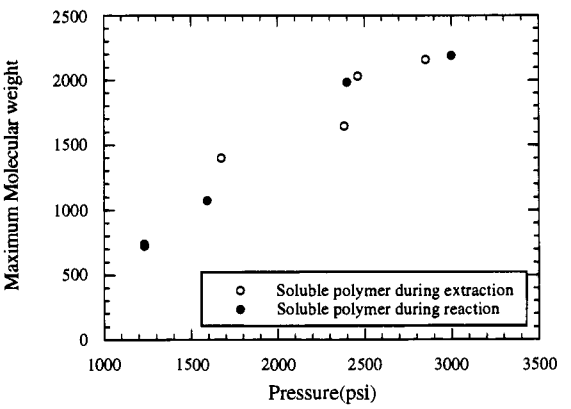

Figure 6. Comparison of molecular weights for extracted (soluble) polymer, and in-situ lipase-synthesized precipitated polymer. The data for this plot are taken from the experiment described by Table 1 (reaction) and Figure 4.

Conclusion

In conclusion, lipase-catalyzed polytransesterification in anhydrous ether and fluoroform were studied. The molecular

weights achieved in ether are limited by the equilibrium of the process. The molecular weight dispersities obtained are also lower than those obtained for a conventional step condensation reaction. Supercritical fluids have been used to separate low-dispersity polymer fractions from the enzyme-synthesized polymer. We have also demonstrated rational and predictable control of polymer dispersity and molecular weight during enzyme-catalyzed polyester synthesis in supercritical fluoroform.

Experimental Procedures

Materials. Porcine pancreatic lipase (crude, L-3126) was purchased from Sigma Chemical Co. (St. Louis, MO). Adipoyl chloride, trichloroethanol, tetrahydrofuran (THF) (HPLC grade), and 1,4-butanediol were purchased from Aldrich Chemical Co. (St. Louis). Anhydrous diethyl ether was purchased from Mallinckrodt (Paris, KY). Carbon dioxide was obtained from Liquid Carbonic, and fluoroform was a kind gift from Air Products. All solvents, substrates, and enzymes were used without further purification.

Methods. (1) Synthesis of Bis(2,2,2-trichloroethyl) Adipate. Bis-(2,2,2-trichloroethyl) adipate was synthesized by the alcoholysis of adipoyl chloride by trichloroethanol.[28] Typically 65.2 g (0.356 mol) of adipoyl chloride were added dropwise to a 250 mL three-necked round bottom flask containing 106 g (0.712 mol) of 2,2,2-trichloroethanol and 4−5 g of PolyDmap. (PolyDmap is a cross-linked styrenic polymer with pendant methylaminopyridine groups used to scavenge the liberated hydrogen chloride.) The reaction was allowed to continue for 24 h at 40 °C. PolyDmap was removed by filtration and the residual adipoyl chloride by washing with 10% sodium hydroxide. After rotary evaporation, the product was dried over anhydrous magnesium sulfate and crystalline product was obtained in yields of about 86%. The melting point of the product is 45−46 °C. ^{1}H NMR (in $CDCl_3$) confirms the structure of substrate: δ 1.8 ppm (4H, -CH_2CH_2), δ 2.5 ppm (4H, -CH_2COO), δ 4.76 ppm (4H, -CH_2CCl_3).

(2) Enzyme Preparation. Porcine pancreatic lipase was used directly after drying over phosphorus pentoxide for about 72 h to suppress potential hydrolysis of polyesters. Over this time period, water content is reduced from 2.25 wt % to 1.3 wt % as determined using a Karl-Fischer titrimeter. Hydrolysis was further suppressed by extensive drying of all solvents prior to use.

(3) General. The course of the reaction in anhydrous ether was studied in both batch and semibatch modes. In the batch system, all of the monomers and enzyme were added to the reactor at the initiation of the reaction, whereas for the semibatch experiment, the same final concentration of enzyme was achieved by adding the enzyme in small increments throughout the time course of the experiment. Samples were withdrawn periodically, and changes in molecular weight and polydispersity index with time were measured as described in detail below.

(4) Lipase-Catalyzed Batch Polyester Synthesis in Anhydrous Ether. In order to avoid the early termination of the polyester synthesis resulting from an excess of one of the bifunctional reacting species, equimolar (0.5 M) quantities of the starting materials were used. Typically, 10.225 g (0.025 mol) of bis(2,2,2-trichloroethyl) adipate, 2.25 g (0.025 mol) of 1.4-butanediol, and 10 g of crude porcine pancreatic lipase (0.4 g of enzyme/mmol diester) were added to 50 mL of anhydrous ether in a sealed reaction bottle. The reaction mixture was stirred continuously using a magnetic stirrer. Prior to sample withdrawal, the reaction vessel was refrigerated briefly to avoid the loss of the solvent due to evaporation. The reaction was stopped after 400 h. The reaction mixture was diluted with THF, and the enzyme was thoroughly washed with THF to prevent loss of any bound polymer. The enzyme was then removed by filtration, and after rotary evaporation, the final product was precipitated from dichloromethane using methanol. Yields of approximately 40% were obtained.

(5) Lipase-Catalyzed Semibatch Synthesis in Anhydrous Ether. Typically, 10.225 g of bis(2,2,2-trichloroethyl) adipate and 2.25 g of 1,4-butanediol were added to 50 mL of anhydrous ether in a sealed reaction bottle. Enzyme was added periodically (about 0.5 g every 20 h) over a period of 400 h. The reaction vessel was refrigerated briefly prior to enzyme addition and sample withdrawal. The same polymer purification strategy was used as described for the batch system.

(6) Supercritical Fluid Extractions of Biosynthesized Polyesters. Supercritical fluids were used to fractionate polymers synthesized as described above. A Hewlett-Packard supercritical fluid extractor was kindly donated for this purpose, and the extraction parameters are given below for each of the fluids used. It should be noted that the instrument is only designed for work with carbon dioxide, and modification of software is necessary before attempting any work with fluoroform.

(i) Carbon Dioxide. Approximately 100 mg of the commercially available poly(1,4-butylene adipate) was added to a 7 mL stainless steel extraction thimble capped at both ends. The sample was placed in CO_2 for 8 min and extracted at a flow rate of 1.0 mL/min for a period of 15 min. Both the equilibration and the extraction steps were carried out at 50 °C. Soluble polymer was deposited on an analyte trap packed with either (a) small stainless steel balls (0.36−0.43 mm diameter) or (b) Hypersil octadecyl silica (30−40 mm diameter). The trap was rinsed with tetrahydrofuran, and samples were collected in vials. The pressure was increased in steps, and the extraction was repeated. The soluble polymer fractions were collected in separate vials and analyzed using GPC.

(ii) Fluoroform. Around 100 mg of the commercially available polymer was extracted using fluoroform as described previously above for CO_2. The enzyme-synthesized polyester (100 mg) was placed in fluoroform for 10 min and extracted for 15 min at a flow rate of 1.0 mL/min with fluoroform. The soluble polymer fractions were recovered from the trap by rinsing with tetrahydrofuran.

(7) Lipase-Catalyzed Batch Polyester Synthesis in Fluoroform. Reactions in fluoroform were carried out in a high-pressure stainless steel reactor in batch mode. The reaction time was 5 days. The residues of reactions in supercritical fluoroform at different pressures were separated into soluble and precipitated fractions by extraction (after depressurization) using a Hewlett-Packard HP 7680A supercritical fluid extractor at the same pressure and temperature.

Molecular Weight Determination. Gel permeation chromatography (GPC) (Waters Model 150 CV) was used to determine the polymer molecular weight averages and the polydispersity index, as has been described previously for many enzyme-catalyzed polymer syntheses[10,24,29−31].

The instrument is equipped with a differential viscometer downstream of the columns (Styragel, pore sizes 500 and 1000 Å), which were placed in series. Tetrahydrofuran was used as the mobile phase solvent, and a calibration curve was obtained using 13 monodisperse polystyrene standards with molecular weights ranging from 162 to 28 500. Most of these standards (9 out of 13) had molecular weights in the range 162 to 7000. Differential viscometry requires accurate flow control (±10 μL/min, 1% of the total flow rate), which helps achieve good reproducibility and accuracy. The molecular weights given are subject to an experimental error of ±5%. Since the basis of separation by size exclusion is molecular size (hydrodynamic volume) and not molecular weight, a correction for the use of polystyrene standards was necessary. A Fisons Analytical LD-MS (TOFSpec) was used as follows. Typically, a mixture containing polyester, silver nitrate, and dithranol (matrix for polyester adsorption) was spotted onto a stainless steel disk. Laser-induced desorbed species were accelerated with a voltage of 25 000 V. The charged ions were analyzed using a time-of-flight analyzer.

Correction strategies for molecular weights of aliphatic polyesters, obtained from GPC include estimation of Mark−Houwink coefficients for the polymer−solvent system[32] or modification of the calibration curve by assignment of peak positions[33] for eluting oligomers. The

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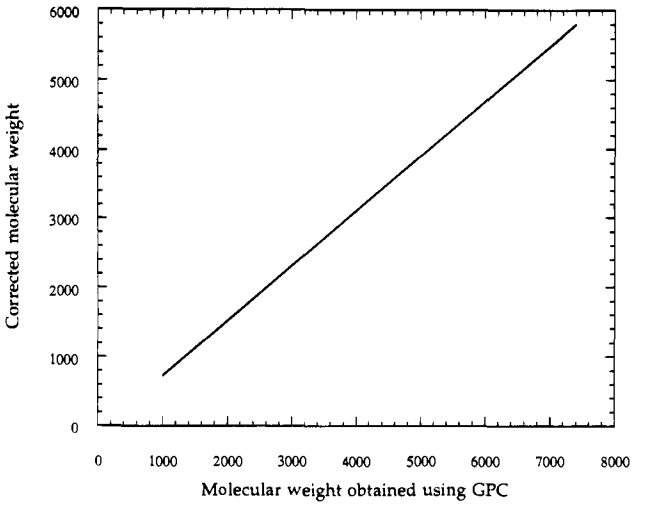

Figure 7. Correction curve for the molecular weights obtained using gel permeation chromatography using LD-MS analysis.

second method involves the risk that an impurity can be assigned a peak. Also, for this method to be effective, the separation of every oligomeric species is absolutely necessary. We therefore also estimated Mark–Houwink coefficients to correct the molecular weight averages (obtained from GPC) using LD-MS data. The correction curve for the molecular weight averages obtained using GPC is given in Figure 7.

Using LD-MS, one can estimate the number average molecular weight (M_n) of the polyester by calculating the centroid of the distribution[17]. Using M_n and the polydispersity index, we estimated the absolute M_w of *some of the* synthesized polyesters. All of these M_w values were around 22–25% less than that obtained using GPC. The basis of separation of GPC is hydrodynamic volume, which is equal to [*n*]M (intrinsic viscosity times the molecular weight). Hence a calibration curve of [*n*]M against retention time is a universal calibration curve. The values of M_w from LD-MS and from GPC, and Mark–Houwink coefficients of polystyrene were used to obtain Mark–Houwink coefficients for the synthesized polyesters using

$$k_1(M_{w_1})^{a_1+1} = k_2(M_{w_2})^{a_2+1}$$

where k_1 and a_1 are Mark–Houwink coefficients for polystyrene, M_{w1} and M_{w2} are weight average molecular weights calculated from GPC and LD-MS, respectively, and k_2 and a_2 are Mark–Houwink coefficients estimated for the synthesized polyester. The estimated values of Mark–Houwink coefficients for the polyester are $k_2 = 0.000319$ dL/g and $a_2 = 0.66$.

Having obtained Mark–Houwink coefficients of synthesized polyesters, values of M_w calculated from GPC are corrected *for all other* readings and are reported.

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