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Enzyme-catalyzed polymerizations at higher temperatures: Synthetic methods to produce polyamides and new poly(amide-co-ester)s

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ABSTRACT

Novozyme-435 (N-435) catalyzed polycondensation reaction between different aliphatic (oligo)esters and diamines by precipitation polymerization at elevated temperatures yields various particulate polyamides and poly(amide-co-ester)s. The reaction between diethylsebacate (DES) and 1,8-diaminooctane (DAO) at 60 °C in dried toluene leads to a low M_n polyamide (NMR: M_n = 520 g mol^{-1} GPC: M_n = 2000 g mol^{-1} with M_w/M_n = 1.3). An improved three-step procedure with an adjusted temperature profile and an optimized amount of enzyme in dried diphenyl ether, yields 97% amide bond formation and a polymer with increased molecular mass (NMR: M_n = 5380 g mol^{-1}, GPC: M_n = 4960 g mol^{-1} with M_w/M_n = 3.5). Three step ring-opening and polycondensation reactions between a cyclic ester (ethylene tridecanedioate) with three different diamines were also performed in dried toluene to obtain the corresponding polyamides. Here, diamine with higher alkyl chain length, i.e. 1,12-diaminododecane shows higher activity towards N-435 catalyzed amide bond formation and therefore the produced nylon-12,13 has higher molecular weight (GPC: M_n = 8250 g mol^{-1} and M_w/M_n = 5.8) compared to the other nylons. By adopting the three step synthetic protocol, a new series of poly(amide-co-ester)s with M_n up to 17,550 g mol^{-1} were produced. Melting and thermal degradation behaviors of these copolymers are compared with pure nylon-8,10 and polyester by DSC and TGA. The microstructure of the synthesized polyamides with different end groups was investigated by MALDI-TOF MS analysis while the particles' morphology was studied by TEM analysis.

1. Introduction

Polyamides, also called nylons, display improved physical properties compared with polyolefins and polyesters due to the directionally specific inter-chain hydrogen bonds and significantly enhanced melting points [1]. Nylons can be synthesized by several ways; for example in lab scale, interfacial polycondensation between hexamethylenediamine (HMD) and adipoyl chloride at room temperature leads to nylon-6,6 [2]. On a technical scale, it is usually produced by reacting adipic acid with HMD at higher temperature (>210 °C) [2]. The second most important polyamide nylon-6 is often formed by ring opening polymerization (ROP) of ε-caprolactam [3].

Since the past decade, enzymatic polymerization has been of growing interest as a new synthetic procedure for producing polymers [4,5]. The in vitro synthesis of polymers through enzyme catalysis has provided an innovative synthetic approach for making useful polymers, most of which are too complicated to be synthesized using conventional chemical catalysts. Furthermore, enzymatic polymerizations can be performed under mild reaction conditions without using toxic reagents. Therefore, in vitro enzymatic syntheses of polymers via non-biosynthetic pathways are recognized as a new area of precision polymer synthesis as well as providing a novel approach to accomplish green chemical technology.

Enzymes, especially lipases, are known for their low cost and great tolerance towards a wide range of unnatural substrates. Many enzymes require a full hydration shell to be active, however immobilized candida antarctica Lipase B (Novozyme 435) is known as an exception because it maintains its activity upon drying over phosphorus pentoxide [6]. Performing non-hydrolytic reactions in polymer synthesis with lipases in the presence of small amounts of water in order to maintain or improve the activity, will nearly always give rise to hydrolytic side reactions. The use of lipase under extreme reaction conditions, such as high pressure (10 MPa) and temperature (150 °C), has also been reported by Lozano et al.

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[7]. Moreover, lipases have been used in a number of different reaction media such as organic solvents [8], ionic liquids, super critical carbondioxide ($scCO_2$) [7] and also in heterophases such as miniemulsions [9]. In our present work, we have chosen to prepare some polyamides (e.g. nylon-8,10 and nylon-8,13) and new series of poly(amide-co-ester)s as model compounds to investigate the ability of lipases to work properly at reaction temperatures between 60 and 150 °C in an organic solvent (diphenylether and toluene). We are highly anticipating that our synthetic methodology will be further transferred to other reaction media such as $scCO_2$, ionic liquids, and biphasic reaction media.

Industrial processes for the nylon production are complicated since thermal degradation reactions lead to cyclization, changes in the balance of reactive end groups, evolution of gaseous degradation products, branching, and eventual gelation [10]. These chemical changes have a harmful effect on the final product quality in terms of processability, physical properties, and the presence of undesirable degradation products in the polymer. One of the main advantages to use an enzyme as a catalyst is to reduce the process temperature of nylons and eventually to prevent the undesired, above mentioned thermal degradation.

Much of the work carried out on lipase catalyzed polymerization is used to produce polyesters by three different polymerization modes such as (i) ROP of lactones, (ii) polycondensation of dicarboxylic acids or their derivatives with diols, and (iii) polycondensation of hydroxyacids or their esters [4,5]. Polymerization using dialkyl esters produced polyesters with relatively low molecular weight. For example, lipase catalyzed polycondensation of dimethyl succinate and 1,6-hexanediol in toluene yields M_n between 300 and 3000 g mol^{-1} [11]. Applying vacuum resulted in a shift of the equilibrium towards the polymer for the lipase-catalyzed polymerization of sebacic acid or its ethylester with 1,4-butanediol in diphenylether or veratrole ($M_w > 4 \times 10^4$ g mol^{-1}), although a long reaction time (>1 week) was required [12–14]. Lipase can also be used to catalyze aminolysis and ammonolysis reactions for the preparation of different amides and for the chiral resolution of esters, amines, and aminoalcohols in organic solvents [15]. Recently, we reported the lipase catalyzed amidation of lactones via the hydrolysis product with a series of amines in aqueous miniemulsion [9]. Gutman et al. [16] reported the intramolecular aminolysis of aminoesters catalyzed by lipase in organic solvents to form lactams. The authors observed some uncyclized oligomers while making macro-size bislactams from diamine and diester.

Novozyme 435 catalyzed polycondensation reaction between dimethyl adipate, diaminosiloxane and 1,8-octanediol was reported by Sharma et al. [17] to synthesize organosilicone polyesteramide. Gu et al. [18] reported the lipase catalyzed synthesis of water soluble poly(aminoamide) and their derivatives in bulk at 50–100 °C using dimethylesters of (phenyl)malonic/fumaric/adipic acids and diethylenetriamine/triethylene glycol diamine as monomers.

However, there is to the best of our knowledge not much known to obtain high melting point (~130–200 °C) polyamides and poly(amide-co-ester)s from polycondensation reactions between diesters with alkyl diamines using enzyme as a catalyst. This could be attributed to the following two reasons: (1) even short oligoamides have a melting point (T_m) near to the corresponding polymer, e.g. T_m of 3-amide nylon-6,6 oligomer is 236 °C thus, intermediates of the reaction solidify before growing to long chains [19]; (2) the polyamides can only be dissolved in strongly acidic solvents such as concentrated H_2SO_4, trifluoroacetic acid, hexafluoroisopropanol, tetrafluoroethanol, and metal salt–solvent mixtures (LiCl in CH_3OH) [20]. Enzymes will normally loose their catalytic activity at high temperatures and they are deactivated by these acidic solvents. Literature reports on the influence of the temperature on the polymerization kinetics, conversion, and molecular weight of lipase catalyzed ROP forming polyesters reveal that the activity of the lipase depends on the substrate as well as on the reaction conditions [5,21–24]. Therefore, temperature dependence of the lipase's catalytic activity needs to be independently determined for each system.

In this paper, we present two and three step synthetic methods to produce different polyamides such as nylon-8,10, nylon-8,13, nylon-6,13, and nylon-12,13 using the lipase Novozyme 435 as a biocatalyst. Kinetic studies were performed to demonstrate the mechanistic aspects of lipase catalyzed polycondensation reactions between 1,8-diaminooctane (DAO) and diethyl sebacate (DES) and to predict the reaction rate. Single step polycondenation reactions were performed between DES and DAO at different temperatures (60–150 °C) to investigate the N-435 activity at different temperatures. In addition, an enzymatic method to produce a new series of poly(amide-co-ester)s is also reported for the first time. The analytical characterization was performed by ^{1}H NMR spectroscopy, GPC, DSC, TGA, MALDI-TOF MS, and TEM.

2. Experimental

2.1. Materials

The monomers diethyl sebacate (DES), ethylene tridecanedioate (ETD), 1,8-diaminooctane (DAO), deuterated trifluoroacetic acid (TFA-d1), diphenylether, and toluene were purchased from Aldrich. 4 nm pore sized molecular sieves and trifluoroacetic acid (TFA) were bought from Acros. Methanol was supplied by Fischer Scientific. 15-Pentadecanolide (PD), 1,6-diaminohexane (DAH), and 1,12-diaminododecane (DADD) were procured from Fluka. Lipase PS (*Pseudomonas cepacia*), Lipase G (Penicillium Camemberti), and Novozyme 435 (N-435) (*candida antarctica*) were purchased from Amano. Lipase from pig pancreas was supplied by Fluka. Lipase A from *candida antarctica* and Lipase from *Pseudomonas fluorescens* were purchased from Fluka.

Toluene was first pre-dried with neutral alumina. Then, it was dried over CaH_2 and vacuum distilled. Diphenyl ether was dried over sodium and vacuum distilled. Molecular sieves were dried using vacuum (~10^{-2} mmHg) and applying frequent heat using a heat gun for 6 h. All other chemicals were used without further purification.

2.2. One step synthesis of nylon-8,10 with N-435 as a catalyst

10 mL of dried toluene or toluene with a few drops of water were added to a mixture of N-435 (0.20 g), DES (1.3 g, 5 mmol), DAO (0.72 g, 5 mmol) and in the case of the reactions performed in dried toluene ~1 g of dried molecular sieves. This mixture was stirred in a vial at 60 °C for 48 h. After 48 h, the toluene was removed in vacuum. The samples were analyzed by ^{1}H NMR spectroscopy and GPC.

2.3. Kinetics of the polycondensation reaction of nylon-8,10 at 100 °C

10 mL of dried diphenyl ether was added to a mixture of N-435 (0.20 g,), DES (1.3 g, 5 mmol), DAO (0.72 g, 5 mmol), and dried molecular sieves (~1 g). The mixture was stirred at 100 °C and 100 mmHg pressure. A small amount of sample was withdrawn from the reaction mixture during the course of the reaction (at 0.17, 0.5, 1, 3, 6, 10, and 24 h) and analyzed by ^{1}H NMR spectroscopy using TFA-d1 as solvent. The concentrations of the reactants [*A*] compared to the product (amide bond) were calculated with respect to reaction time by ^{1}H NMR analysis.

2.4. Two step enzymatic synthesis of nylon-8,10

10 mL of dried diphenyl ether was added to a mixture of N-435 (0.20 g), DES (1.3 g, 5 mmol), DAO (0.72 g, 5 mmol) and dried molecular sieves (~1 g). The following sequence of reaction conditions was performed: (i) 60 °C for 20 h at 500 mmHg pressure, (ii) elevated temperature (90, 100, 110, 120, or 130 °C, respectively) at 100 mmHg pressure for 24 h. After polymerization, the reaction mixture was added to 100 mL of methanol and filtered. The solid particles were analyzed by ^{1}H NMR and GPC.

2.5. Three step enzymatic synthesis of nylon-8,10

10 mL of dried diphenyl ether was added to a mixture of N-435 (0.4 g), DES (1.3 g, 5 mmol), DAO (0.72 g, 5 mmol), and dried molecular sieves (~1 g). The subsequent series of reaction conditions was performed: (i) 60 °C for 20 h at 500 mmHg pressure, (ii) 100 °C for 24 h at 100 mmHg, (iii) 110, 120, 130 °C (3 h at each temperature), and 150 °C for another 12 h at 100 mmHg. After polymerization, the reaction mixture was treated as described in the previous section.

2.6. Three step enzymatic synthesis of nylon-8,10 with discontinuous vacuum

A synthetic procedure similar to Section 2.5 was followed except for the continuous vacuum. Here, 100 mmHg vacuum was applied only for 10 min in total 10 times during the second and third step of polymerization.

2.7. N-435 catalyzed ring-opening polymerization of cyclic diester ETD

A mixture of dried toluene (5 mL), N-435 (0.10 g), ETD (1.3 g, 5 mmol), and about 0.5 g of dried molecular sieves was stirred in a Schlenk tube at 90 °C for 24 h in argon atmosphere. After 24 h, the toluene was removed and the sample was analyzed by ^{1}H NMR and GPC.

2.8. N-435 catalyzed ring-opening polymerization of PD

A mixture of dried diphenylether (5 mL), N-435 (0.10 g,), PD (1.2 g, 5 mmol), and about 0.5 g of dried molecular sieves was stirred in a Schlenk tube at 90 °C for 24 h in argon atmosphere. After 24 h, the reaction mixture was dissolved in $CHCl_3$ and re-precipitated by methanol. The sample was analyzed by DSC and TGA.

2.9. Two step enzymatic synthesis of nylon-8,13

10 ml of dried toluene was added to a mixture of N-435 (0.20 g), ETD (1.35 g, 5 mmol), DAO (0.72 g, 5 mmol), and dried molecular sieves (~1 g). This mixture was magnetically stirred in a vial with 500 rpm speed. The following sequence of reaction conditions was performed: (i) 60 °C for 5 h, (ii) 80 °C for 20 h. After the polymerization reactions, the toluene was removed under vacuum. The samples were analyzed by ^{1}H NMR spectroscopy, GPC, and MALDI-TOF MS.

2.10. Three step enzymatic synthesis of nylon-6,13, nylon-8,13, and nylon-12,13

10 mL of dried toluene was added to a mixture of N-435 (0.20 g), ETD (1.35 g, 5 mmol), DAH/DAO/DADD (0.58/0.72/1 g, 5 mmol), and dried molecular sieves (~1 g) in a round bottom flask connected to a reflux condenser. This mixture was magnetically stirred in a vial with 500 rpm speed. The following sequence of reaction conditions was performed: (i) 60 °C for 5 h, (ii) 100 °C for 20 h, (iii) 110, 120, 130 °C (3 h at each temperature), and 140 °C for 12 h. After polymerization, the toluene was removed and the samples were analyzed by ^{1}H NMR spectroscopy.

2.11. Three step enzymatic synthesis of poly(amide-co-ester)s

In a first step, one of the following combinations of monomers (i) PD (1.2 g, 5 mmol) and DAO (0.72 g, 5 mmol), (ii) PD (1.2 g, 5 mmol) and DADD (1.0 g, 5 mmol), and (iii) PD (1.2 g, 5 mmol), DAO (0.36 g, 2.5 mmol) and DADD (0.5 g, 2.5 mmol), dried molecular sieves, and N-435 (0.2 g) were mixed with 10 mL of dried diphenyl ether and stirred at 90 °C for 20 h. During a second step the other co-monomer, i.e. DES (1.3 g, 5 mmol) and N-435 (0.2 g) were added to the reaction mixture. The reaction temperature was increased to 110 °C for another 24 h. In a third step, the reaction temperature was further increased to 120 °C (3 h) and 130 °C (12 h) at 100 mmHg pressure for 12 h. Without further purification the samples were analyzed by ^{1}H NMR spectroscopy to determine M_n.

Nylon-8,10 and poly(amide-co-ester)s were purified by re-precipitation method (solvent: TFA, non-solvent: methanol) and analyzed by DSC and TGA.

2.12. Analytical techniques

^{1}H NMR spectroscopy was performed on a Bruker DRX400 with TFA-d1 as solvent and the obtained signals are referenced to tetramethylsilane (δ = 0.00 ppm). The molecular weight M_n was calculated by using the following equations:

$$M_n = 310 \times \left[\frac{I_{-CH_2-NH-CO-}}{\sum I_{\text{end groups}}}\right] [\text{g mol}^{-1}] + M_{\text{end group}}[\text{g mol}^{-1}]$$

where 310 is the molecular weight of the repeating unit in g mol^{-1} and I the intensity of the signal. The end group molecular weight $M_{\text{end group}}$ has been calculated by the following equation:

$$M_{\text{end group}} = M_{\text{amine}} \times \text{fraction amine} + M_{\text{ester}} \times \text{fraction ester} + M_{\text{acid}} \times \text{fraction acid}$$

where M_{amine}, M_{ester}, and M_{acid} are the molecular weights of the monomers. Fraction is obtained from the ratio of end group signals by ^{1}H NMR analysis.

Polymer molecular weights and their distribution of the synthesized polyamides were determined by gel permeation chromatography (GPC) in hexafluoroisopropanol with 0.05% trifluoroacetic acid-potassium salt solution using a combination of three different columns (PL HFIPgel Precolumn, HTS PL HFIPGel, and PL HFIPgel) at 40 °C. The flow rate was maintained at 1.0 mL min^{-1}. Detection was performed with a differential refractometer Agilent G1362A. Calibration was done by narrowly distributed poly(methyl methacrylate) standards supplied by PSS.

Polymer molecular weights and their distribution of the synthesized polyester [i.e. poly(ethylene tridecanedioate)] were determined by GPC in tetrahydrofuran with a combination of three different columns (SDV 10e6 Å, SDV 10e4 Å, and SDV 500 Å) at 30 °C. The flow rate was maintained at 1.0 mL min^{-1}; the detection was achieved by an ERC differential refractometer. Calibration was performed by narrowly distributed polystyrene standards supplied by PSS.

Differential scanning calorimetry (DSC) measurements were carried out on a Mettler Toledo DSC823 in the scanning mode. Sample masses were in the range of 10–20 mg. Cooling and heating rates of 10 K min^{-1} were used for all measurements.

The thermal stability was measured by thermal gravimetrical analysis (TGA). The samples were analyzed by a Mettler Toledo TGA 851 apparatus under a nitrogen atmosphere and a heating rate of 10 °C min^{-1}.

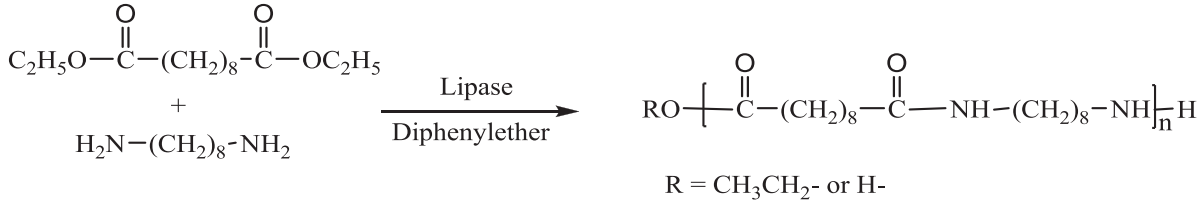


Fig. 1. Lipase catalyzed polycondensation reaction between DES and DAO.

MALDI-TOF MS was performed on a Bruker Biflex III spectrometer at an acceleration voltage of 19 kV, accumulating 100 laser shots in the reflectron mode. Samples were prepared mixing the matrix 2-(4-hydroxy-phenylazo)benzoic acid (HABA) as a 20 g L^{-1} solution in 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) 1:1 with a 2 g L^{-1} solution of the respective polymer in HFIP. Sodium trifluoroacetate was added for cationization. The masses of the oligomers (nylon-8,10) correspond to $M = x + (n \times 310.3\ \text{Da}) + Na^+$, where n is the degree of polymerization, x is the mass of end groups (46.0 Da for amine/ester, 18.0 Da for amine/acid, 144.2 Da for amine/amine and 258.2 Da for ester/ester). From masses at the peaks the corresponding degrees of polymerization can be calculated, for example: $n = 4$ (with amine/ester end group): 1310.1 Da; $n = 4$ (with amine/acid end group): 1282.1 Da; $n = 4$ (with amine/amine end group): 1408.4 Da; $n = 3$ (with ester/ester end group): 1212.1 Da (Table S1). Similarly, molecular weights corresponding to nylon-8,13 oligomers can also be calculated (Table S2).

Transmission electron microscopy (TEM) was performed with a TEM Zeiss 902 operating at 80 kV. The synthesized polyamide sample was redispersed in water and 0.25 μL of the dispersion was applied to a 400 mesh carbon-coated copper grid and left to dry; no further contrasting was applied.

3. Results and discussion

3.1. N-435 catalyzed polycondensation reactions in toluene

Two N-435 catalyzed reactions between DES and DAO (see Fig. 1) were carried out at 60 °C in dried toluene (nylon-8,10a) as well as in toluene with a few drops of water (nylon-8,10b) for a reaction time of 48 h. After the reaction time, the purified samples were analyzed by ^{1}H NMR using TFA-d1 as a solvent. The ^{1}H NMR of DEDAO-T1 is given in Fig. 2 with the peak assignments. The signals at 3.6 and 2.8 ppm (—**CH**$_2$—NH—CO— and —NH—CO—**CH**$_2$) corresponding to the amide group, indicate the product formation. Interestingly, ^{1}H NMR clearly shows the three different end groups CH_3—**CH**$_2$—O—CO— (4.3 ppm), H_2N—**CH**$_2$— (3.3 ppm), and HOOC—**CH**$_2$— (2.6 ppm). M_n of nylon-8,10a is calculated (see Section 2) to be 520 g mol^{-1}. In the other reaction, i.e. nylon-8,10b, the addition of a small amount of water in order to enhance the activity of N-435 for the amide formation leads to hydrolysis of the ester yielding the corresponding acid predominantly, which immediately forms a salt with the amine. Therefore, the reaction does not yield even oligoamides.

By increasing the reaction temperature and applying vacuum in order to remove the byproducts H_2O and CH_3CH_2OH, the equilibrium of the polycondensation reactions can be shifted towards the amide formation and thus the molecular weight of the polymers can be increased [8]. In some cases, molecular sieves which adsorb the byproducts, were also used to increase the molecular weight of polyesters synthesized using lipase as a biocatalyst [25]. The next following sections are committed to fully understand this enzymatic polycondensation reaction especially at different reaction temperatures with reduced pressure to deliver a modified reaction protocol to further increase M_n of the polyamides.

3.2. N-435 catalysis at different reaction temperatures

In literature, N-435 catalyzed polymer forming reactions have been reported to be performed maximum at ~100 °C [5,18,24]. However, a process of kinetic resolution of *rac*-1-phenylethanol with vinyl propionate [26] has been reported at 120 and 150 °C in both ionic liquids and $scCO_2$. Here, the increase in temperature from 120 to 150 °C only produced a slight decrease in enzymatic

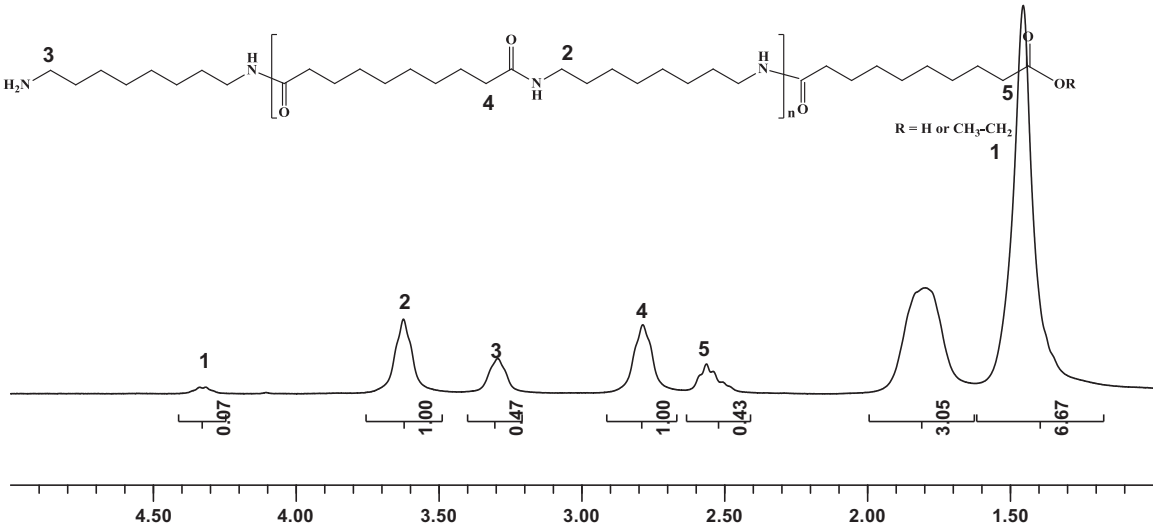


Fig. 2. ^{1}H NMR spectrum of nylon-8,10 synthesized in dried toluene (nylon-8,10a).

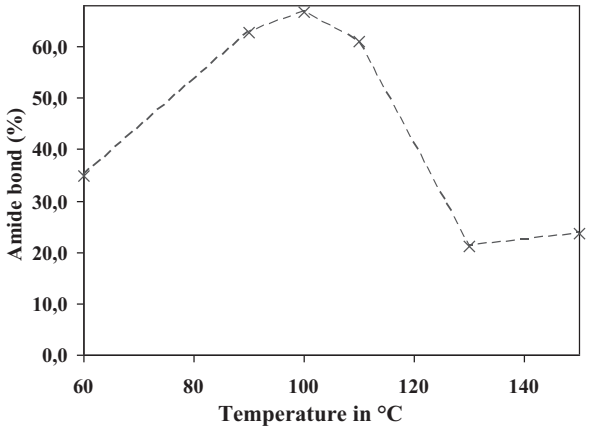


Fig. 3. Single step N-435 catalyzed polycondensation reactions between DES and DAO at different reaction temperatures; formation of amide bonds; the dashed line is added as guide for the eye.

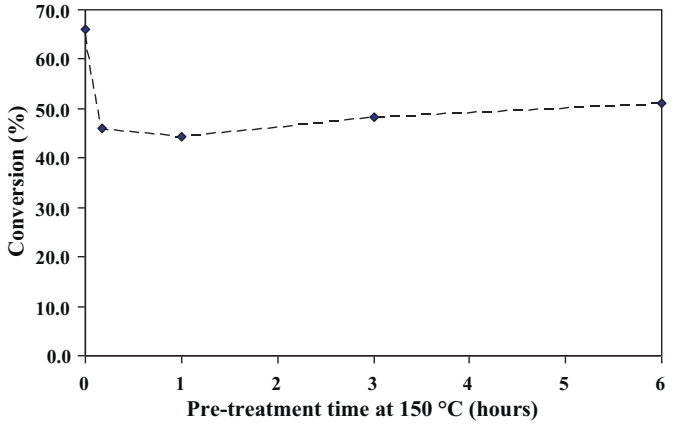


Fig. 4. Pre-treating time of the enzyme and monomers at 150 °C versus amide bond formation between DES and DAO at 100 °C; the dashed line is added as guide for the eye only.

activity, but the maximum level of enantiomeric excess for product purity was observed at 150 °C [26].

In order to reveal the optimum temperature range with respect to maximum enzymatic activity and highest molecular weight, N-435 catalyzed single step polycondensation reactions between DES and DAO were performed at different temperatures between 60 and 150 °C for 24 h with 100 mmHg vacuum in dried diphenyl ether.

Among the temperatures studied, the maximum conversion was obtained at 90–110 °C with ~65% ester into amide bond conversion (Fig. 3). At 60 °C, the enzyme yields the conversion of 35% ester into amide bonds. Interestingly, even at 150 °C, the enzyme still shows activity with 24% amide bond formation. At the same time, the non-enzymatic reaction shows only 4% amide product formation. Thus, the formation of the amide bonds is significantly promoted by the enzyme and not by the high temperature.

To further ensure that the catalytic activity of N-435 is maintained up to 150 °C, a series of control experiments were performed. Here, the reaction mixture with enzyme and monomers was first stirred at 150 °C for a defined time and after quenching with liquid nitrogen stirred at 100 °C for another 24 h. All these experiments were carried out with 100 mmHg vacuum for the removal of the byproducts ethanol and water. ^{1}H NMR analysis was used to calculate the ester into amide bond conversion (Fig. 4). It should be noted that pre-heating the reaction mixture at 150 °C should also lead to the formation of amide bonds to a certain extent (maximum 24% after 24 h heating, see above).

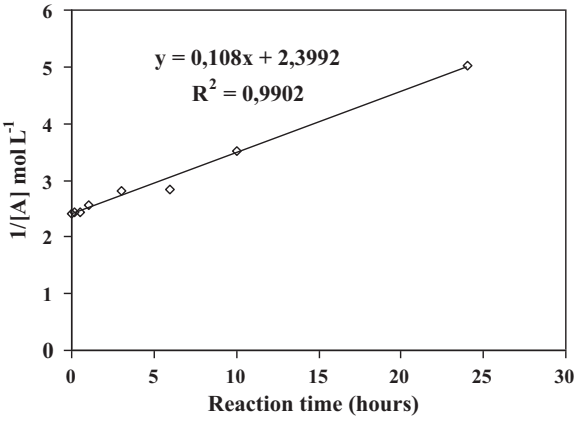


Fig. 5. Kinetic investigation of polycondensation reaction between DES and DAO at 100 °C.

Even, pretreating the enzyme up to 6 h at 150 °C without the presence of any substrate, the enzyme displays a significant catalytic activity at 100 °C with ~50% amide bond formation. This shows that the enzyme stays still active even after treatment at this higher temperature.

The kinetics of the N-435 catalyzed polycondensation reaction between DES and DAO was investigated at 100 °C at 100 mmHg vacuum. Gross et al. [5] reported that the kinetics of PD polymerization in bulk varies from first to second order at different stages of the reaction, i.e. 3–13% and 13–40% consumption of the monomer, respectively. The fact that the concentration of polymer chains increased [i.e. the concentration of —OH and —COOH increases] with increasing monomer (PD) conversion may explain the increased sensitivity of the reaction rate to monomer concentration as the reaction progresses.[5] A plot of $1/[A]$ (where $[A]$ is the average concentration of the reactants) versus reaction time shows linear dependence with a rate constant of $k = 0.1077\ \mathrm{L\,mol^{-1}\,h^{-1}}$ (Fig. 5), which clearly indicates a second order reaction in accordance with the experiments of Gross et al. [5] at high monomer conversion. In our case the presence of two different monomers (DES and DAO) in the rate determining step makes the polymerization rate to follow the second order directly from the beginning.

NMR experiments during the kinetic investigation clearly show that no acid product (absence of signal at 2.6 ppm) is formed during the course of the reaction, therefore the amine directly attacks the enzyme activated monomer (EM) and there is no initiation by water which is in accordance with the proposed reaction mechanism [5,8] for lipase catalyzed polymerization where first an acyl-enzyme intermediate EM is formed in the rate determining step.

3.3. Two step polycondensation reactions in diphenyl ether

In order to maximize the conversion of the ester and the amine groups to amide bonds resulting in higher molecular weight (i) a higher reaction temperature and (ii) lower pressure should be advantageous as both will lead to a more efficient removal of the volatile byproducts. On the other side care has to be taken for the optimum temperature range for the enzymatic activity and the volatility of the monomers (especially DAO). Recently, Gross et al. [24] successfully performed N-435 catalyzed polycondensation reactions to produce low melting temperature silicone containing polyesteramides with M_n up to 13,360 g mol^{-1}. Here, the authors used two stage polymerizations (step 1: ambient pressure and 70 or 80 °C; step 2: 2 mmHg pressure and 95 °C). In this case, the produced polymers show low melting points (<50 °C) and are observed to be even sticky at room temperature if one of the co-monomers [α,ω-(diaminopropyl)polydimethylsiloxane]

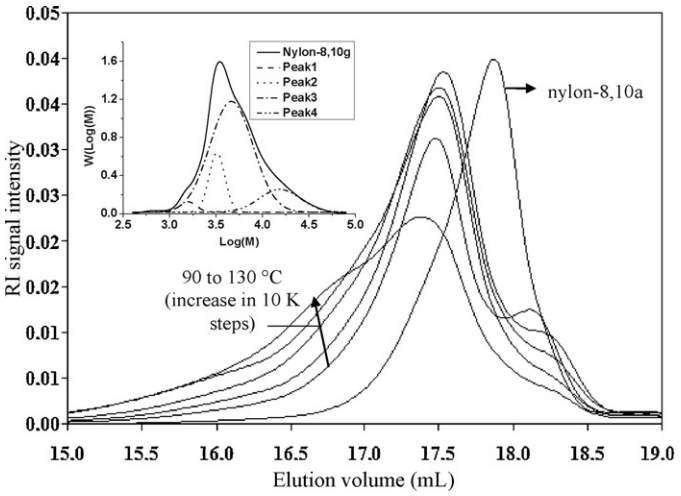


Fig. 6. GPC chromatograms of purified nylon-8,10 using two-step and single-step polymerizations. The inset shows exemplary the calibrated chromatogram of nylon-8,10g with four subpeaks.

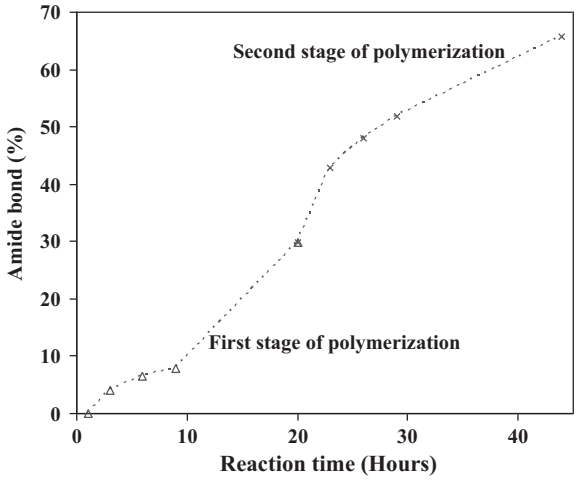


Fig. 7. Conversion of ester into amide bonds observed from N-435 catalyzed two stage polycondensation reactions between DES and DAO; (△) first step (60 °C and 500 mmHg), (×) second step (130 °C and 100 mmHg); the dashed line is shown as guide for the eye.

is ≥50 mol%. In our case, the synthesized nylon-8,10 displays a much higher melting transition (200 °C). However, a temperature and vacuum modified two-stage protocol for the N-435 catalyzed polycondensation reactions between DES and DAO might be also advantageous to increase the molecular weight of nylon-8,10 significantly compared to the single step polymerization (Section 3.1). In a first step, the reaction mixtures were stirred at 60 °C and 500 mmHg pressure for 20 h and in the second step at 90 °C and 100 mmHg for 24 h. The end group analysis of the purified sample shows a slightly increased molecular weight of M_n = 890 g mol^{-1}; the polymer contains predominantly amine end groups (72%) with 9 and 19% of ester and acid end groups, respectively.

An increase of the temperature up to 130 °C in the second step led to an increase of the molecular weight of nylon-8,10 to M_n = 1240 g mol^{-1} (Table 1). Although stoichiometric amounts of DES and DAO were used in all reactions, the purified nylon-8,10 contains predominantly amine end groups (∼75%) and only low amounts of the ester and acid end groups (∼10% and ∼15%, respectively; see Table 1). While performing kinetic investigations by ^{1}H NMR spectroscopy in TFA-d1 (see Section 2.3), there is no signal at 2.6 ppm, which corresponds to the acid product. Therefore, the formation of the hydrolysis product, the acid, might be attributed to the moisture from the workup with non-dried methanol.

To differentiate potential cyclization and linear growth [4,16], which both affect the end group analysis similarly, all the samples were analyzed by GPC in hexafluoroisopropanol (Fig. 6). The number average molecular weights (M_n) and the polydispersities are given in Table 1. Nylon-8,10 synthesized in dried toluene at 60 °C clearly shows the monomodal distribution at 17.8 min (corresponding to M_n = 2000 g mol^{-1}). However, the polymers synthesized from a two-step temperature and vacuum varied method show a bimodal distribution. With increasing the reaction temperature in the second step from 90 to 130 °C, a decrease of the peak intensity corresponding to M_n = 2000 g mol^{-1} was observed. At the same time, an increase of the peak intensity at 17.3 min (corresponding to M_n = 3000 g mol^{-1}) was found. In addition, a shoulder appears at 16.7 min (corresponding to M_n = 6000 g mol^{-1}) and its intensity increases for the polymers synthesized at high temperatures. Since the chromatograms clearly indicate the increase of hydrodynamic radii of the polyamides synthesized at higher reaction temperatures in the second step of the polymerization, the apparent increase of M_n derived from ^{1}H NMR analysis cannot predominantly be caused by the formation of cyclic oligo-amides. Along with the increase of the reaction temperature in the second step of the polymerization and the subsequent increase of molecular weight, the polydispersity values also increase from 1.3 to 1.7 as expected for a step-growth polymerization.

Since the chromatograms show multimodal peaks they were deconvoluted with up to four subpeaks using OriginPro8.1 SR3 software. The respective peak positions (calibrated against PMMA standards) and fractional areas in percent are given in Table 1. An exemplary deconvoluted elugram is shown as inset in Fig. 6. The deconvolution reveals clearly that there is a fraction with M_p centered at around 1500 g mol^{-1} (2000 g mol^{-1} for nylon-8,10a) which keeps its position but decreases steadily from 96% to 1% with increasing T_{react}. A similar trend is found for the second peak at around 3000 g mol^{-1}, going through a maximum with increasing reaction temperature. The third and fourth peak increases in M_p with increasing T_{react}. It shall be noted that the development of the peak positions give only the tendency of the development of the average molecular weight of each peak but do not directly reflect M_n or M_w.

It is worth mentioning that the high reaction temperature does not deactivate the enzyme N-435 (see above); other enzymes as Lipase PS from Pseudomonas cepacia, Lipase G, Lipase from hog pancreas, Lipase from pseudomonas fluorescens, or Lipase A from candida antarctica, do not lead to a significant amount of polymer but rather showed degradation of the enzymes at elevated temperature as indicated by a color change of the reaction mixture. ^{1}H NMR analysis of these samples revealed only minor amounts of amide bonds with a maximum for Lipase A from candida antarctica at ∼8%. The reactions performed without enzyme even at 150 °C during the second step yield only 4% amide. These findings identify the enzyme N-435 is the preferred candidate of those studied enzymes for polycondensation reactions between DES and DAO even at high temperatures.

In order to get insight to the time-dependent evolution of the polymer formation and to get a better understanding of the high amount of amine end groups of the purified polymer, a kinetic investigation on polycondensation reactions between DES and DAO was performed. The corresponding reaction conditions were maintained (first step 60 °C and second step 130 °C). A small amount of the sample was removed from the reaction mixture during the course of the reaction and dissolved in TFA-d1 and analyzed by ^{1}H NMR. The conversion of ester into amide bond was calculated and is shown in Fig. 7.

Table 1
Molecular weight and end group characterization of nylon-8,10 by ^{1}H NMR spectroscopy and GPC (with respect to PMMA standards, subpeaks from deconvolution) of purified nylon-8,10 samples obtained in one-step and two-step temperature reactions.

Sample	T_{react} (2nd step) (°C)	M_n (g mol^{-1})[a]	Content of end groups (%)[a]			Molecular weight (g mol^{-1})[b]					M_w/M_n [b]
			Amine	Ester	Acid	M_n	M_p [c] (fractional peak area in %)				
							Peak 1	Peak 2	Peak 3	Peak 4	
Nylon-8,10a	–	520	52	8	40	2000	2040 (96)	3320 (4)	–	–	1.3
Nylon-8,10c	90	890	72	9	19	2500	1500 (5)	3020 (11)	3300 (57)	6800 (27)	1.5
Nylon-8,10d	100	940	79	11	11	2900	1560 (2)	2970 (19)	3380 (49)	4410 (30)	1.5
Nylon-8,10e	110	1000	76	8	16	3100	1650 (3)	3040 (30)	3900 (47)	8470 (20)	1.6
Nylon-8,10f	120	1010	70	14	16	3500	1670 (1)	3030 (16)	3860 (32)	6400 (51)	1.7
Nylon-8,10g	130	1240	72	10	17	3900	1580 (1)	3240 (5)	4630 (76)	15,370 (18)	1.7

[a] From ^{1}H NMR analysis.
[b] From GPC.
[c] By deconvolution.

During the first stage of polymerization (60 °C and 500 mmHg pressure), after 1 h inhibition time, ^{1}H NMR analysis shows that 20 h reaction time lead to 30% ester consumption yielding nylon-8,10 in the performed N-435 catalyzed reaction. Increasing the reaction temperature and applying a higher vacuum (130 °C and 100 mmHg pressure) during the second stage polymerization increased the consumption of the reactants (DAO and DES) yielding the amide bonds up to 66% conversion compared to unreacted ester after 24 h. In addition, ^{1}H NMR analysis is indicating that about 19% DAO (∼1 mmol) was removed due to vacuum (which was necessary to remove the byproducts water and ethanol) during the second step of polymerization. Hence, a further modification of the protocol to increase the molecular weight of nylon-8,10 without removing significant amounts of monomer by vacuum is required.

3.4. Three temperatures step polymerizations with higher amount of enzyme in diphenyl ether

A further increase of the molecular weight can be obtained if in an early stage of the reaction a high amount of DAO reacts with DES, so that the resulting oligoamide cannot be removed by vacuum and if the high conversion at 100 °C is used. Therefore, in order to increase the high conversion in the first step of polymerization and at the same time to decrease the DAO evaporation, the amount of N-435 was doubled (i.e. 400 mg). Additionally, the temperature was adjusted in three steps: (i) 60 °C for 20 h with 500 mmHg vacuum, (ii) 100 °C for 24 h and the pressure reduced to 100 mmHg, and (iii) stirring the reaction mixture for 3 h at each of the following higher temperatures, i.e. 110, 120, and 130 °C and finally increasing to 150 °C for another 12 h upholding the same reduced pressure. ^{1}H NMR analysis of the reaction mixture after the first step of reaction shows that 56% of ester was converted into amide bond, which is 1.9 times higher than what we achieved with 200 mg of N-435 during two step reactions (see previous section, Fig. 7). After the second and third steps of the reaction, 77 and 89% of ester is observed to be converted into amide bonds, respectively. After three steps, ^{1}H NMR analysis of a purified sample (nylon-8,10h) shows M_n = 2120 g mol^{-1} while GPC analysis gives M_n = 4650 g mol^{-1} with a polydispersity of 1.7. The molecular weight is significantly higher compared to what was achieved under two step reaction conditions. Still, 9% of DAO was removed by vacuum.

To completely avoid the DAO evaporation and subsequently to increase M_n further, a similar three step reaction was performed with non-continuous vacuum. In this reaction, 100 mmHg vacuum was used for 10 min in total 10 times during second and third step of polymerization. After the third step, 97% of ester was converted into amide bonds and the purified sample (nylon-8,10i) gives M_n = 5380 g mol^{-1}. GPC examination of pure nylon-8,10 gives a M_n of 4960 g mol^{-1} with a polydispersity M_w/M_n of 3.5.

From the conversions obtained from ^{1}H NMR analysis, the number of repeating units in synthesized nylon-8,10i was calculated using Carothers equation (X_n = 16), from which the calculated M_n is 4960 g mol^{-1} (excluding the end group molecular weight). This is in good agreement with the M_n observed from GPC measurement.

3.5. Enzyme N-435 catalyzed ring-opening and polycondensation reactions

Ethylene tridecanedioate (ETD) is a cyclic diester, which was polymerized in bulk using lipase as a catalyst by Müller et al. [27] The authors were able to synthesize polyesters up to M_n = 4100 g mol^{-1}; the structure of this polyester was the same as that from dicarboxylic acids (or their derivatives) and ethylene glycol. The same monomer in dried toluene using enzyme N-435 as a catalyst at 90 °C for 24 h, yields a polyester with $M_n = 51.8 \times 10^3$ g mol^{-1} and a polydispersity of 2.94. The increase of the molecular weight might be due the fact that the solution polymerization increases the oligomeric/polymeric chain mobility, which allows the reaction between the end groups of the oligomers/polymers repeatedly.

ETD might act as a diester if it is polymerized with equal molar amounts of a diamine because of higher reactivity of the amine compared to the hydroxy group and therefore the reaction produces polyamides (Fig. 8). The enzyme N-435 catalyzed ring opening and polycondensation of ETD and DAO in dried toluene was performed to obtain the corresponding polyamide, i.e. nylon-8,13. The reaction was initially started at 60 °C, after 2 h the reaction mixture starts to precipitate. After 5 h, the reaction temperature was increased to 80 °C and continued for another 20 h. ^{1}H NMR analysis confirms the product formation and shows that 75% of ester was converted into amide. This is a relatively higher conversion compared to the percentage of amide bond formation for any of the two step polycondensation reactions between DES and DAO. The end group analysis of this polymer to determine M_n by ^{1}H NMR analysis is complicated due to ambiguity in identifying the end groups (—O—CH_2—CH_2—O—, —CH_2—CH_2—OH and OH—CH_2—CH_2—OH). GPC analysis of this polyamide (nylon-8,13a) in HFIP shows M_n = 2200 g mol^{-1} with a polydispersity of 1.8.

As seen from the N-435 polycondensation reactions, the conversion of ester into amide bond are efficiently increased using multi step polymerization at different reaction temperatures. Therefore, ring-opening and polycondensation reactions of ETD with different diamines such as 1,6-diaminohexane (DAH), DAO, and 1,12-diaminododecane (DADD) were also performed using the three step reaction conditions (60 °C for 5 h, 100 °C for 20 h, 140 °C for 20 h) with 200 mg N-435. ^{1}H NMR analysis shows that 90, 87,

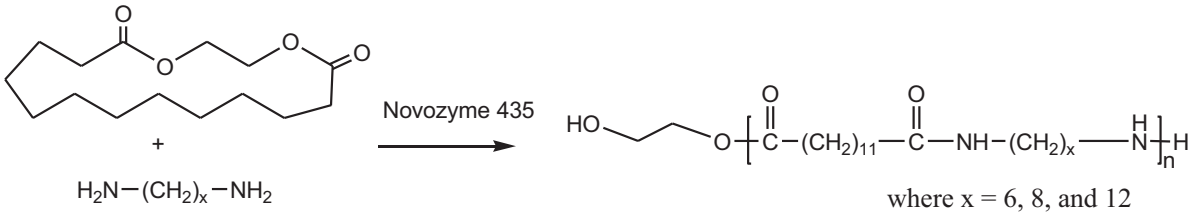


Fig. 8. Lipase catalyzed ring opening and polycondensation reaction between ethylene tridecanedioate and DAO.

Table 2
Molecular characterization of polyamides synthesized from ETD and diamines (with respect to PMMA standards).

Polymer	M_n (g mol^{-1})	M_w/M_n
Nylon-6,13	5560	1.4
Nylon-8,13b	6420	2.4
Nylon-12,13	8250	5.8

and 85% amide bond formation was observed for the reactions performed with DADD, DAO, and DAH, respectively, which we relate to the polarity of the diamines. The hydrophobic nature of diamine increases with increasing alkyl chain length, which increases the chance of diamine to access the catalytic active site of enzyme N-435.

Molecular weights and their distributions of these polymers were calculated from GPC and are given in Table 2. The calculated M_n and polydispersity is increased by increasing alkyl chain length of the diamines, as evidenced by NMR analysis. The highest M_n (8250 g mol^{-1}) polyamide with a large polydispersity (5.8) is produced from the reaction between ETD and DADD.

3.6. Synthesis of poly(amide-co-ester)s

As given in Scheme 1, poly(amide-co-ester)s were synthesized by three steps using enzyme N-435 as a catalyst. In a first step, an equal molar ratio of PD and diamine and enzyme N-435 are mixed with dried diphenyl ether and stirred at 90 °C for 20 h. ^{1}H NMR analysis in d-TFA (Fig. 9) after the first step shows that the predominant product is the amide product (e.g. N-dodecylamino-15-hydroxypentadecanoylamide). In addition, the ^{1}H NMR spectrum indicates the presence of a small amount of either unreacted PD or polyester formation (peak at 4.18 ppm). In a second step, 5 mmol of the other co-monomer, i.e. DES, and 0.2 g N-435 were added and the reaction temperature was increased to 110 °C for another 24 h. The ^{1}H NMR analysis after this step confirms the quantitative conversion of amine into amide and the product shows still reactive —OH and —COO—CH_2—CH_3 end groups. In step three, a further increase of the reaction temperature to first 120 °C (3 h) and then to 130 °C (12 h) with 100 mmHg pressure allow the trans-esterification reactions between these two end groups, leading to poly(amide-co-ester)s.

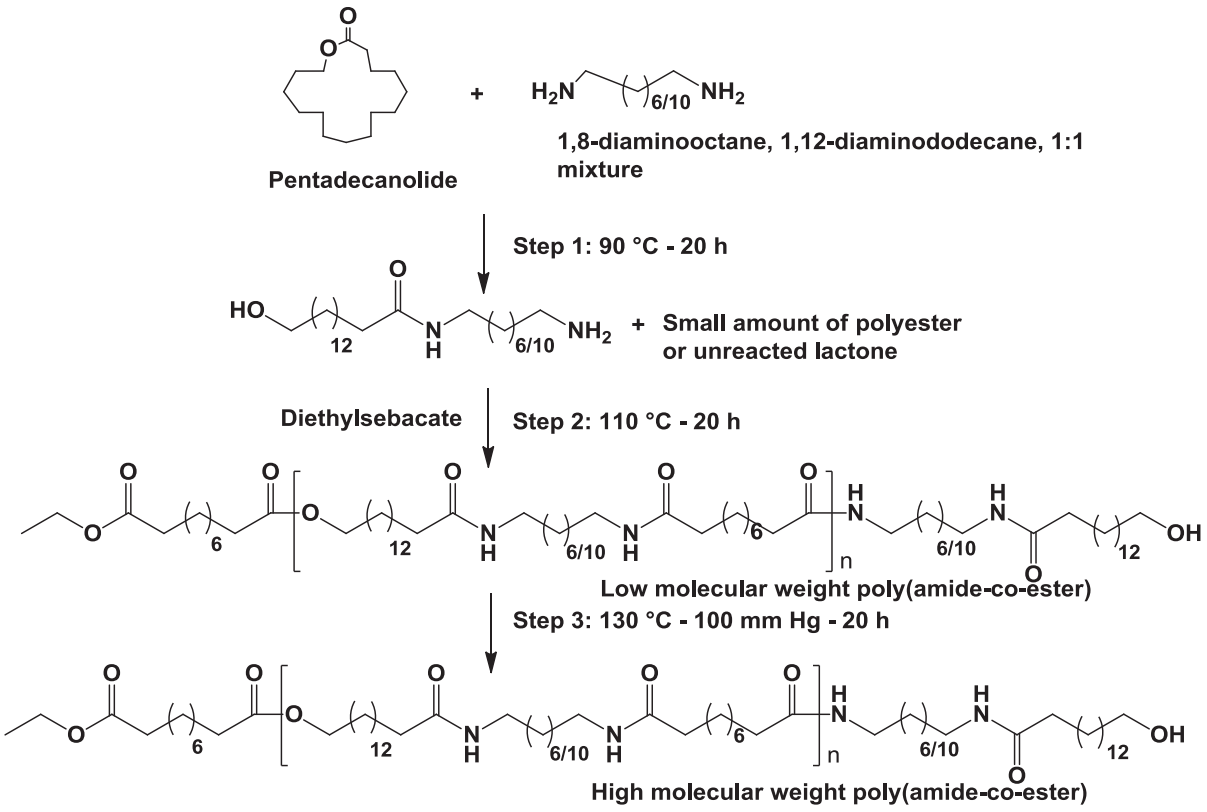
Three poly(amide-co-ester)s were synthesized using the following combinations of monomers: (i) PD, DAO, and DES, (ii) PD, DADD, and DES, and (iii) PD, DAO, DADD, and DES. The end group analysis from ^{1}H NMR measurements shows that the copolymer synthesized using both the diamines (DAO and DADD) as co-monomers, yields the highest molecular weight poly(amide-co-ester) (PAE1) with M_n 17,550 g mol^{-1}. The NMR end group analysis of the copolymer synthesized with DADD as comonomer (PAE2) shows a molecular weight of M_n = 12,930 g mol^{-1}. In contrast, the copolymer produced using DAO as a comonomer only yields low molecular weight polymer (PAE3) with M_n = 2050 g mol^{-1}. Since the polymers are not soluble in common solvents for GPC, GPC analysis could not be performed.

The thermal behavior of these poly(amide-co-ester)s is compared with the one of pure nylon-8,10 and poly(pentadecanolide) by DSC analysis (see Fig. S1 in Supporting information). Pure polyamide and polyester show peak melting points (T_m) at 200 and 94 °C, respectively. The poly(amide-co-ester), which was synthesized using DAO as comonomer, shows a main T_m at 164 °C, whereas the copolymer produced with DADD, displays a T_m at 153 °C. The copolymer obtained from both the diamines shows a main endothermic peak at 134 °C. In addition, copolymers synthesized using either DAO or DADD show an additional small melting peak at around 120 °C while copolymer produced via both the diamines displays a additional small melting peak at around 95 °C. The main and shoulder melting peaks can be attributed to the polyamide and the polyester rich portions of copolymers, respectively.

TGA analysis was also performed from all these polymers (Fig. S2 in Supporting information). Here, the thermal degradation behavior of poly(amide-co-ester)s is compared with pure nylon-8,10 and poly(pentadecanolide). All these polymers display a sharp degradation temperature of around 430–460 °C. Polyamide has a relatively higher degradation temperature (460 °C) while the polyester has a relatively lower degradation temperature (430 °C). Poly(amide-co-ester)s possess an intermediate degradation temperature at 450 °C. In addition, polyester and poly(amide-co-ester)s have a small degradation step (1–3 wt%) at around 160 and 230 °C, respectively (see the inset in Fig. S2, Supporting information). This could be either due to the non catalytic thermal esterification between the end groups (OH and COOH) of the polymer leading to the loss of trapped solvents, i.e. diphenylether/methanol/trifluoroacetic acid, which were used during synthesis and purification.

3.7. MALDI-TOF MS analysis

It has become an important tool for the analysis of complex polymer systems as it is a fast, accurate and non-averaging technique that offers detailed structural information about the individual molecules contained in a polymer sample [28,29]. ^{1}H NMR analysis of the synthesized polyamides indicates that nylon-8,10 (from DES and DAO) possess amine, ester, and acid as end groups whereas nylon-8,13 (from ETD and DAO) only shows amine and ester end groups. To corroborate and further elucidate this, we performed MALDI-TOF MS analysis of some of the representative polyamide samples synthesized using N-435 as a catalyst. First, MALDI-TOF MS analysis was performed with one of the lower M_n nylon-8,10 sample [nylon-8,10c, obtained by the two step procedure (see Table 1 for NMR and GPC molecular weights)]. As given in Fig. S3, MALD-TOF mass spectra of this sample extends up to m/z 1900 and shows a distribution of ions corresponding to sodium adducts ($M+Na^+$). The peak-to-peak mass increment is 310.3 Da, which exactly matches the difference between m/z values of species belonging to consecutive oligomers. The spectrum also shows that the oligoamide series are terminated with four different identifiable end group configurations. They are amine/ester, amine/acid, amine/amine and ester/ester. In addition, oligoamides with an unknown additional end group configuration (x = 270 Da), which



Scheme 1. Synthesis of poly(amide-co-ester)s by three step N-435 catalyzed polymerization.

certainly does not come from the reactants, are also observed in the MALDI-TOF MS spectrum. A possible end group corresponding to this mass was determined as C_3H_5O/ester, where C_3H_5O could be either CH_2=CH–CH_2–O or CH_3–CH_2–CO, which might be derived from the acrylic resin used for immobilization of the enzyme. It is interesting to note that no cyclic oligoamide (e.g. 1265.3 Da for cyclic nylon-8,10 with four repeating units) is detected in the MALDI-TOF mass spectrum (Table S1).

One of the higher M_n nylon-8,10 (nylon-8,10g) was also analyzed by MALDI-TOF MS (Fig. S4 in Supporting information). As

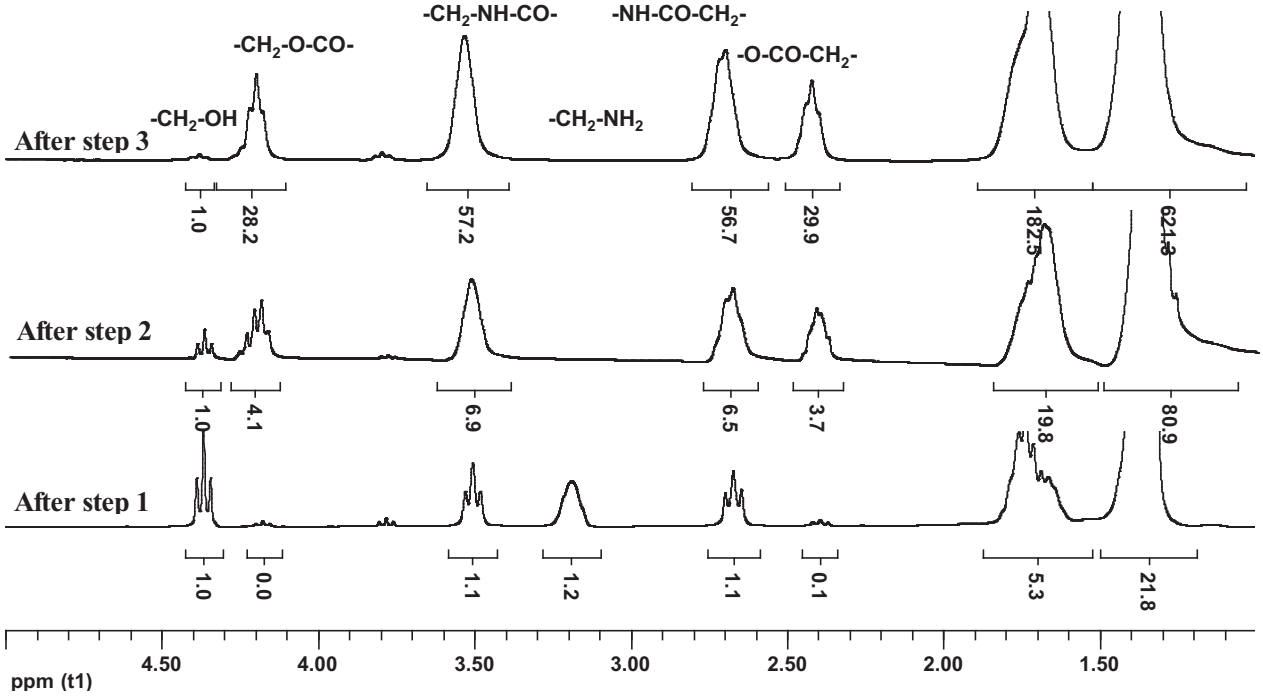


Fig. 9. Synthesis of poly(amide-co-ester) by three step was investigated by ^{1}H NMR analysis after each step of polymerization.

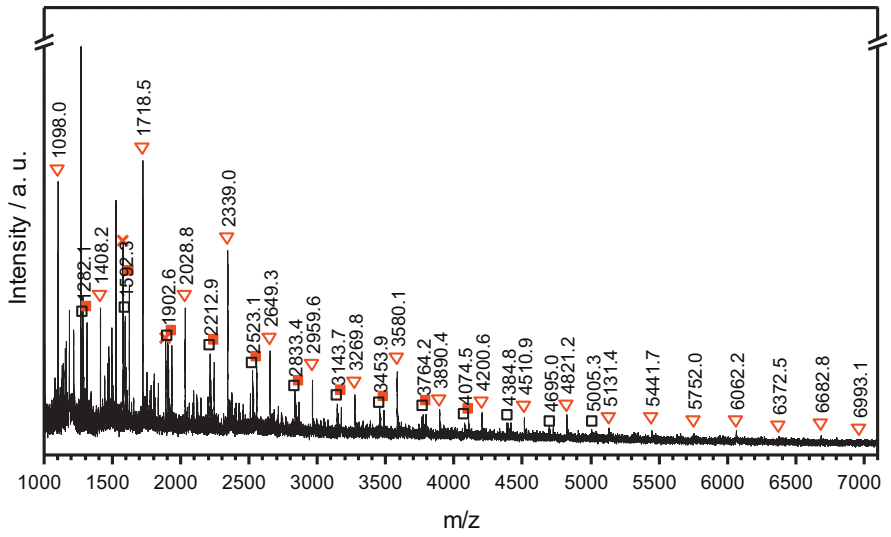


Fig. 10. MALDI-TOF MS spectrum of the highest M_n sample (nylon-8,10i) synthesized by enzymatic catalysis.

shown already, ^{1}H NMR and GPC measurements of this sample (nylon-8,10g) indicate a definite increase of the molecular weight of nylon-8,10 compared to the previous sample (see Table 1). The MALDI-TOF mass spectrum here extends up to *m*/*z* 2700 in good agreement with our NMR and GPC results. Similar end groups as discussed above (i.e. four readily identifiable and one unexpected) are observed (spectrum not shown). This sample was further purified with $CHCl_3$ to be again analyzed by MALDI-TOF mass spectroscopy as shown in Fig. 8. This spectrum is identical to the parent sample except for the peaks corresponding to the unexpected endgroup configuration of $x = 270$ Da, which are not present any more.

The purified highest M_n nylon-8,10 synthesized by three step polymerization (nylon-8,10i), was also examined by MALDI-TOF MS. Remarkably, the mass spectrum here widens up to *m*/*z* 7000 g mol^{-1} (Fig. 10) in good agreement with the NMR and GPC results. Polymers with the above four identifiable end groups were seen in this spectrum. Moreover, the spectrum also predicts the presence of cyclic oligoamides (x in Fig. 10), which was not observed in the previous two lower M_n nylons (see Fig. 11 and Tables 3 and 4).

Nylon-8,13a (synthesized by the two-step procedure) was also analyzed by MALDI-TOF MS. The spectrum contains a number of equidistant peaks of different intensity and extends up to *m*/*z* 2300. The peak-to-peak mass increment is 352.8 Da and exactly equals the mass of the repeating unit in the nylon-8,13 chain. The main peaks are due to the intact M+Na$^+$ molecular ions, which are formed by attachment of ubiquitous Na$^+$ to the oligomers. The spectrum also shows that this polymer is terminated with three different end group configurations such as amine/ester, amine/amine, and ester/ester. The spectrum (Fig. S5 in Supporting information) does not show any peaks corresponding to oligoamides with an acid end group, which is in good agreement with ^{1}H NMR predictions. Here also the reaction does not yield cyclic oligoamides (e.g. 1433.7 Da for cyclic nylon-8,10 with four repeating units) and therefore no such peaks are observed in the MALDI-TOF mass spectrum (Table S2). The mass spectrum of nylon-8,13b here widens up to *m*/*z* 5500 in good agreement with our NMR (see Fig. 9) and GPC measurements. Polymers with the above three end groups were observed in the spectrum. In addition, here also the spectrum predicts the presence of cyclic polyamides, which was not observed in the previous nylon-8,13 sample.

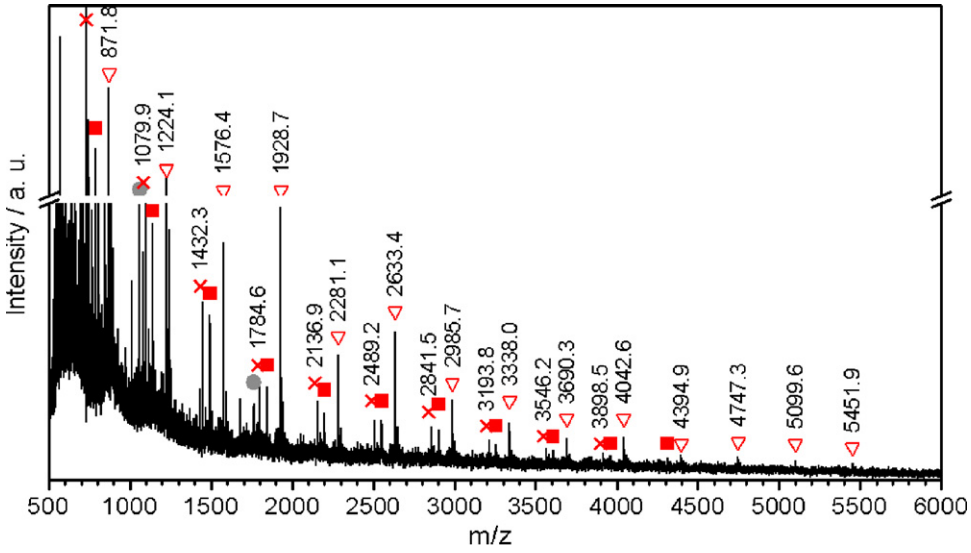
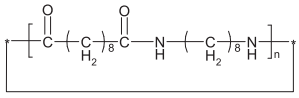
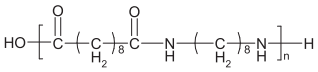
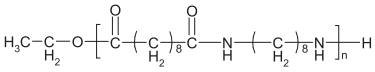
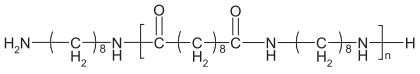
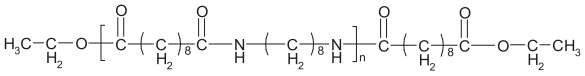


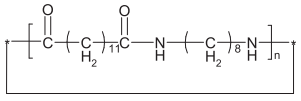
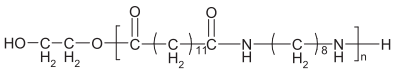
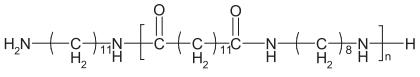
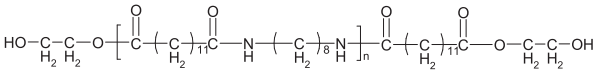
Fig. 11. MALDI-TOF MS spectrum of the highest M_n sample (nylon-8,13b) synthesized from cyclic diester and diaminooctane by enzymatic catalysis.

Table 3
Nylon-8,10 microstructures with different end groups.

x		Cycles, RM = 0 amu
□		Acid/amine, RM = 18 amu
■		Ester/amine, RM = 46 amu
▽		Diamine, RM = 144.2 amu
●		Diester, RM = 258.2 amu
◆	?	(?), RM = 270 amu

RM, remaining mass.

Table 4
Nylon-8,13 microstructures with different end groups.

x		Cycles, RM = 0 amu
■		Ester/amine, RM = 62 amu
▽		Diamine, RM = 144.2 amu
●		Diester, RM = 332.2 amu

RM, remaining mass.

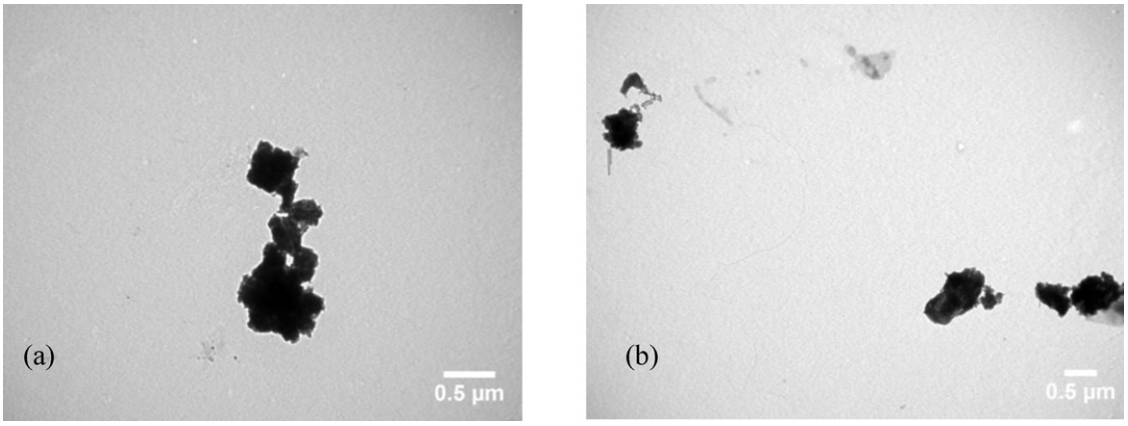


Fig. 12. TEM picture of (a) nylon-8,10 and (b) nylon-8,13.

3.8. Morphology of polyamide particles

The produced nylons are particulate and presumably highly crystalline as found in DSC (Fig. S1). Therefore, they were analyzed by transmission electron microscopy to get insight to their morphology. As shown in Fig. 12, most of the particles were observed in TEM are aggregates with a size ranging from 0.5 to 1 μm (see also Fig. S6 in Supporting information) and relatively sharp edges supporting the high crystallinity found in DSC. It should be noted that the employed enzyme N-435 is an immobilized material with 315–1000 μm particle size, 130 m^2 g^{-1} surface area, and 150 Å pore diameter, respectively [30]. Thus, it can be ruled out that the particles present in Figs. 12 and S6 are support material from the immobilized enzyme.

4. Conclusions

Single step enzymatic reactions between DES and DAO at different temperatures (60–150 °C) with 100 mmHg pressure in dried diphenyl ether led to the enzyme's highest catalytic activity at

90–110 °C (~65% amide bond). Unexpectedly, even at 150 °C, the enzyme does not lose its catalytic activity completely and shows ~22% amide bond formation. Further control experiments show that even pre-treating the enzyme at 150 °C up to 6 h, does not drop the enzyme's catalytic activity significantly at 100 °C and shows 50% amide bond formation. A kinetic investigation between the same monomers at 100 °C shows that this reaction follows a second order and the initiation is due to the nucleophilic attack by amine.

To further increase the molecular weight of nylon-8,10, a series of two step reactions with lower pressure and higher reaction temperature reveal by end group analysis as well as by GPC analysis that the molecular weight increases. Similar two step reactions with other lipases and a control reaction without enzyme show that only minor amounts of amide bonds (4–8% at most) are formed indicating that N-435 is by far the best enzyme in the investigated series.

Using three step reactions with reduced pressure and 400 mg of N-435, 97% of ester were observed to be converted into amide bond with a molecular weight M_n of about 5000 g mol^{-1} as detected by ^{1}H NMR and GPC analysis. A three step ring-opening and polycondensation reaction between ETD and DAH/DAO/DADD in dried toluene leads to >90% conversion of ester into amide bond formation. Among the polyamides produced, nylon-12,13 has the highest M_n of more than 8000 g mol^{-1} and a polydispersity of 5.8.

A new sequence of poly(amide-co-ester)s were synthesized by three step reactions using N-435 as a catalyst. ^{1}H NMR measurements show that the copolymer synthesized using both the diamines (DAO and DADD) as co-monomers, yields the highest molecular weight poly(amide-co-ester) M_n with about 17 kg mol^{-1}. The melting and thermal degradation behaviors of these poly(amide-co-ester)s were investigated and compared with the pure polyamide and polyester by DSC and TGA analysis showing the expected behavior of a statistical copolymer.

MALDI-TOF MS analysis of nylon-8,10 indicates that the polymer contains four identifiable end groups (amine/ester, amine/acid, amine/amine, and ester/ester) whereas in nylon-8,13 only three identifiable end groups (amine/ester, amine/amine and ester/ester) are found. The spectra also prove the molecular weight increase while changing the reaction conditions. TEM analysis of the polyamides shows that the majority of the particles are aggregates with a size ranging from 0.5 to 1 μm.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2011.11.019.

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