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Full paper

Enzymatic synthesis of multi-component copolymers and their structural characterization

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Summary

The use of enzymes in synthetic applications has increased dramatically in the recent years and the field of polymer science is part of this trend. Synthesis of a variety of polymers using lipase catalyzed (*Candida antarctica*) polymerization reactions has led to a variety of new materials with interesting properties in our laboratories. This paper describes the synthesis of multi-component polyesters and mixed polymers having polyester and polyamide linkages under solvent-less conditions using *Candida antarctica* lipase B. The effect of a third component, i.e. a series of 1,ω-alkanediols (1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 1,12-dodecanediol, 1,14-tetradecanediol and 1,16-hexadecanediol) on the copolymerization reaction of dimethyl 5-hydroxyisophthalate with poly(ethylene glycol 600) has been studied and the mechanism for the incorporation of the third component is proposed. We have also studied the effect of different functional groups during terpolymerization reaction of dimethyl 5-hydroxyisophthalate with poly(ethylene glycol) by adding a third component having different functionalities (1,6-hexanediol, 1,6-hexanediamine or 1,6-hexanedithiol) and compared the effect of hydroxyl, amine and thiol groups on the polymerization reactions.

Abbreviations: PEG, Poly(ethylene glycol); BDL, 1,4-butanediol; HDL, 1,6-hexanediol; ODL, 1,8-octanediol; DDL, 1,10-decanediol; DDDL, 1,12-dodecanediol; TDL, 1,14-tetradecanediol; HDDL, 1,16-hexadecanediol; HDA, 1,6-hexanediamine.

Introduction

A variety of materials based on multi-component copolymers are being produced and used in various applications. Among these, aliphatic polyesters, e.g. poly-L-lactide (PLA) and polyglycolide, and polyethers, e.g. polyethylene glycol (PEG) are the most important biodegradable polymers used clinically in wound closure [1,2], tissue repair and regeneration [3] and/or drug delivery [4].

The past 15 years have seen great progress in the use of enzymes in organic media to catalyze a wide variety of chemical transformations on small molecules [5-8]. The rationale for using enzymes as cata-

lysts in polymer synthesis and their modifications has been documented in various comprehensive reviews [9,10]. The chemical catalytic processes of condensation polymerizations between diols and diacids normally require high temperatures (e.g. > 200 °C) that prohibit the incorporation of thermally unstable moieties into the products. Other challenges of chemically catalyzed polymerizations between alcohols and acids include the preparation of linear polymers when using monomers having multiple functional groups. Normally for the latter, protection-deprotection protocols are needed. Furthermore, chemically catalyzed condensation polymerizations most often give products with Mw/Mn values > 2[11]. Lipase-catalyzed condensation polymerizations between diols and diacids have the potential to overcome some or all of these difficulties. This is largely due to the ability of enzymes to substantially lower the activation energy of condensation reactions and to provide enantio-, chemo- and regioselectivity.

Earlier studies on lipase-catalyzed synthesis of polyesters focused primarily on the use of activated esters [12], which are expensive and not practical for commercial processes. Furthermore, previous reports of lipase-catalyzed condensation polymerizations of diols and diacids may be perceived as discouraging due to long reaction times, excessive catalyst concentrations, and the formation of products of low molecular weights [13–15]. Recently, we have demonstrated the enzyme-catalyzed co-polymerization of isophthalic acid with polyethylene glycols of various sizes under solvent-less conditions [16–19]. We have also functionalized these polymers by attaching various long alkyl chains to phenolic hydroxyl group to construct branched amphiphilic polymers. These polymeric systems self organize in aqueous media to form micelles having particle sizes between 20–80 nm, depending upon the side chain functionality [20, 21]. This paper describes a small part of a large effort in our laboratory to better understand the lipase-catalyzed condensation polymerizations of multi-component systems, in particular the effect of size and nature of blocks on the polymer properties (such as molecular weight and self-organization of these systems in aqueous and organic solvents). It is well known that NMR spectroscopy is the appropriate methodology for the determination of the sequence distribution and randomness of copolymers like copolyamides or PET copolyesters [22]. Therefore the microstructure of polyethylene glycol-based terpolymers was analyzed from a detailed study of their ^{1}H NMR and ^{13}C NMR spectra. To our knowledge, studies addressing the synthesis of polyesters having aromatic and aliphatic moieties have not been carried out so far by enzymatic catalysis.

Experimental

Materials and methods

Candida antarctica lipase B (Novozyme-435, 10% immobilization of enzyme on solid support), an immobilized enzyme was a gift from Novozymes Inc., USA. All other chemicals and solvents were of analytical grade and were used as received unless otherwise noted.

Characterization

Gel permeation chromatography (GPC) was used to determine the molecular weights and molecular weights distributions, Mw/Mn of polymer samples. Light scattering data were determined by a laser light scattering photometer (Wyatt Technology DAWN Model F) equipped with a 632 nm He-Ne laser as a light source. ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H NMR correlation spectra were recorded on a Bruker Instrument Inc. 250 MHz ARX spectrometer equipped with Silicone Graphics station.

General method for terpolymerization

In a typical experiment, equimolar amounts of the monomers dimethyl 5-hydroxyisophthalate (**1**, 2.5 mmol, 0.525 g), poly(ethylene glycol 600) (**9**, 1.5 mmol, 0.900 g) and the appropriate 1,ω-alkanediol/diamine/dithiol (1.0 mmol) [0.090 g (1,4-butanediol, **2**), 0.118 g (1,6-hexanediol, **3**), 0.146 g (1,8-octanediol, **4**), 0.174 g (1,10-decanediol, **5**), 0.202 g (1,12-dodecanediol, **6**), 0.230 g (1,14-tetradecanediol, **7**), 0.258 g (1,16-hexadecanediol, **8**), 0.116 g (1,6-hexanediamine, **17**) and 0.150 g (1,6-hexanedithiol, **18**)] were taken in a round-bottom flask under nitrogen, 10% by weight of the enzyme Novozyme-435 (immobilized *Candida antarctica* lipase B) with respect to the weights of the monomers was added. The resulting reaction mixture was kept at 85 °C for a predetermined time and vacuum was pulled. Aliquots were taken at different time periods. After completion, chloroform was added to quench the reaction and the enzyme was removed by filteration. The solvent was removed under reduced

Table 1. ^{1}H NMR spectral data, number average molecular weights (M_n) and % yields of terpolymers **10-16**

Terpolymer	^{1}H NMR ($CDCl_3$) ppm values, δ	M_n	Yield %
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthal-oyl-co-butanediol], **10**	2.1 (bm, 2xCH_2 of BDL), 3.3 (bs, CH_2OH end gp of BDL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.80 (brs, C-9H), 3.90 (s, OCH_3), 4.40 (bs, C-7H), 4.50 (bs, C-8H), 7.75 (s, C-3H & C-5H) and 8.26 (s, C-1H).	2000	20
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co-hexanediol], **11**	1.58 (brs, 2xCH_2 of HDL), 1.88 (brs, 2xCH_2 of HDL), 3.30 (bs, CH_2OH end gp of HDL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.81 (brs, C-9H), 3.90 (s, OCH_3), 4.32 (t, C-7H), 4.45 (bs, C-8H), 7.65 (s, C-3H & C-5H) and 8.17 (s, C-1H).	6000	60
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co-octanediol], **12**	1.38 (m, 4xCH_2 of ODL), 1.78 (brs, 2xCH_2 of ODL), 3.30 (bs, CH_2OH end gp of ODL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.81 (brs, C-9H), 3.91 (s, OCH_3), 4.35 (t, C-7H), 4.51 (bs, C-8H), 7.78 (s, C-3H & C-5H) and 8.21 (s, C-1H).	8000	80
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co-decanediol], **13**	1.38 (m, 6xCH_2 of DDL), 1.74 (brs, 2xCH_2 of DDL), 3.30 (bs, CH_2OH end gp of DDL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.81 (brs, C-9H), 3.91 (s, OCH_3), 4.31 (t, C-7H), 4.50 (bs, C-8H), 7.74 (s, C-3H & C-5H) and 8.20 (s, C-1H).	10000	75
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co-dodecanediol], **14**	1.38 (m, 8xCH_2 of DDDL), 1.77 (brs, 2xCH_2 of DDDL), 3.31 (bs, CH_2OH end gp of DDDL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.81 (brs, C-9H), 3.91 (s, OCH_3), 4.30 (t, C-7H), 4.50 (bs, C-8H), 7.74 (s, C-3H & C-5H) and 8.20 (s, C-1H).	16800	70
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co tetradecanediol], **15**	1.25 (m, 10xCH_2 of TDL), 1.71 (brs, 2xCH_2 of TDL), 3.31 (bs, CH_2OH end gp of TDL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.81 (brs, C-9H), 3.91 (s, OCH_3), 4.29 (t, C-7H), 4.45 (bs, C-8H), 7.74 (s, C-3H & C-5H) and 8.20 (s, C-1H).	3000	30
Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co-hexadecanediol], **16**	1.25 (m, 12xCH_2 of HDDL), 1.71 (brs, 2xCH_2 of HDDL), 3.31 (bs, CH_2OH end gp of HDDL), 3.62-3.65 (brs, 24xCH_2 of PEG main chain), 3.81 (brs, C-9H), 3.91 (s, OCH_3), 4.29 (t, C-7H), 4.45 (bs, C-8H), 7.74 (s, C-3H & C-5H) and 8.20 (s, C-1H)	1200	20

pressure from the filterate to yield the terpolymers **10–16 (Table-1)** and **19** as viscous oils, which were analyzed by spectroscopic means.

Spectral data of Poly[poly(ethylene glycol 600)-oxy-5-hydroxyisophthaloyl-co-hexanediamine], 19: ^{1}H NMR ($CDCl_3$): δ 1.20 (m, 2xCH_2 of HDA), 1.50 (m, 2xCH_2 of HDA), 3.40 (t, C-7H), 3.50-3.70 (brs, 24xCH_2 of PEG main chain), 3.80 (brs, C-9H), 3.90 (s, OCH_3), 4.50 (bs, C-8H), 7.74 (s, C-3H & C-5H) and 8.20 (s, C-1H).

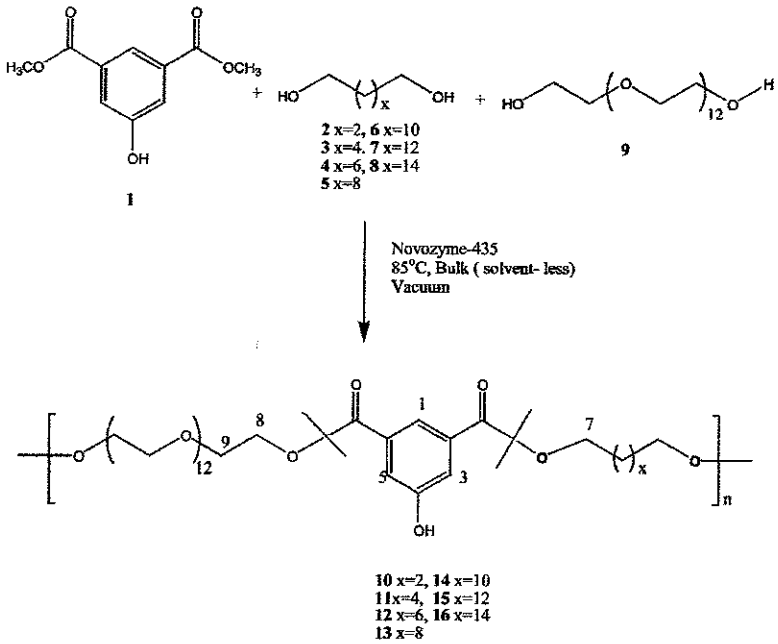
IR (cm^{-1}): 3167(NH), 2872, 1723 (COO), 1639 (CONH, band I), 1542 (CONH, band II).

Transesterification reaction

Novozyme-435 (10% catalyst w/w of the total monomers) was added to a round bottom flask containing 1.0 g of poly[poly(oxyethylene 600)-oxy-5-hydroxyisophthaloyl] (**21**)[18]and 0.25 g of 1,6-hexanediol (**3**). The resulting reaction mixture was kept at 85 °C for a predetermined time and vacuum was pulled. After completion, chloroform was added to quench the reaction and the enzyme was removed by filteration. The solvent was removed under reduced pressure from the filterate to yield the terpolymer **11**.

Synthesis of poly(hexanoxy-5-hydroxyisophthaloyl), 22: A round bottom flask containing equimolar amounts of dimethyl 5-hydroxyisophthalate (**1**, 2 mmol, 0.420 g) and 1,6-hexanediol (2 mmol, 0.236 g), and 65 mg of Novozyme 435 (10% w/w w.r.t. monomer weights) was kept in an oil bath maintained at 85 °C for a predetermined time and vacuum was pulled. After completion, chloroform was added to quench the reaction and the enzyme was removed by filteration The solvent was removed under reduced pressure from the filterate to yield the terpolymer **22**.

^{1}H NMR ($CDCl_3$): δ 1.40 (t, 4H, C-9H & C-10H), 1.80 (t, 4H, C-8H & C-11H), 3.70 (t, 2H, C-12H), 3.80 (s, OCH_3), 4.37 (t, 2H, C-7H), 7.75 (s, 2H, C-3H & C-5H) and 8.26 (s, 1H, C-1H).



Scheme 1. Candida antarctica Lipase B catalyzed synthesis of multicomponent polyesters.

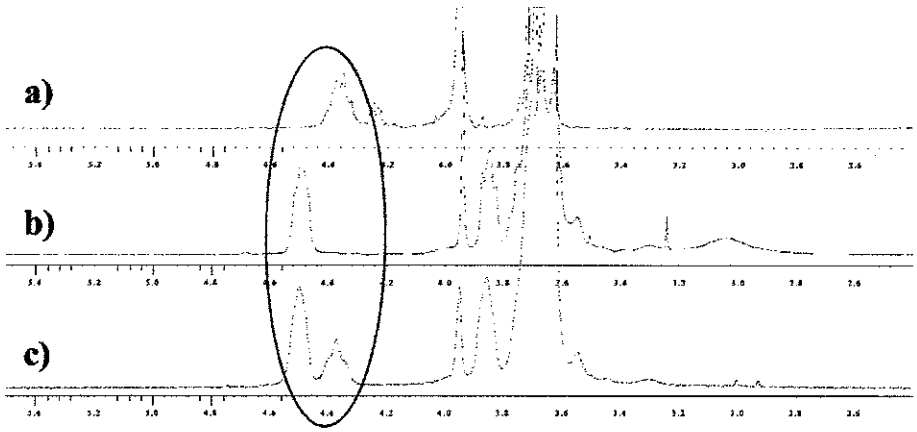
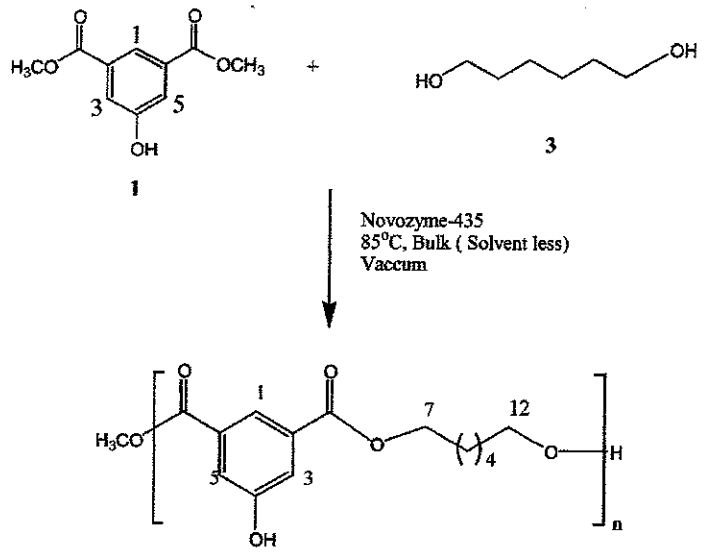


Figure 1. a) ^{1}H NMR spectrum of dimethyl 5-hydroxyisophthalate-co-hexanediol, **22** (top), b) ^{1}H NMR spectrum of dimethyl 5-hydroxyisophthalate-co-poly(ethylene glycol 600), **21** (middle); c) ^{1}H NMR spectrum of terpolymer, **11** (bottom).

^{13}C NMR ($CDCl_3$): δ 25.85 (C-9 & C-10), 32.90 (C-8 & C-11), 52.92 (OCH_3), 63.29 (C-12), 65.0 (C-7), 121.32 (C-3 & C-5), 122.98 (C-1), 132.14 (C-2 & C-6), 156.97 (C-4) and 166.82 (COO).

Results and discussion

The goal of this work was to investigate the incorporation of a third component during the copolymerization of dimethyl 5-hydroxyisophthalate with poly(ethylene glycol 600) using *Candida antarctica* lipase B (immobilized form, Novozyme-435). The co-polymerization reaction was carried out at 85 °C



Scheme 2. Synthesis of poly(hexanoxy-5-hydroxyisophthaloyl) catalyzed by *Candida antarctica* lipase B.

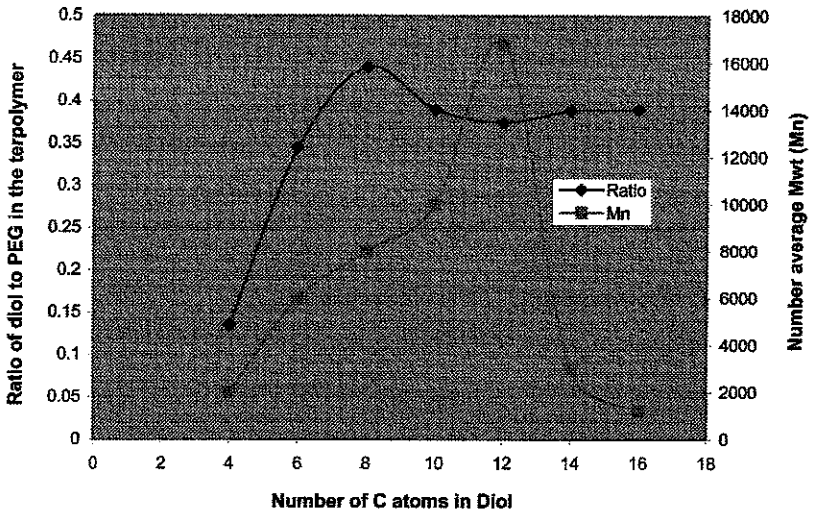


Figure 2. Effect of chain length of diol on the number average molecular weight of terpolymer and % incorporation of the diol.

in bulk (solvent-less) (Scheme-1). Seven different 1,ω-alkanediols, *i.e.* 1,4-butanediol, 1,6-hexanediol, 1,8-octanediol, 1,10-decanediol, 1,12-dodecanediol, 1,14-tetradecanediol and 1,16-hexadecanediol were screened along with poly(ethylene glycol) keeping the other monomer, *i.e.* dimethyl 5-hydroxyisophthalate constant in the reaction mixture. The product terpolymers **10–16** (Table 1) were analyzed from their ^{1}H NMR spectra. Figure 1 represents the ^{1}H NMR spectrum of the terpolymer formed by the copolymerization of dimethyl 5-hydroxyisophthalate, 1,6-hexanediol and poly(ethylene glycol 600), comparison of the spectra with the copolymer **22** of 1,6-hexanediol and dimethyl 5-hydroxyisophthalate (Scheme 2, Figure 1a) showed that the signal at δ 4.36 is due to the 1,6-hexanediol repeat unit $COOCH_2$ protons, this peak is a signature for the trans-esterification reaction between 1,6-hexanediol and dimethyl 5-

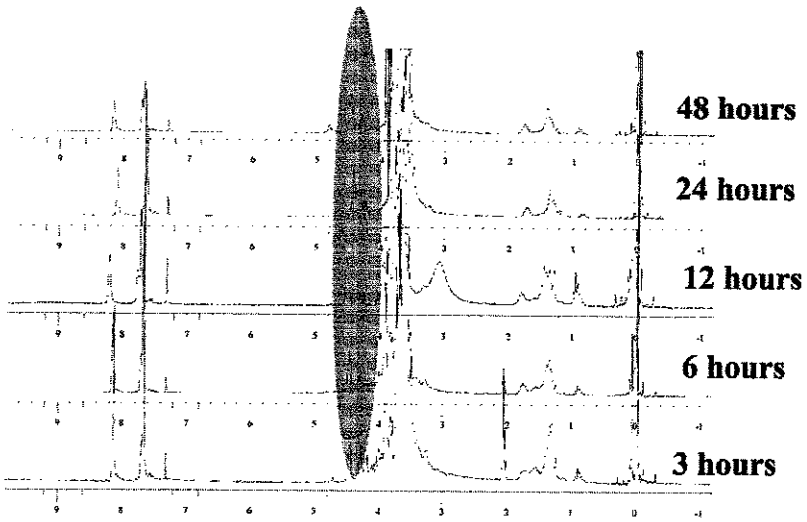
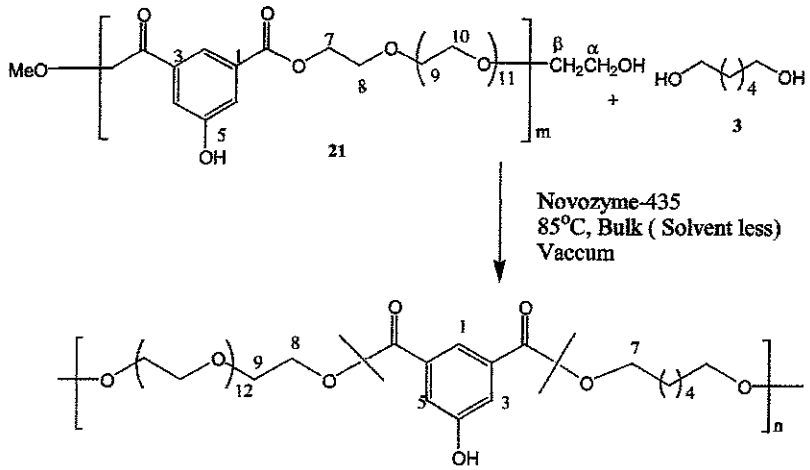


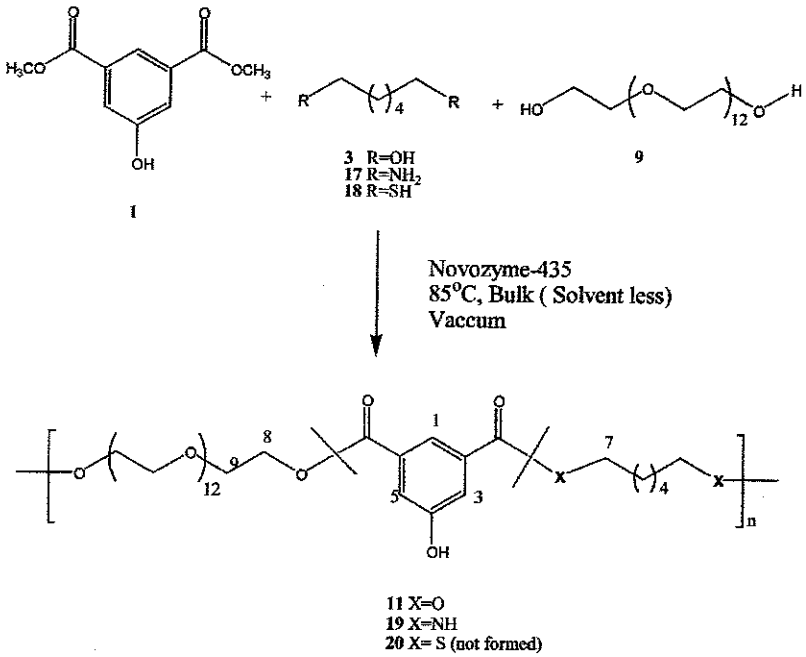
Figure 3. ^{1}H NMR spectra of terpolymers containing dimethyl 5-hydroxyisophthalate, poly(ethylene glycol) and 1,6-hexanediol at different times of the reaction.



Scheme 3. Novozyme-435 catalyzed transesterification reaction.

hydroxyisophthalate. The signal at δ 4.50 (Figure 1b) in the ^{1}H NMR spectrum of **21** was assigned to the poly(ethylene glycol) repeat unit $COOCH_2$protons, which undergoes transesterification reaction with the methoxycarbonyl of the isophthalate moiety, this assignment was in agreement with our earlier published results [18]. The signals at δ 4.36 and 4.50 (Figure 1c) were used to calculate the percentage of each monomer in the terpolymer **11**.

By varying the third component (diol) and keeping the ratio of other two monomers (PEG diol 600 and dimethyl 5-hydroxyisophthalate) in the reaction mixture constant, the reaction was carried out at 85 °C for different time periods. It was observed that the percentage incorporation of the diol in the terpolymer increases in the order 1,4-butanediol < 1,6-hexanediol < 1,8-octanediol > 1,10-decanediol ≥ 1,12-dodecanediol ≥ 1,14-tetradecanediol ≥ 1,16-hexadecanediol (Figure 2). From these results, it was concluded that with the increase in the number of spacer methylene units, the percentage of the diol in the terpolymer increases irrespective of the reac-



Scheme 4. Synthesis of multifunctional copolymers catalyzed by Novozyme-435.

tion time period and after 10 methylene spacer, it levels off. Thus, maximum incorporation of the diol was achieved with 1,8-octanediol and least incorporation was obtained with 1,4-butanediol, this trend is in agreement with earlier reported results on CAL B-catalyzed condensation copolymerization reactions of aliphatic diacids and 1,ω-alkanediols [23].

The time dependent incorporation of the diol in the terpolymer was also studied. It was observed that in the early stages of the reaction, the % incorporation of the diol is more as compared to poly(ethylene glycol) irrespective of the diol used in the reaction. But with progress of time, the % incorporation of the poly(ethylene glycol) in the terpolymer increases steadily. This indicates that poly(ethylene glycol) acts as a solvent in the early stages of the reaction, and as diol gets consumed, it start reacting. The time dependent studies of terpolymerization also indicated that the monomer reactivity of the diol is more than that of poly(ethylene glycol) (Figure 3). These studies also revealed the possible mechanism of the terpolymerization reaction. We propose that the terpolymerization reaction occurs by the combination of condensation reactions, followed by transesterification reactions between the polymer and monomers as well as between the two polymer chains. In order to substantiate this hypothesis, we studied the transesterification reaction between a preformed copolymer of dimethyl 5-hydroxyisophthalate and poly(ethylene glycol 600) (**21**) with 1,6-hexanediol (**3**) at 85 °C in bulk using *Candida antarctica* lipase B for 48 hours (Scheme 3). The ^{1}H NMR spectrum of the product was identical to that obtained by the terpolymerization of the three monomers, which further proved the point that during these polymerization reactions, there is a substantial amount of transesterification reactions between the polymer chains and monomers and between two polymers.

The potential of terpolymerization reactions was further demonstrated by the synthesis of terpolymers with multiple functionalities. For this purpose, we studied the incorporation of 1,6-hexanediamine and 1,6-hexanedithiol during the copolymerization of dimethyl 5-hydroxyisophthalate with poly(ethylene glycol 600) using *Candida antarctica* lipase B (Scheme-4). The ^{1}H NMR spectrum of the terpolymer **19** with 1,6-hexanediamine showed a broad triplet at δ 3.40 assigned to the CONHCH_2 protons, thus indicating the formation of amide linkages. The formation of amidic linkages was also confirmed by the

appearance of a band at 1639 cm^{-1} along with a band for the ester linkage at 1723 cm^{-1} in the IR spectrum of the terpolymer **19**. However the addition of 1,6-hexanedithiol during the terpolymerization reaction (Scheme-4) did not exhibit any thioester linkages in the product polymer, as no such signal was observed in its ^1H NMR or IR spectrum. The product polymer isolated in this case was **21**, i.e. the copolymer of just two components (dimethyl 5-hydroxyisophthalate and PEG 600). This indicates that thiols may not be good substrates for the enzyme-catalyzed polymerization reaction, it may be because thiols are considered as soft nucleophiles and may not readily attack a hard electrophilic center such as the carbonyl carbon of the acyl enzyme complex. This has been supported by the observation of Ohrner *et al.* [24] who have reported facile CAL-B catalyzed acyl transfer reaction of alcohols and amines with esters, while no such reaction occurred with thiols. Extensive work is in progress in our laboratory to establish the comprehensive mechanism of the terpolymerization reactions, and on further functionalization of these novel multicomponent polymers.

Conclusions

It has been demonstrated for the first time that multicomponent polyesters having aromatic and aliphatic moieties can be synthesized by the enzyme-catalyzed polymerization reactions. The methodology developed is very efficient and has a tremendous potential in terms of tailoring the microstructure of the polymer or to introduce the desired components in the polymeric system for improved properties and applications. The flexibility in the present methodology also permits the introduction of various functionalities in the polymeric system for any specific applications. The use of enzymes under the solventless conditions, viz. 'Green Chemistry' in this methodology further opens new avenues for its industrial applications.

References

1. Frazza, E. J. and Schmitt, E. E., *New absorbable suture*, J. Biomed. Mater. Res., 1 (1970) 43–49.
2. Kobayashi, S., Hyon, H. and Ikada, Y., *Progress in medical imaging and surgery of liver cancer*, J. Biomed. Mater. Res., 25 (1991), 1481–1489.
3. Daniels, A. U., Chang, M. K. D. and Andriano, K. P., *Mechanical properties of biodegradable polymers and composites proposed for internal fixation of bone*, J. Appl. Biomater., 1 (1990), 57–64.
4. Chasin, M. and Langer, R., (eds.), Biodegradable Polymers as Drug Delivery Systems, Marcel Dekker, New York (1990).
5. Kumar, R. and Gross, R. A., *Biocatalytic route to well-defined macromers built around a sugar core*, J. Am. Chem. Soc., 124 (2002) 1850–1851.
6. Sharma, S. K., Roy, S., Kumar, R. and Parmar, V. S., *Novel diastereoselective acylation of 4-C-hydroxymethyl-1,2-O-(1-methylethylidene)-3-O-(phenylmethyl)-D-pentofuranose: effect of lipases and acylating agents on stereoselectivity*, Tetrahedron Lett., 40 (1999) 9145–9146.
7. Parmar, V. S., Prasad, A. K., Pati, H. N., Kumar, R., Azim, A., Roy, S. and Errington, W., *Enzyme-catalyzed chemoselective transesterification reactions on hydroxymethylated phenolic compounds*, Bioorg. Chem., 27 (1999) 119–134.
8. Kumar, R., Azim, A., Kumar, V., Sharma, S. K., Prasad, A. K., Howarth, O. W., Olsen, C. E., Jain, S. C. and Parmar, V. S., *Lipase-catlyzed chemo- and enantioselective acetylation of 2-alkyl/aryl-3-hydroxypropiophenones*, Bioorg. Med. Chem., 9 (2001) 2643–2652.
9. Gross, R. A., Kumar, A. and Kalra, B., *Polymer synthesis by in vitro enzyme catalysis*, Chem. Rev., 101 (2001) 2097–2124.
10. Kobayashi, S., Uyama, H. and Kimura, S., *Enzymatic polymerizations*, Chem. Rev., 101 (2001) 3793–3818.
11. Binns, F., Roberts, S. M., Taylor, A. and Williams, C. F., *Enzymic polymerization of unactivated diol/diacid system*, J. Chem. Soc., Perkin Trans. 1 (1993) 899–900.
12. Wallace, J. S. and Morrow, C. J., *Biocatalytic synthesis of polymers. II. Preparation of [AA-BB]x polyesters by porcine pancreatic lipase catalyzed transesterification in anhydrous, low polarity organic solvents*, J. Polym. Sci. Part A: Poly.Chem., 27 (1989) 3271–3278.
13. Linko, Y. Y., Wang, Z. L. and Seppala, J., *Lipase biocatalysis in the production of esters*, J Biotechnol., 40 (1995) 133–135.
14. Uyama, H., Inafda, K. and Kobayashi, S., *In vitro enzymatic synthesis and applications of poly(amino acid)s*, Kagaku Kogaku Ronbun, 54 (2003) 112–117.
15. Binns, F., Harffey, P., Roberts, S. M. and Taylor, A., *Studies of lipase-catalyzed polyesterification of an unactivated diol/diacid system*, J. Polym. Sci. Part A: Polym. Chem., 36 (1998) 2069–2078.
16. Watterson, A. C. and Chen, M.-H., *Observing the aggregation of a novel amphiphilic polymer in solution using ^1H-NMR relaxation times (T1)*, Polymer Preprints, 42 (2001) 189–200.
17. Kumar, R., Shakil, N. A., Chen, M.-H., Parmar, V. S. and Watterson, A. C., *Chemo-enzymatic synthesis of novel functionalized amphiphilic polymer*, Polymer Preprints, 43 (2002) 578–579.
18. Kumar, R., Shakil, N. A., Chen, M.-H., Parmar, V. S., Samuelson, L. A., Kumar, J., Watterson, A. C., *Chemo-enzymatic synthesis and characterization of novel functionalized amphiphilic polymers*, J. Macromol. Sci. – Pure Appl. Chem., 39 (2002) 1137–1149.
19. Kumar, R., Shakil, N. A., Chen, M.-H., Parmar, V. S., Samuelson, L. A., Kumar, J. and Watterson, A. C., *Synthetic and mechanistic studies on biocatalytic reactions generating novel amphiphilic polymers*, Polymer Preprints, 43 (2002) 879–880.
20. Kumar, R., Shakil, N. A., Chen, M.-H., Parmar, V. S., Samuelson, L. A., Kumar, J. and Watterson, A. C., *Novel self-assembled polymeric nano-particles for drug delivery: Chemoenzymatic synthesis, characterization and applications*, J. Am. Chem. Soc., communicated.

21. Chen, M.-H., Kumar, R., Yang, K., Parmar, V. S., Samuelson, L. A., Kumar, J. and Watterson, A. C., *Studies on the supramolecular organization of amphiphilic copolymers into nano-micelles by light scattering techniques*, Polymer Preprints, 44 (2003) 1199–1200.
22. Karayannidis, G. P., Sideridou, I. D., Zamboulis, D. N. and Bikiaris, A. J., *Thermal behavior and tensile properties of poly(ethylene terephthalate-co-ethylene isophthalate)*, J. Appl. Polym. Sci., 78 (2000) 200–208.
23. Mahapatro, A., Kalra, B., Kumar, A. and Gross, R. A., *Lipase-catalyzed polycondensations: effect of substrates and solvent on chain formation, dispersity, and end-group structure*, Biomacromolecules, 4 (2003) 544–551.
24. Ohrner, N., Orrenius, C., Mattson, A., Norin, T. and Hult, K., *Kinetic resolutions of amine and thiol analogues of secondary alcohols catalyzed by the Candida antarctica lipase B*, Enzyme Microb. Technol., 19 (1996) 328–331.