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Lipase-Catalyzed Synthesis of Polymeric Prodrugs of Nonsteroidal Anti-Inflammatory Drugs

Xueqi Qian, Qi Wu, Fangli Xu, Xianfu Lin
Department of Chemistry, Zhejiang University, 38 Zheda Road, Hangzhou 310027, People's Republic of China
Correspondence to: X. Lin (E-mail: llc123@css.zju.edu.cn)

ABSTRACT: Because of the potential application of prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), *Candida antarctica* lipase B (CAL-B) catalyzed polycondensation of profen-containing diol monomers and diesters were designed to prepare a series of biodegradable polymeric prodrugs composed of NSAID branches and poly(amide-*co*-ester) backbone. The structure of the products was confirmed by Fourier transform infrared spectroscopy, NMR, and gel permeation chromatography (GPC). The reaction conditions of polymerization, such as the enzyme source, amount of catalyst, and temperature, were optimized. The molecular weights of resultant copolymers were 2170–13,270 g/mol, with corresponding polydispersities from 1.17 to 2.4. The copolymers had relatively high drug loadings of 44.7–59.7 wt % because every repeat unit contained one drug molecule. The strategy of enzymatic polymerization appeared to be quite general and accommodated a large number of comonomer substrates with various chain lengths and substituents. The optically pure (*R*)-naproxen monomer was demonstratively incorporated into the corresponding copolymers with the developed synthesis strategy. The *in vitro* study showed that the polyester could release the drug effectively under physiological conditions with enzyme, which indicated that the obtained product could be a promising prodrug for extending pharmacological effects by delayed drug release. With GPC analysis, we confirmed that the prodrug was completely degradable in aqueous solution. The attractive features of the copolymer were its high drug loading, biodegradability, and biocompatibility. The high tolerance of the CAL-B toward drug groups, as described in this article, provides a new route for synthesizing polymeric drugs with potential biomedical applications in mild conditions and for reducing environmental impact. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Polymeric prodrugs, which have been evaluated and developed in preclinical[1] and clinical studies for many years, are widely approved for clinical applications because of their obvious benefits, such as the control of drug release, the reduction of drug resistance, and the improvement of therapeutic effectiveness.[2–4] These advantages have become a strong incentive for scientists and entrepreneurs to prepare specific polymeric prodrugs with different structures and different drug-delivery formulations.[5] Examples of these polymers, such as poly(lactic acid), poly(ethylene imine), poly(4-hydroxy-L-proline ester), poly[α-(4-aminobutyl)-L-glycolic acid], poly(β-amino esters), poly(L-lysine), chitosan, poly(dimethyl aminoethyl methacrylate), and poly(trimethyl aminoethyl methacrylate), have been widely used for drug-delivery systems. Among them, biodegradable main chains, such as polyesters and polypeptides, are particularly promising because of their biocompatibility, low cytotoxicity, and outstanding transfection efficacy. For example, poly(lactic acid) and poly(phosphate ester) have extensive applications in drug-delivery systems and other biomedical fields.[6–8] However, these polymers have generally been synthesized by chemical methods, which need critical conditions such as high pressures/temperatures for the synthesis of the main chain and complicated protection/deprotection steps for the incorporation of drugs. Wang and Paul[9] reported a chemical process for the synthesis of a precisely engineered amphiphilic polyester drug bearing combretastatin A4 (CA4) and paclitaxel for the treatment of cancers. Marilena and Miguel[10] developed a potential anticancer drug by chemically conjugating Daunorubicin to a gonadotropin-releasing hormone III derivative through an oxime bond. The drugs were designed on a PEG or polypeptide basis by end-group conjugation. Yang et al.[11] reported the preparation of a biodegradable poly(amino-*co*-ester) for drug and DNA delivery using sebacoyl chloride to form the polyester. This process generated much HCl, and a large excess of triethylamine was used to remove it. Furthermore, diacyl halides, which are expensive and sensitive to moisture, would render them undesirable for serving

as comonomers for polyester synthesis. So far, few efficient synthetic methods are currently available for the preparation of amino-containing polyesters, primarily because the metal catalysts required for conventional polyester synthesis are often sensitive to and deactivated by amino groups. It is believed that enzymes will provide another significant route for forming these polyester drugs.

Enzymes (e.g., lipase) have been widely accepted as nontoxic catalysts since the early 21st century because they replaced potentially toxic metal-type catalysts in polymerization.[12] Thus, enzymatic polymerization is fully environmental friendly compared to traditional chemical routes. Furthermore, other advantages of enzymatic polymerization, such as their high stereoselectivity, regioselectivity, and chemoselectivity, and good recyclability and biocompatibility, are also very important. Particularly, lipase represents a high tolerance of functional organic groups and excellent reaction activity and versatility for the transesterification polymerization of diesters and diols.[13] It is easy to remove the catalyst and solvents completely, and thus, this is widely applied in biomedical and pharmaceutical fields.[14] Various functional polyesters are ideally prepared via lipase-catalyzed polymerization. Jiang[15] successfully used *Candida antarctica* lipase B (CAL-B) to catalyze the copolymerization of dialkyl diester with diol and lactone to form aliphatic polyesters in two steps. Dai et al.[16] first synthesized thermoplastic block copolymers using lipase. Clarson[17] also prepared silicone aromatic polyesters and silicone aromatic polyamides by lipase-catalyzed polymerization. To our knowledge, although many researches have focused on the lipase-catalyzed synthesis of polyesters, few studies on the enzymatic synthesis of biodegradable polyester prodrugs have been reported.

Our group focused on the chemoenzymatic synthesis of polymer drugs through the radical polymerization of enzymatically prepared polymerizable monomers containing drug branches. We reported the synthesis of optically active polymeric prodrug of profens[18] and functional polymeric prodrugs of acyclovir with high drug payload.[19] Because the main chain of poly(vinyl alcohol)-based polymer drugs is hard to biodegrade *in vivo*, we hoped to synthesize biodegradable polymeric prodrugs using fully enzymatic approaches, aiming to increase the biocompatibility and biological activity of the prodrug system. Particularly, the poly(amino-*co*-ester) backbone is more interesting because it can be used as a codelivery carrier of drugs and DNA.

Herein, we report the facile preparation of a poly(amide-*co*-ester) prodrug with a high drug loading via a CAL-B catalyzed two-step reaction of acylation and polycondensation. In the first step, profen vinyl esters were acylated by 2,2′-azanediyldiethanol to form *N, N*-bis(2-hydroxyethyl)-2-arylpropanamide. In the second step, diols containing drug groups were reacted with dimethyl diesters to build poly(amide-*co*-ester) prodrugs. Furthermore, a copolymer with a chiral drug branch was demonstratively prepared from (*R*)-naproxen. The polymer structures were characterized and confirmed by IR, NMR spectroscopy, and gel permeation chromatography (GPC). The hydrolysis of poly[*N, N*-bis(2-hydroxyethyl) naproxenamide-*co*-sebacate] (PNSC) was carried out in an aqueous solution; this demonstrated that the prodrug was biodegradable. The *in vitro* study showed that the polyester could release naproxen effectively under physiological conditions; this indicated that the polyester could be a promising prodrug.

EXPERIMENTAL

Materials

Dimethyl succinate, dimethyl adipate, dimethyl azelate, and dimethyl sebacate were prepared from dicarboxylic acid and methanol with thionyl chloride as the catalyst. Diphenyl ether (99%), methanol (99%), tetrahydrofuran (THF; 99%), and hexane were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Lipozyme immobilized from *Mucor miehei* (MML; EC 3.1.1.1, 42 U/g) and lipase from *Candida cylindraceal* (CCL; 2.8U/mg) were purchased from Fluka (Shanghai, China). Lipase type VII from *Candida rugosa* (CRL; EC 3.1.1.3, 706 U/g) and lipase immobilized on acrylic resin from *Candida antarctica* (CAL-B; EC 3.1.1.3, $\geq$ 10,000 U/g) were purchased from Sigma (Shanghai, China). The lipase catalyst was dried at 25°C under 2.0 mmHg for 24 h before use. Ketoprofen was purchased from Zhejiang Jiuzhou Pharmaceutical Co., Ltd. (Zhejiang, China). Naproxen was purchased from Zhejiang Chariotteer Pharmaceutical Co., Ltd. (Zhejiang, China). Ibuprofen was purchased from Juhua Corp. Pharmaceutical Factory (Zhejiang, China). Profen vinyl ester was synthesized as described by Cai et al.[18]

Instrumental Methods

Infrared spectra were measured with a Nicolet Nexus FT-IR 470 spectrophotometer (Massachusetts, USA). NMR spectra were recorded with a Bruker DRX 400 NMR spectrometer (Rheinstetten, Germany). The number- and weight-average molecular weights (M_n and M_w, respectively) of polyester drugs were measured by GPC with a system equipped with a refractive-index detector (Waters 2414) and Waters Styragel GPC columns (Massachusetts, USA). The GPC columns were standardized with narrow-dispersity polystyrene in molecular weights ranging from 1×10^5 to 162. The mobile phase was THF at a flow rate of 1.0 mL/min. The samples analysis was performed with an Agilent 1100 system (Agilent, California, USA) with a reversed-phase Shim-Pack VP-ODS column (150×4.6 mm^2) equipped with a UV detector at 210 nm. For the analysis of ibuprofen diol, 65:35 v/v methanol/water was used as the eluent (flow rate = 1.0 mL/min).

Enzymatic Preparation of Polymerizable *N, N*-bis(2-hydroxyethyl)-profenamide (Profen Diol)

As is known, polymeric prodrugs play an essential role. In this study, we synthesized three polymeric prodrugs bearing different profen groups. The reaction of 1 mmol of profen vinyl ester with 2,2′-azanediyldiethanol in a molar ratio of 1:3 was catalyzed by CAL-B (10 wt % profen vinyl ester) in 3 mL of cosolvent [isopropyl ether (IPE)/2-methylbutan-2-ol (2MB2L) = 2:1 v/v]. The mixture was incubated at 25°C under shaking at 200 rpm for 24 h. We terminated the reaction by filtering off the enzyme. The product was purified by silica gel column chromatography with petroleum/pure ethyl acetate as the eluent. The reaction conditions, including the reaction time, solvents, enzyme, and reaction temperature, were screened to get diol monomers containing a chiral profen drug. The enantiomer

excess of products was determined by chiral HPLC analysis. Finally, a low enantiomeric excess value of about 33% was obtained after the optimization of all of these conditions. The structures of the products were characterized by Fourier transform infrared (FTIR) spectroscopy, high resolution mass spectrometer (HRMS), ^{1}H-NMR, and ^{13}C-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for *N, N*-bis(2-hydroxyethyl)-naproxenamide (**2 a**): 7.70 (m, 2 H), 7.62 (s, 1 H), 7.38 (m, 1 H), 7.14−7.09 (m, 2 H), 4.15 (m, 1 H), 4.07 (m, 2 H), 3.90 (s, 3 H), 3.72 (m, 2 H), 3.61 (m, 3 H), 3.44 (m, 1 H), 3.11 (m, 2 H), 1.48 (d, J = 7.8, 3 H). ^{13}C-NMR (400 MHz, $CDCl_3$, δ, ppm): 176.3, 157.6, 136.7, 133.5, 129.1, 127.6, 126.0, 125.6, 119.0, 105.6, 61.4, 60.7, 55.3, 51.8, 50.8, 43.1, 20.7. IR (cm^{-1}): 3430, 3306, 2938, 1630, 1609, 1462, 1064, 853. HRMS: 317.1634. Calcd. mass: 317.1627.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for *N, N*-bis(2-hydroxyethyl)-ketoprofenamide (**2 b**): 7.75−7.69 (m, 3 H), 7.58−7.52 (m, 3 H), 7.46−7.36 (m, 3 H), 4.40−4.20 (s, 2 H), 4.16 (m, 1 H), 3.76 (m, 2 H), 3.59 (m, 3 H), 3.50 (m, 2 H), 3.23 (m, 1 H), 1.41 (d, J = 7.2, 3 H). ^{13}C-NMR (400 MHz, $CDCl_3$, δ, ppm): 196.9, 175.5, 142.2, 138.0, 137.2, 132.7, 131.5, 130.1, 128.8, 128.3, 60.9, 60.4, 51.8, 50.6, 42.6, 20.4. IR (cm^{-1}): 3405, 2933, 1649, 1622, 1284, 1069, 720. HRMS: 341.1625. Calcd. mass: 341.1627.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for *N, N*-bis(2-hydroxyethyl)-ibuprofenamide (**2 c**): 7.11 (d, J = 7.6, 2 H), 7.05 (d, J = 8.0, 2 H), 3.98 (m, 2 H), 3.81 (m, 2 H), 3.65 (m, 2 H), 3.59 (m, 3 H), 3.40 (m, 1 H), 3.10 (d, J = 14, 1 H), 2.40 (d, J = 7.6, 2 H), 1.80 (m, 1 H), 1.37 (d, J = 6.8, 2 H), 0.86 (d, J = 6.4, 6 H). ^{13}C-NMR (400 MHz, $CDCl_3$, δ, ppm): 176.3, 140.3, 138.7, 129.6, 126.9, 61.0, 60.6, 51.8, 50.8, 44.9, 42.7, 30.1, 22.3, 20.7. IR (cm^{-1}): 3385, 2954, 2869, 1622, 1466, 1063, 850. HRMS: 293.1998. Calcd. mass: 293.1991.

Enzymatic Polycondensation of *N, N*-Bis(2-hydroxyethyl)-2-arylpropanamide and Diesters

The polymerization reactions were performed in a diphenyl ether solution with a parallel synthesizer connected to a vacuum line. In a typical experiment, reaction mixtures containing equal numbers of moles of diester and amide-substituted diol monomers, Novozym 435 (20 wt % vs drug monomer), and diphenyl ether solvent were transferred to reaction tubes that were placed in an oil bath. The polymerization reactions were carried out after pressure was reduced to 1–2 mmHg, and the reactions were maintained at 85°C for 48 h. At the end of the reactions, the formed polymers were dissolved in THF and filtered to remove the enzyme catalyst. The polymer products were not fractionated by precipitation before the analysis of the molecular weight and structure. The filtrates containing whole products were analyzed by GPC with polystyrene standards to measure the polymer molecular weights. To determine the polymer structures, the product mixtures were dissolved in chloroform-d. The resultant solutions were filtered to remove catalyst particles and then analyzed by IR and ^{1}H-NMR spectroscopy.

Because of the high solubility of the polyester drugs in some polar organic solvents, such as THF, chloroform, and dichloromethane, except methanol, special purification procedures were used to isolate the polyesters from their corresponding product mixtures. Thus, hexane was added as a nonsolvent to the viscous mixture solutions three times to precipitate the polyesters and remove the diphenyl ether. Then, the oily polymeric drugs were dissolved in THF and filtered to remove the CAL-B particles. The residual high-boiling oligomers and monomers in the mixture were subsequently removed by repeated precipitation after the addition of methanol into their THF solution three times. Thereafter, purified poly(*N, N*-bis (2-hydroxyethyl)-2-arylpropanamide-*co*-esters) were obtained upon complete removal of the solvents at 50°C under high vacuum ($<$ 1.0 mmHg pressure) for 20 h. The structures of the products were characterized by FTIR spectroscopy, GPC, and ^{1}H-NMR.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-naproxenamide-*co*-succinate] (PNSN): 7.69 (d, 2 H), 7.60 (s, 1 H), 7.35 (d, 1 H), 7.10−7.13 (d, 2 H), 4.28 (s, 1 H), 4.14−4.20 (m, 2 H), 4.00−4.07 (m, 2 H), 3.88 (s, 3 H), 3.76 (s, 1 H), 3.59 (s, 1 H), 3.38 (s, 1 H), 3.26 (d, 1 H), 2.12−2.20 (d, 4 H), 1.62 (s, 4 H), 1.47 (s, 3 H). IR: 3455, 2971, 1733, 1645, 1606, 1158, 1031, 855, 814.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-naproxenamide-*co*-adipate] (PNAP): 7.69 (d, 2 H), 7.60 (s, 1 H), 7.35 (d, 1 H), 7.13−7.10 (d, 2 H), 4.28 (s, 1 H), 4.20−4.14 (m, 2 H), 4.07−4.00 (m, 2 H), 3.88 (s, 3 H), 3.76 (s, 1 H), 3.59 (s, 1 H), 3.38 (s, 1 H), 3.26 (d, 1 H), 2.20−2.12 (d, 4 H), 1.62 (s, 4 H), 1.47 (s, 3 H). IR: 2953, 1735, 1647, 1606, 1171.

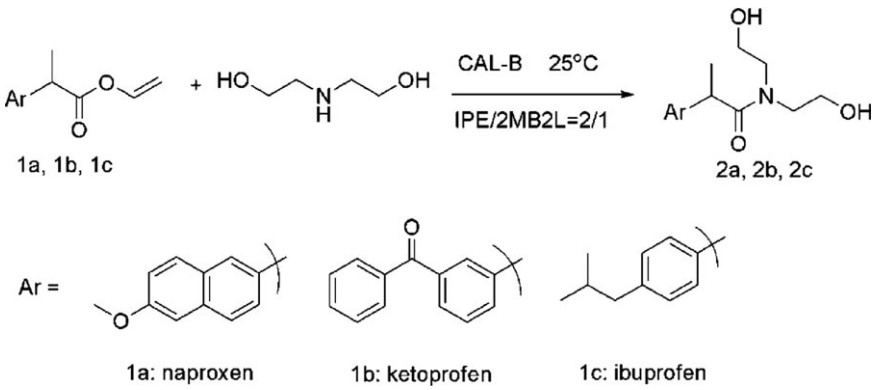
^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-naproxenamide-*co*-azelate] (PNAL): 7.68 (d, 2 H), 7.59 (s, 1 H), 7.35 (d, 1 H), 7.13−7.09 (d, 2 H), 4.29 (s, 1 H), 4.21−4.14 (m, 2 H), 4.08−4.02 (m, 2 H), 3.89 (s, 3 H), 3.77 (s, 1 H), 3.60 (s, 1 H), 3.38 (s, 1 H), 3.24 (d, 1 H), 2.20−2.12 (d, 4 H), 1.63 (s, 4 H), 1.48 (m, 3 H), 1.28−1.24 (m, 6 H). IR: 2937, 2857, 1740, 1437, 1198, 1172.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for PNSC: 7.67 (d, 2 H), 7.60 (s, 1 H), 7.36 (m, 1 H), 7.14−7.10 (m, 2 H), 4.29 (m, 1 H), 4.22−4.14 (m, 2 H), 4.08−4.01 (m, 2 H), 3.90 (s, 3 H), 3.77 (s, 1 H), 3.60 (s, 1 H), 3.39 (s, 1 H), 3.25 (d, 1 H), 2.25−2.12 (d, 4 H), 1.64 (s, 4 H), 1.49 (m, 3 H), 1.29−1.23 (m, 8 H). IR: 2931, 2855, 1736, 1648, 1606, 1170.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-ketoprofenamide-*co*-succinate] (PKSN): 7.74−7.70 (m, 3 H), 7.60−7.37 (m, 6 H), 4.17−4.04 (m, 4 H), 3.73−3.32 (m, 5 H), 2.55−2.43 (m, 4 H), 1.49−1.42 (s, 3 H). IR: 3454, 2972, 1736, 1651, 1158, 722.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-ketoprofenamide-*co*-adipate] (PKAP): 7.74−7.70 (m, 3 H), 7.60−7.37 (m, 6 H), 4.17−4.04 (m, 4 H), 3.73−3.32 (m, 5 H), 2.55−2.43 (m, 4 H), 1.49−1.42 (s, 3 H). IR: 2951, 1734, 1651, 1447, 1283, 1173, 722.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-ketoprofenamide-*co*-azelate] (PKAL): 7.76−7.71 (m, 3 H), 7.63−7.41 (m, 6 H), 4.17 (m, 3 H), 4.10−3.96 (m, 2 H), 3.79−3.70 (m, 2 H), 3.41−3.28 (m, 2 H), 2.25−2.16 (m, 4 H), 1.52 (m, 4 H), 1.44 (d, 3 H), 1.25−1.20 (m, 6 H). IR: 2951, 1734, 1651, 1283, 1173, 1075, 722.



Scheme 1. CAL-B catalyzed synthesis of profen monomers.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-ketoprofenamide-*co*-sebacate] (PKSC): 7.74−7.68 (m, 3 H), 7.61−7.37 (m, 6 H), 4.10−4.04 (m, 3 H), 4.04−3.96 (m, 2 H), 3.77−3.66 (m, 2 H), 3.37−3.26 (m, 2 H), 2.23−2.14 (m, 4 H), 1.52−1.46 (m, 4 H), 1.42 (d, 3 H), 1.20 (m, 8 H). IR: 2931, 2857, 1737, 1654, 1283, 1170, 721.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-ibuprofenamide-*co*-succinate] (PISN): 7.12 (m, 2 H), 7.06 (m, 2 H), 4.22 (s, 1 H), 4.16−4.12 (m, 2 H), 3.93 (m, 2 H), 3.79−3.65 (m, 2 H), 3.40 (m, 1 H), 3.25 (m, 1 H), 2.43 (d, J = 6.8, 1 H), 2.30 (s, 2 H), 2.21 (s, 2 H), 1.82 (m, 1 H), 1.61−1.58 (m, 4 H), 1.39 (m, 3 H). IR: 3442, 2956, 2865, 1737, 1649, 1168.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis (2-hydroxyethyl)-ibuprofenamide-*co*-adipate] (PIAP): 7.12 (m, 2 H), 7.06 (m, 2 H), 4.22 (s, 1 H), 4.15−4.11 (m, 2 H), 3.94 (m, 2 H), 3.79−3.64 (m, 2 H), 3.41−3.38 (m, 1 H), 3.27−3.22 (m, 1 H), 2.42 (d, J = 6.8, 1 H), 2.29 (m, 2 H), 2.21 (m, 2 H), 1.83 (m, 1 H), 1.63−1.58 (m, 4 H), 1.39 (m, 3 H), 0.87 (m, 6 H). IR: 2955, 2869, 1737, 1649, 1169, 1076.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis (2-hydroxyethyl)-ibuprofenamide-*co*-azelate] (PIAL): 7.12 (m, 2 H), 7.05 (m, 2 H), 4.21 (s, 1 H), 4.16–4.12 (m, 2 H), 3.94 (m, 2 H), 3.77 (m, 1 H), 3.64 (m, 1 H), 3.40 (m, 1 H), 3.25 (m, 1 H), 2.41 (d, J = 6.8, 1 H), 2.27 (m, 2 H), 2.18 (m, 2 H), 1.83 (m, 1 H), 1.62−1.57 (m, 4 H), 1.40 (m, 3 H), 1.27 (m, 6 H), 0.86 (m, 6 H). IR: 2930, 2865, 1737, 1650, 1461, 1168.

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm) for poly[*N, N*-bis(2-hydroxyethyl)-ibuprofenamide-*co*-sebacate] (PISC): 7.13 (m, 2 H), 7.06 (m, 2 H), 4.22 (s, 1 H), 4.16−4.12 (m, 2 H), 4.08 (m, 2 H), 3.93 (m, 2 H), 3.80 (m, 1 H), 3.65 (m, 1 H), 3.39 (m, 1 H), 3.23 (m, 1 H), 2.42 (d, J = 6.8, 1 H), 2.28 (m, 2 H), 2.19 (m, 2 H), 1.82 (m, 1 H), 1.60−1.56 (m, 4 H), 1.39 (m, 3 H), 1.27 (m, 8 H), 0.87 (m, 6 H). IR: 2929, 2858, 1737, 1649, 1462, 1167.

Enzymatic Synthesis of the Optically Active Polymeric Drug PNAL

Enzymatic acylation of 3 g of (*R*)-naproxen vinyl ester with 2,2′-azanediyldiethanol at a molar ratio of 1:3 was performed under the catalysis of 0.3 g of CAL-B in 9 mL of cosolvent of IPE/2MB2L = 2:1 v/v. The mixture was incubated at 25°C under shaking at 200 rpm for 24 h. The product was purified by silica gel column chromatography by elution with petroleum pure ethyl acetate. Then, 1.6 g of drug monomer and dimethyl adipate in an equal molar ratio was added to 2 mL of diphenyl ether containing 0.32 g of Novozym 435. The condensation reaction was performed under a pressure of 1–2 mmHg for 48 h at 85°C. Then, the oily mixture was dissolved in THF and filtered to remove the CAL-B particles. The residual high-boiling oligomers and monomers were subsequently removed by repeated precipitation after the addition of methanol into their THF solution three times. The obtained chiral polyester drug had an $[\alpha]_D^{25}$ of 2.2°.

In Vitro Release Studies

The chemical and enzymatic *in vitro* hydrolysis of the polyester drug PNSC was studied with pH 7.4 phosphate buffer, pH 7.4 phosphate buffer with CAL-B, and pH 1.2 HCl/KCl aqueous solution. The polyester samples for hydrolysis were suspended in 20 mL of buffer solution and shaken at 37°C. The samples were withdrawn at a regular time intervals and diluted with mobile phase for analysis by UV spectroscopy.

Degradation of PNSC

The chemical degradation of PNSC was studied in a cosolvent of THF/H_2O (pH 0) = 2/1 v/v. The polyester sample (20 mg of PNSC) was dissolved in 3 mL of cosolvent and shaken at 50°C. GPC was used to monitors the degradation process.

RESULTS AND DISCUSSION

Enzyme-Catalyzed *N*-Acylation between 2,2′-Azanediyldiethanol and Profen Vinyl Ester

The enzymatic synthesis of *N, N*-bis(2-hydroxyethyl) profenamide is shown in Scheme 1. The acylation of profen vinyl ester with 2,2′-azanediyldiethanol was catalyzed by CAL-B in cosolvent, where the diol can be well dissolved and CAL-B showed good activity. The products were purified by silica gel chromatography and characterized by FTIR spectroscopy, HRMS, and NMR spectrometries. The structural analysis confirmed the successful synthesis of the drug monomers containing profen drugs. For example, the FTIR spectra in Figure 1 clearly showed the *N*-acylation from the peak of 1622 cm^{-1} assigned to –C(O)-N. Meanwhile, the peaks at 3381 and 1063 cm^{-1} also

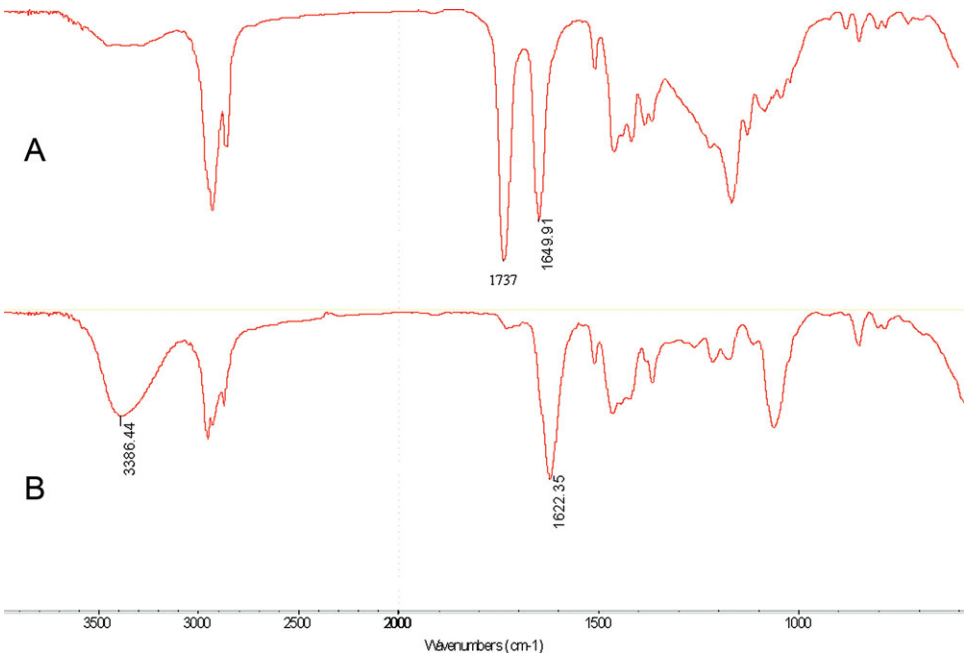
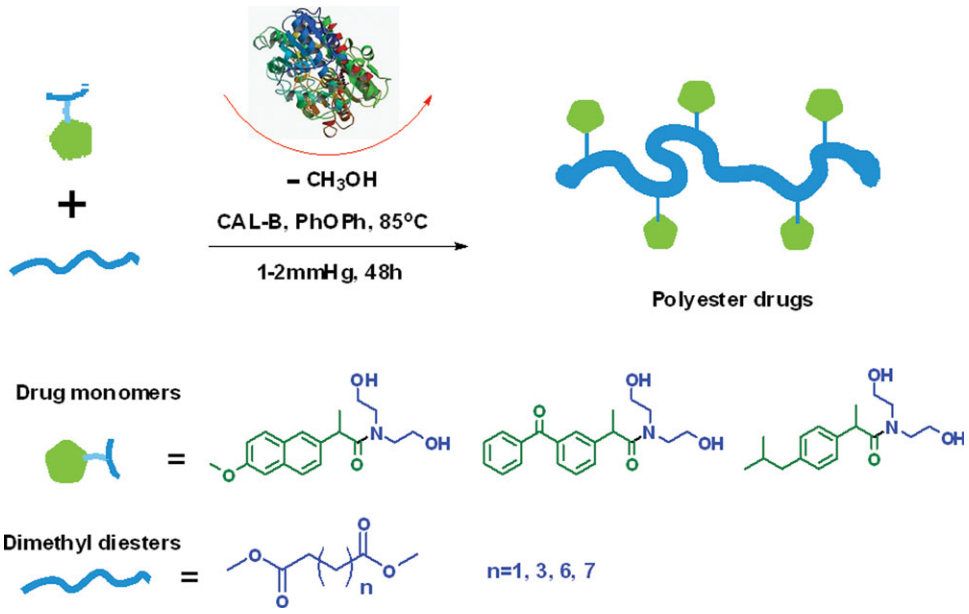


Figure 1. FTIR spectra of (A) PISC and (B) *N, N*-bis(2-hydroxyethyl) ibuprofenamide. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

indicated the incorporation of 2,2′-azanediyldiethanol into the polymerizable prodrug.

Enzyme-Catalyzed Polycondensation for the Synthesis of Copolymers

Three types of polymerizable prodrugs were obtained as described previously and were used as effective monomers for enzymatic polymerization. Four fatty acids (C4, C6, C9, and C10) were chosen to form the polyester chain because they were biocompatible both *in vitro* and *in vivo;*[20] this could be useful for drug-delivery systems and tissue engineering applications. The CAL-B catalyzed polycondensation reactions of polymerizable monomers and diesters are shown in Scheme 2. The reactions were carried out at 85°C at a molar ratio of 1:1 for 48 h with diphenyl ether as the solvent. The yields and molecular weight of the polymers are shown in Table I. Furthermore, (*R*)-naproxen was demonstratively introduced into the polyester by the enzymatic method, and the stereo configuration was retained; this prevented racemization in the



Scheme 2. CAL-B catalyzed condensation polymerization of *N, N*-bis(2-hydroxyethyl)-2-ary propanamide with diesters PhOPh: diphenyl ether. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. M_n and PD (M_w/Mn) Values for the CAL-B Catalyzed Copolymerization of *N, N*-Bis(2-hydroxyethyl)-2-arylpropanamide with Dimethyl Ester

Substrate			Polyester as formed			
Dimethyl diester	Diol	Name[a]	M_n	M_w/M_n	DP_w[b]	Drug payload (%)
Succinate	**2 a**	PNSN	2170	1.25	6	57.4
Adipate	**2 a**	PNAP	3760	1.17	10	53.6
Azelate	**2 a**	PNAL	9070	1.60	40	48.8
Sebacate	**2 a**	PNSC	13,270	1.64	58	47.4
Succinate	**2 b**	PKSN	3270	1.20	9	59.7
Adipate	**2 b**	PKAP	4070	1.20	11	54.9
Azelate	**2 b**	PKAL	8390	1.44	24	51.3
Sebacate	**2 b**	PKSC	6490	1.34	17	49.9
Succinate	**2 c**	PISN	5520	1.56	23	54.6
Adipate	**2 c**	PIAP	2820	1.38	10	50.8
Azelate	**2 c**	PIAL	5830	1.66	22	46.1
Sebacate	**2 c**	PISC	6740	2.24	34	44.7

Reaction conditions: 1:1 molar ratio of diester to *N, N*-bis (2-hydroxyethyl)-2-arylpropanamide (**2 a**-**2 c**), CAL-B 20 wt % of diol, diphenyl ether as the solvent, 85°C, 1–2 mmHg vacuum, and 48 h for polymerization.

[a]The abbreviations used for naming the polymers are described in the Experimental section.

[b]Weight-average degree of polymerization.

polymerization reactions catalyzed by metal catalysts under harsh conditions.

After the synthesis of a series of copolymers, their molecular structures were analyzed and confirmed by both IR and ^{1}H-NMR spectroscopy. For typical examples, Figure 1 shows the FTIR spectra of *N, N*-bis(2-hydroxyethyl) naproxenamide and PISC. The peak at 3386 cm^{-1} was assigned to –OH of *N, N*-bis(2-hydroxyethyl) naproxenamide, whereas this signal was hardly visible in PIAL. The peak appearing at 1737 cm^{-1} in PIAL due to –C(O)O also confirmed the formation of polyester. The disappearing peak at 3386 cm^{-1}, attributed to –OH of the monomer, was more proof of polymerization. Figure 2 indicates that the ibuprofen monomer was incorporated into the polyester. In this spectrum, a small triplet (signal d) at 3.64 ppm were attributed to –C(O)O-;CH_3 end groups, whereas signals a, b, and c were assigned to hydrogens of the diester chain.

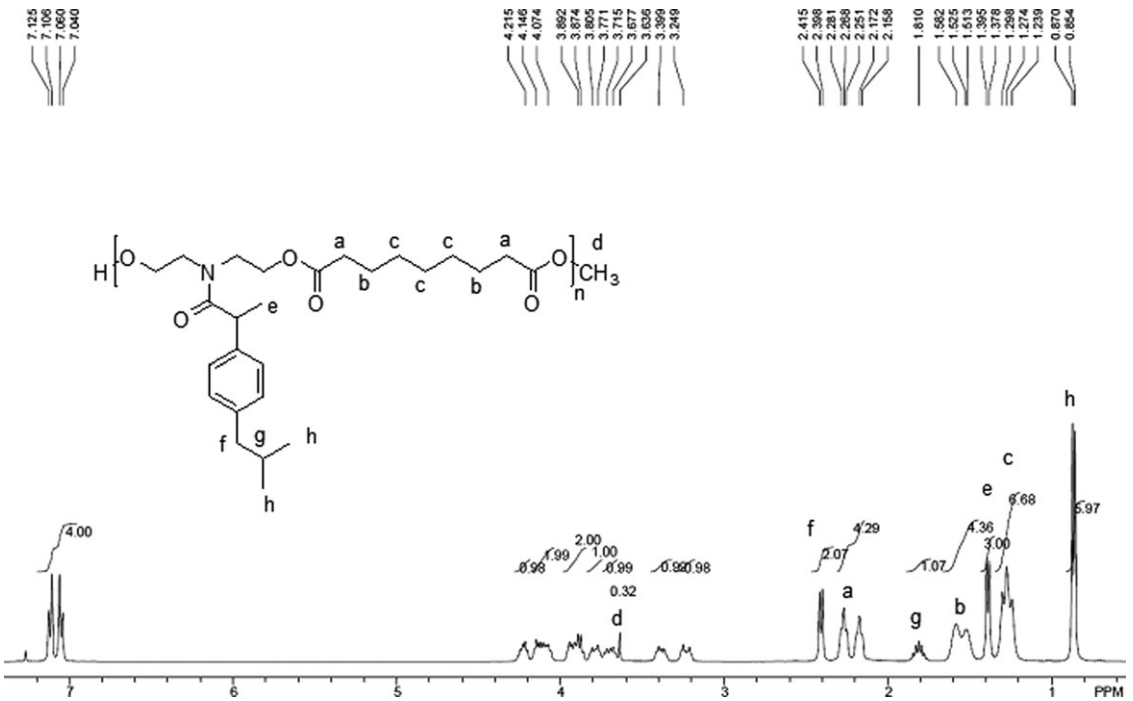


Figure 2. ^{1}H-NMR (400-MHz) spectrum of PIAL recorded in chloroform-d.

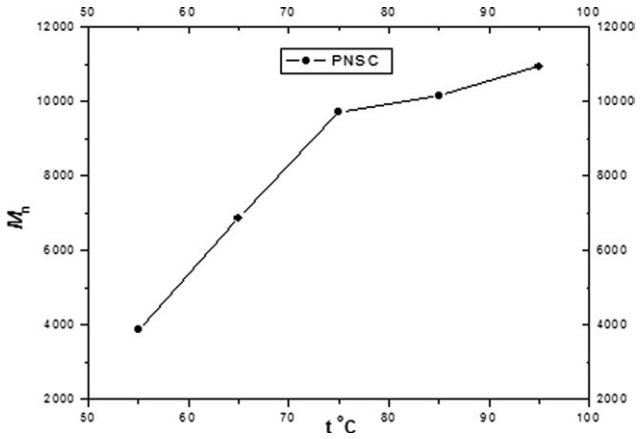


Figure 3. Product molecular weight versus the reaction temperature for the Novozym435-catalyzed copolymerization of *N, N*-bis(2-hydroxyethyl) naproxenamide with dimethyl sebacate to form PNSC. Reaction conditions: 1:1 molar ratio of sebacate to *N, N*-bis(2-hydroxyethyl) naproxenamide, CAL-B 20 wt % of diol, diphenyl ether as the solvent, 1–2 mmHg vacuum, and 48 h for polymerization, "t" for tempterature.

Furthermore, the signals f, g, h, and e belonged to the hydrogens of the ibuprofen group.

After similar structure analysis with FTIR spectroscopy and NMR as PIAL, a series of polyester drugs with different chain structures were confirmed. Table I summarizes the results of enzymatic polymerizations between the C4–C10 diesters and *N, N*-bis(2-hydroxyethyl)-2-arylpropanamide. The formed polymers had M_n's in the range from 2170 to 13,270 and polydispersities (PDs) in the range 1.17−2.24. Among all of the synthesized polyester drugs, PNSC gave the highest M_n of 13,270, whereas PNSN gave the lowest M_n of 2170. Most PD values were below 2 except PISC. More than half of polyester drugs have drug payloads higher than 50%. These results clearly show that the enzymatic synthesis of polyester drugs described previously appeared quite general; this accommodated both short-chain (e.g., C4) and long-chain (e.g., C10) diester monomers and diol substrates with different profen substituents (naproxen, ibuprofen, and ketoprofen).

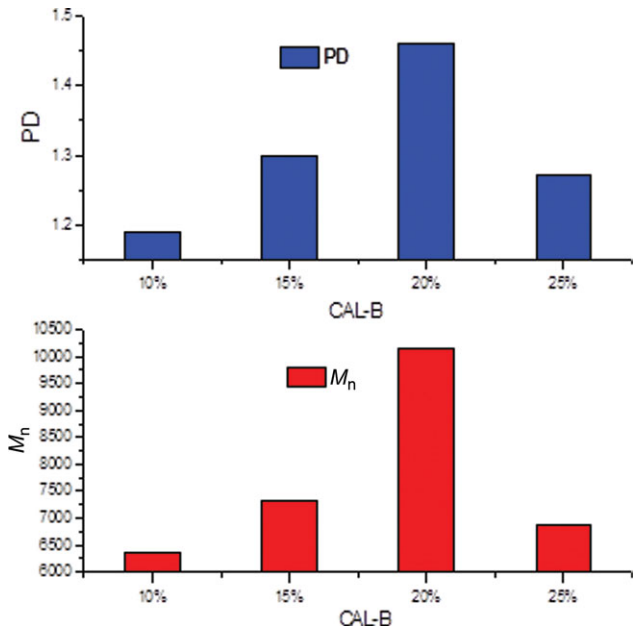


Figure 4. Polymeric M_n and PD versus the enzyme amount for the CAL-B catalyzed copolymerization of *N, N*-bis(2-hydroxyethyl) naproxenamide with dimethyl sebacate to form PNSC. Reaction conditions: 1:1 molar ratio of sebacate to *N, N*-bis(2-hydroxyethyl) naproxenamide, diphenyl ether as the solvent, 85°C, 1–2 mmHg vacuum, and 48 h for polymerization. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Enzyme Screening of Lipase-Catalyzed Copolymerization of *N, N*-bis(2-hydroxyethyl) Naproxenamide with Dimethyl Sebacate to form PNSC

Entry	Lipase	M_n
1	CAL-B	13,274
2	MML	<1000
3	CCL	<1000
4	CRL	<1000

Reaction conditions: 1:1 molar ratio of sebacate to *N, N*-bis(2-hydroxyethyl) naproxenamide, 85°C, lipase 20 wt % of diol, diphenyl ether as the solvent, 1–2 mmHg vacuum, and 48 h for polymerization.

Effect of the Reaction Temperature on the Copolymerization of Anti-Inflammatory Drug Monomers and Diesters

The effects of the reaction temperature on the polymer molecular weight and PD were studied with the copolymerizations of dimethyl sebacate with *N, N*-bis(2-hydroxyethyl) naproxenamide as a model. The polycondensation temperatures were changed from 55 to 95°C. The reactions were carried out in diphenyl ether with a 1:1 molar ratio of the diesters to *N, N*-bis(2-hydroxyethyl) naproxenamide and 20 wt % CAL-B catalyst under a 1–2 mmHg vacuum for 48 h. Figure 3 summarizes the M_n values of PNSC formed at different temperatures. We found that increases in molecular weight in the temperature range 55–75°C were faster than those at 75–95°C, and the desirable reaction temperature for the copolymerizations was in the range 75–95°C, where products with M_n's above 9000 were easily formed. The polymerization reaction of the profen diols and diesters tended to give a higher molecular weight at the higher tested temperature.

Effect of the Enzyme Amount on the Enzymatic Copolymerization

Figure 4 summarizes the M_n values (PNSC) and PD values of the copolymers formed under different catalyst amounts ranging from 10 to 25%. The molecular weights could be improved when the catalyst amount increased from 10 to 20%. The highest molecular weight of 10,150 was obtained when 20 wt % CAL-B was added. However, when the catalyst amount reached 25%, the polyester drug molecular weight decreased quickly to 6860. The result implied that 20 wt % CAL-B (vs drug monomer) was a suitable catalyst loading. The PDs of the four copolymers were in the range 1.13–1.46; this showed a similar change tendency to the molecular weight when different catalyst

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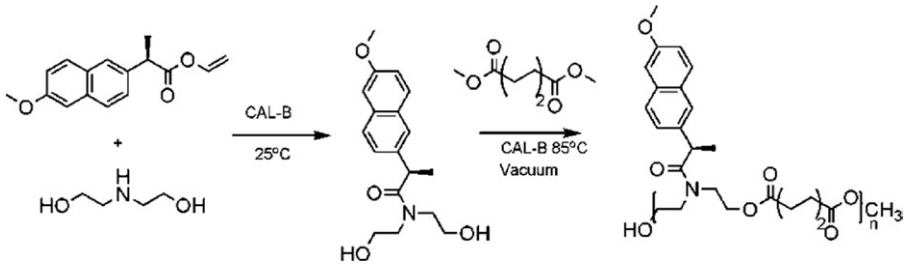

Figure 5. CAL-B catalyzed synthesis of optically active PNAL.

loadings were used. The copolymers with high molecular weights had a high viscosity, which resulted in a high PD.

Effect of the Enzyme on the Copolymerization of the Drug Monomer and Diester

Table II summarizes the molecular weight of the copolymers formed by four different lipases. CAL-B showed a remarkable catalytic efficiency. However, in other polymerization reactions catalyzed by MML, CRL, and CCL, no precipitation was generated after the addition of methanol into the mixture, and all of the molecular weights were below 1000. To determine whether the polymerization reactions were indeed catalyzed by CALB, a control experiment with dimethyl sebacate and *N, N*-bis(2-hydroxyethyl) naproxenamide as representative monomers were performed without CAL-B in diphenyl ether under identical conditions (equal molar ratio of the diester to the drug monomer at 85°C under 1–2 mmHg of pressure for 48 h). No precipitation was detected when methanol was added to the reaction solvent and the polymer M_n was below 1000. This proved that CALB indeed catalyzed the condensation polymerization of dimethyl sebacate and profen diols.

Enzymatic Synthesis of the Optically Active Polymeric Drug PNAL

As we know, the enantiomers of a chiral drug candidate often have differences in their physiological, pharmacokinetic, and metabolic activities, and toxicology. After the preparation of all of the copolymers with racemic drugs, an optically active polymeric drug containing (*R*)-naproxen was also successfully synthesized with the enzymatic method, as shown in Figure 5. The process was mild and environmental friendly. Also, the catalyst was nontoxic and recyclable. Therefore, the enzymatic method of introducing the enantiomeric purity of chiral drugs into certain polymers may play an important role in the pharmaceutical industry.

In Vitro Hydrolysis of the Polyester Drug

The *in vitro* hydrolysis behavior of the polyester drug PNSC was investigated with pH 7.4 phosphate buffer, pH 7.4 phosphate buffer with CAL-B, and pH 1.2 HCl/KCl aqueous solution As shown in Figure 6the release amount of naproxen was 17.5% within 23 h in pH 7.4 phosphate buffered saline (PBS) because of the slow rate of polyester degradation. To study the enzymatic hydrolysis of the polyester drug, CAL-B was introduced in the pH 7.4 PBS, and we observed releases of 70% over 1.2 h and 98% over 23 h. In the study, PNSC showed a release of 13% for 2.2 h in a pH 1.2 HCl/KCl aqueous solution, which was better than that at pH 7.4. Drug release followed thereafter became slow: less than 30% after 23 h.

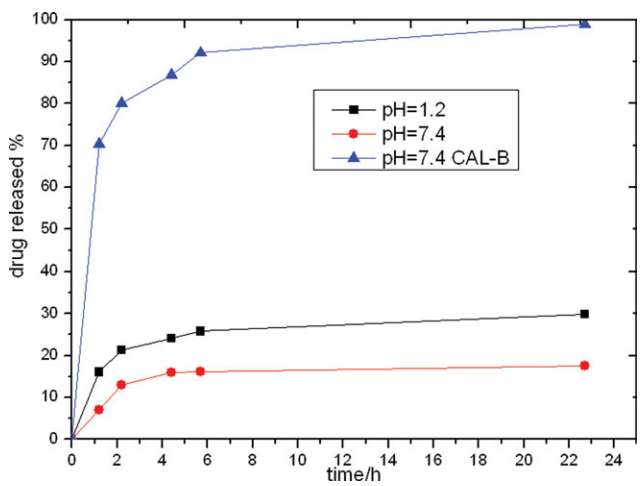

Figure 6. Release of the drug from PNSC into pH 7.4 phosphate buffer or pH 1.2 KCl/HCl buffer (with or without CAL-B added) at 37°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

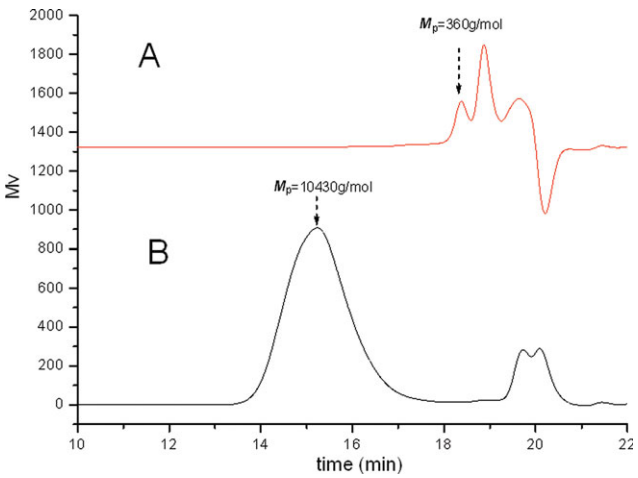

Figure 7. GPC data of (B) prodrug PNSC and (A) its products after degradation in 2 mol/L HCl for 24 h, M_p: peak molecular weight, M_v is the value of voltage which is interpreted by the instrument from the refractive index differentials between sample and reference source. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Degradation of PNSC

The degradation of the polymeric prodrug was investigated with a 2 mol/L HCL aqueous solution at 50°C. As shown in Figure 7compared with M_n of PNSC (9940 g/mol), no PNSC was left in the solution, and the molecular weight of degraded products was below 500 g/mol after 24 h of hydrolysis. The results demonstrate that these kind of polymeric prodrugs are biodegradable in nature.

CONCLUSIONS

Twelve polymeric prodrugs bearing profen moieties were successfully prepared via CAL-B. Their structures were clearly verified by FTIR spectroscopy, NMR, and GPC. The effects of different reaction conditions, such as the enzyme source, amount of catalyst, and temperature, were investigated. The resulting copolymers had moderate to high molecular weights (2170–13,270 g/mol) and small PDs. A high drug loading (44.7–59.7 wt %) was one important advantage of these copolymers bearing one drug molecule for every repeat unit. Also, because of the mild reaction conditions, the copolymer containing optical pure naproxen prevented side reactions. The degradation of PNSC illustrated that the prodrugs are totally biodegradable in nature. The hydrolysis under physiological conditions was investigated, and the drug release from the polyester was slow. CAL-B accelerated the drug release, which indicated that this polyester could be a promising nonsteroidal anti-inflammatory drug (NSAID) prodrug with extended pharmacological effects by delayed release of the parent drug. The poly(amide-*co*-ester) prodrugs of NSAIDs could be further functionalized with PEG, targeting groups, and quaternary ammonium compound; this endowed some special properties, such as amphiphilic micellization, a targeting drug-delivery system, and DNA transfection. Further studies concerning polymeric prodrugs are in progress.

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