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Synthesis and properties of a naproxen polymeric prodrug

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Abstract

A water-soluble polymeric prodrug containing a naproxen moiety was synthesized. The carboxylic groups of naproxen were condensed with the hydroxyl groups of 2-hydroxyethyl methacrylate (HEMA) to produce a drug-linked monomer, denoted HN. The polymeric prodrug was prepared by copolymerization of HN with methacrylic acid. The molar percentage of HN in the polymeric prodrug was 26 mol %, as determined by ^{1}H NMR. To investigate the pertinence of this polymeric prodrug, the hydrolysis was studied in-vitro with or without esterase or lipase. The kinetics of enzymatic catalysis was calculated from a Lineweaver–Burk plot. The anti-inflammatory activity was evaluated using the carrageenan-induced oedema test. The polymeric prodrug released a major fraction of the free naproxen and a significant fraction of the hydroxyethyl ester derived-naproxen. The maximum hydrolysis rate V_{max}, and the Michaelis constant K_m were calculated to be 2.16×10^{-5} equiv. mol L^{-1} min^{-1} and 5.11×10^{-2} equiv. mol L^{-1}. The maximum anti-inflammatory inhibition of free naproxen appeared at 2 h and quickly decreased thereafter. In contrast, the polymeric prodrug showed a maximum at around 2~3 h and then slowly decreased. This indicates that the polymeric prodrug displays greater potency than free naproxen in the inhibition of acute inflammatory processes over long periods.

Introduction

Naproxen is a non-steroidal anti-inflammatory drug (NSAID) associated with gastrointestinal side-effects, particularly stomach ulceration, bleeding and perforation (Guslandi 1997). The action of NSAIDs is thought to involve the inhibition of cyclooxygenases, responsible for prostaglandin synthesis, which controls pain and inflammation in rheumatic diseases (Heyneman et al 2000). The main disadvantage of NSAIDs is a relatively short plasma half-life, which results in a short duration of activity, and a pronounced ulcerogenic potency. To reduce the gastrointestinal symptoms and prolong the drug activity, a recent approach has been the concept of retrometabolic drug design that incorporates targeting and metabolic considerations into the design processes. Accordingly, the carboxylic group of the NSAID can be temporarily masked and its direct effect on the gastric mucosa will be prohibited. Ester prodrugs of naproxen have been designed using *N*-hydroxymethylsuccinimide and *N*-hydroxymethylisatin as pro-moieties to reduce their gastrointestinal toxicity and improve bioavailability (Mahfouz et al 1999). Similarly, naproxen has been bonded to dextran polymer with varying degrees of substitution (Harboe et al 1988). The hydrolytic degradation of naproxen–dextran ester prodrug follows strict first-order kinetics (Larsen 1989). The bioavailability of the naproxen–dextran ester prodrugs after oral administration of aqueous solutions to pigs has been studied (Harboe et al 1989; Larsen et al 1989). Recently, polyoxyethylene esters of ketoprofen, naproxen and diclofenac were prepared (Bonina et al 2001). Naproxen was also coupled via its carboxylic group directly to lysinic amino groups of human serum albumin (HAS) and mannosylated HAS (Albrecht 1996).

To summarize the above literature, various NSAID prodrugs have been studied with the aim of sustaining site-specificity, delaying release of the drug, improving the anti-inflammatory effect or reducing unwanted side-effects. In this work, the synthesis of a

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water-soluble prodrug containing the naproxen moiety is described. The water-soluble methacrylic acid is introduced as the counter monomer in the synthesis of polymer–drug conjugate. The interest in methacrylic ester and its methacrylic acid copolymer stems largely from its pH sensitivity in the digestive tract. A typical example using carboxylic acid groups in drug-controlled release is Eudragit S-100, which needs to contain 27.6∼30.7 mol% of methacrylic acid for it to be dissolved in the intestinal tract. Drugs that produce side-effects in the stomach (e.g. by irritating the gastric mucosa or by inducing nausea and vomiting) could be coated with, or embedded in, this acrylic resin so that they could pass through the acidic gastric region without undergoing any change. The same strategy was adapted to our polymeric prodrug to prevent ulcers arising from the use of NSAIDs. To pursue this characteristic property, the drug content was designed to be less than 30 mol%, to maintain solubility in the intestine. This article describes the synthesis of a polymeric prodrug with a 26 mol% drug-linked monomer content. The dissolution of this polymeric prodrug in buffer solutions of differing pH was tested, and the hydrolysis kinetics with or without enzymes were also described. The therapeutic effect of the polymeric prodrug was evaluated using a carrageenan-induced oedema test.

Materials and Methods

Materials and purification

2-Hydroxyethyl methacrylate (HEMA) and 1, 3 dicyclohexyl carbodiimide (DCC) were purchased from Aldrich Chemical Company, Inc. Azobisisobutyronitrile (AIBN) was obtained from Aldrich Chemical Company, Inc., and recrystallized twice from methanol. Methacrylic acid and thionylchloride and triethylamine from Janssen Chimica were respectively distilled before use. Ethylene glycol was purchased from Janssen Chimica and dried with sodium powder before distillation. Naproxen from ICI Chemical Company was recrystallized from acetone–hexane, 1:1 v/v. Methylene chloride obtained from Fisher Scientific was refluxed with calcium hydride overnight before distillation. 4-Dimethylamino pyridine (DMAP) was obtained from E. Merck. Esterase from porcine liver suspension in 3.2 M $(NH_4)_2SO_4$ solution and lipase (type II) from porcine pancreas were purchased from Sigma. All other reagents were purchased from Fisher Scientific unless otherwise stated.

Compound characterization

^{1}H NMR spectra were recorded on a Varian, Germini-200 VT spectrometer in d_6-DMSO or $CDCl_3$. FT-IR spectra were performed using a Perkin-Elmer System 2000 apparatus. Sixteen scans were single-averaged at a resolution of 4 cm^{-1}. UV-VIS spectra were obtained using a Shimadzu UV-160A. Elemental analysis was conducted using a Heraeus CHN-O-Rapid Analyzer. Mass spectral analysis was carried out with a VG Biotech Quattro 5022 using electron ionization with an energy of 70 eV. HPLC analysis of naproxen and its derivative was recorded on a Hewlett-Packard 1050 system containing a quaternary pump, online degassing, autosampler and HP 1100 photodiode array detector, and equipped with a C_{18} column (HP Spherisob ODS-2 column). Samples were filtered with 0.45 μm Millipore filters and eluted with phosphoric acid solution (pH 3.0)–acetonitrile, 55:45 v/v at 1 mL min^{-1}. The eluent was monitored at 245 nm and the column oven was set at 50°C. The molecular weight of the polymeric prodrug was measured by gel permeation chromatography (GPC) using a Waters Model 501 containing 4 Shodex KF-800 series columns with the exclusion limit from 1.5×10^3 to 4×10^5. Tetrahydrofuran of HPLC grade was used as the eluent at a flow rate of 1 mL min^{-1}. The column setting was calibrated by using 10 monodisperse polystyrene standards obtained from Polyscience.

Synthesis of naproxen-linked 2-hydroxyethyl methacrylate (HN)

HN was prepared by dissolving 11.5 g (0.05 mol) of naproxen and 0.61 g (0.005 mol) of DMAP in 250 mL of ethyl acetate. A solution of 10.3 g (0.05 mol) of DCC in 100 mL of ethyl acetate was added drop-wise to the above solution at −20°C under nitrogen in 60 min, and then a solution containing 5 mL HEMA in 50 mL ethyl acetate was added slowly within 60 min. The reaction mixture was warmed up to room temperature and stirred overnight. The precipitate was filtered and the organic layer was sequentially extracted by 10% w/w of $NaHCO_3$ three times, 2 M HCl twice, deionized water once, and a saturated solution of NaCl once. The extracted solution was dried over $MgSO_4$. A white solid product was obtained by evaporating ethyl acetate in the vacuum to yield 10.5 g (61%). The solid was purified by column chromatography (70–130 mesh silica gel purchased from Merck as a stationary phase and ethyl acetate–hexane, 1:4 v/v, as a mobile phase). After removal of solvents, the white solid was collected with a 30% yield (m.p. 66±0.5°C). Analyses: calculated for $C_{20}O_5H_{22}$: C, 70.09; H, 6.43; found: C, 70.21; H, 6.47. IR (KBr, cm^{-1}): 2990 (C—H), 1739 (C═O), 1711 (C═O), 1633 (C═C), 1605 (C═C), 1189 (C—O). ^{1}H NMR ($CDCl_3$, δ): 7.00–8.00 (m, 6H, ArH); 5.85 (s, H, trans-H); 5.55 (s, 1H, cis-H); 4.27 (m, 4H, -OCH_2CH_2O-); 3.93 (q, 1H, -Φ-$CH(CH_3)$-); 3.86 (s, 3H, -OCH_3); 1.73 (s, 3H, C═C-CH_3); 1.47 (d, 3H, -(Φ)CH-CH_3). UV/VIS (dioxane): λ_{max} = 243.8 nm.

Synthesis of naproxen hydroxyethyl ester (Nap-EtOH)

A solution of 0.0048 mol of naproxen and 0.0097 mol of thionylchloride in 10 mL of methylene chloride was refluxed for 2 h. The excess thionylchloride and solvent was removed under a vacuum line. The dried residue was dissolved in 10 mL of methylene chloride and added drop-wise to a solution containing 0.0388 mol of ethylene glycol and 0.0048 mol of triethylamine in 15 mL of methylene chloride at 0°C. The reaction mixture was slowly returned to room temperature and continued for 3 h. The organic

layer was sequentially extracted by 1 M HCl twice, 10% w/w of $NaHCO_3$ twice, deionized water once and a saturated solution of NaCl once. The extracted solution was dried over $MgSO_4$ and the solvent was removed by a rotary vacuum evaporator. The yellow liquid was purified by column chromatography (70–130 mesh silica gel purchased from Merck as a stationary phase and ethyl acetate–hexane, 1:4 v/v, as a mobile phase). The final product was a white solid with a yield of 46% with a mass spectrum of 274 (molecular ion), 185 (M^+ -$CO_2CH_2CH_2OH$). Analyses: calculated for $C_{16}O_4H_{18}$: C, 70.06; H, 6.61; found: C, 69.87; H, 6.64. IR (NaCl, cm^{-1}): 2976 (sp^3 C-H), 1726 (C═O), 1607 (ring C═C), 1197 (C—O). ^{1}H NMR ($CDCl_3$, δ): 1.60 (d, 3H, -ΦCHCH_3), 3.75 (m, 3H, ΦC$\underline{H}$CH_3 and C$\underline{H_2}$OH), 3.90 (s, 3H, -OCH_3), 4.20 (m, 2H, CO_2CH_2), 7.10–7.75 (m, 6H, ArH). UV/VIS (dioxane): λ_{max} = 245 nm.

Copolymerization

The 1:3 molar ratio of HN–methacrylic acid in feed was used for copolymerization. The 33% w/w monomer concentration in dioxane was prepared. After deoxygenating by alternate connection of the polymerization vessel to vacuum and nitrogen gas, 2% w/w of AIBN to the total weight of monomers was added. Copolymerization was carried out for 3 h at 65°C under nitrogen. HN–methacrylic acid copolymer was precipitated into methanol–water, 1:2 v/v. The copolymer was filtered and dried with a 40% yield.

Polymeric prodrug hydrolysis without enzyme

Each sample (5–30 mg) was dissolved in 10 mL of 0.1 M pH 7.4 phosphate buffer solution at 37°C. At certain intervals, the solvent was removed by freeze-drying under vacuum. The drug and drug-EtOH were extracted from the polymer residue with 20 mL of acetone. The supernatant acetone solution was then withdrawn and evaporated using a rotary vapor evaporator to produce a white solid. The white solid was dissolved in 1 mL of ethanol and analysed by HPLC. Quantities of the hydrolysates in the solution were determined by comparing the HPLC areas with the calibration curves, which were obtained from known concentrations of standards. The correlation coefficients of standard curves were ~ 0.999.

Polymeric prodrug hydrolysis with enzymes

The prodrug hydrolysis with enzymes was conducted using a procedure similar to that stated above, except that the enzyme was added at a concentration of 50 μL containing 23 U esterase, or 1 mg of lipase containing 61 U activities.

Anti-inflammatory activity

The polymeric drug anti-inflammatory activity was evaluated using carrageenan-induced oedema test on rat paws according to the technique reported by Winter et al (1962). Female Wistar rats (150–200 g) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Rats were divided into three groups of 8 each. Group I served as a control group without using drugs. Groups II and III received naproxen (7.0 mg kg^{-1}) and the polymeric prodrug (42 mg kg^{-1}), respectively, where the dose was molecularly equivalent to the free drug. Drugs were administered as a homogeneous microsuspension in an aqueous solution of Tween-80 (10% w/v) intraperitoneally. Thirty minutes after administration, each rat received in its right hind paw a subplantar injection of a 0.8% carrageenan in normal saline (λ-carrageenan, type IV, Sigma, 0.1 mL/rat). The measurement of the hind-paw volume was carried out using a Ugo Basile Plethysmometer model 7150, before any treatment (V_o) and in any intervals (V_t) after the injection of the drugs. All results were expressed as means ± s.e.m. Statistical evaluations were performed using analysis of variance followed by the Newman–Keul's test for subgroup comparison (level of significance $P < 0.01$).

Results and Discussion

Synthesis of monomer and polymeric prodrug

In the HN synthesis, the white precipitate was isolated when DCC was added into the drug-contained solution. This precipitate was assigned to be a proton-ionized DCC intermediate by ^{1}H NMR spectroscopy. Since it was difficult to remove the cyclohexylurea from the final product HN by simple recrystallization, column chromatography was used for purification. The same method was adapted to synthesize Nap-EtOH, but the naproxen was linked to OH groups of ethylene glycol at two ends (i.e., Nap-Et-Nap). The Nap-EtOH was thus prepared by modifying the reported method (Weber & Meyer-Trûmpener 1994). The acyl derivative of naproxen was prepared and then esterification with ethylene glycol was added in the presence of triethylamine. The Nap-EtOH was obtained with a quantitative yield comparable with that reported in the literature. However, the excess ethylene glycol was required to prevent the disubstitution of naproxen on the two-hydroxyl groups. Also, the reverse addition of equal molar ratio of ethylene glycol into acyl naproxen led to the formation of disubstituted drug compound.

The HN molar percent in polymeric prodrug was calculated from ^{1}H NMR spectral data, as shown in Figure 1. The ratio of the peaks around 7–8 ppm, corresponding to six aromatic protons from HN to the total area between 0.4 and 2.2 ppm, which were attributed to 8 protons in HN and five protons in methacrylic acid (marked by an asterisk in the structural formula as depicted in Figure 1). The molar percent of HN can be calculated from the following equations:

$$I_a/I_b = 6x/(8x+5y) \quad (1)$$

$$x + y = 1 \quad (2)$$

where I_a is the area of aromatic protons, I_b is the area of aliphatic protons marked by an asterisk in Figure 1, x is

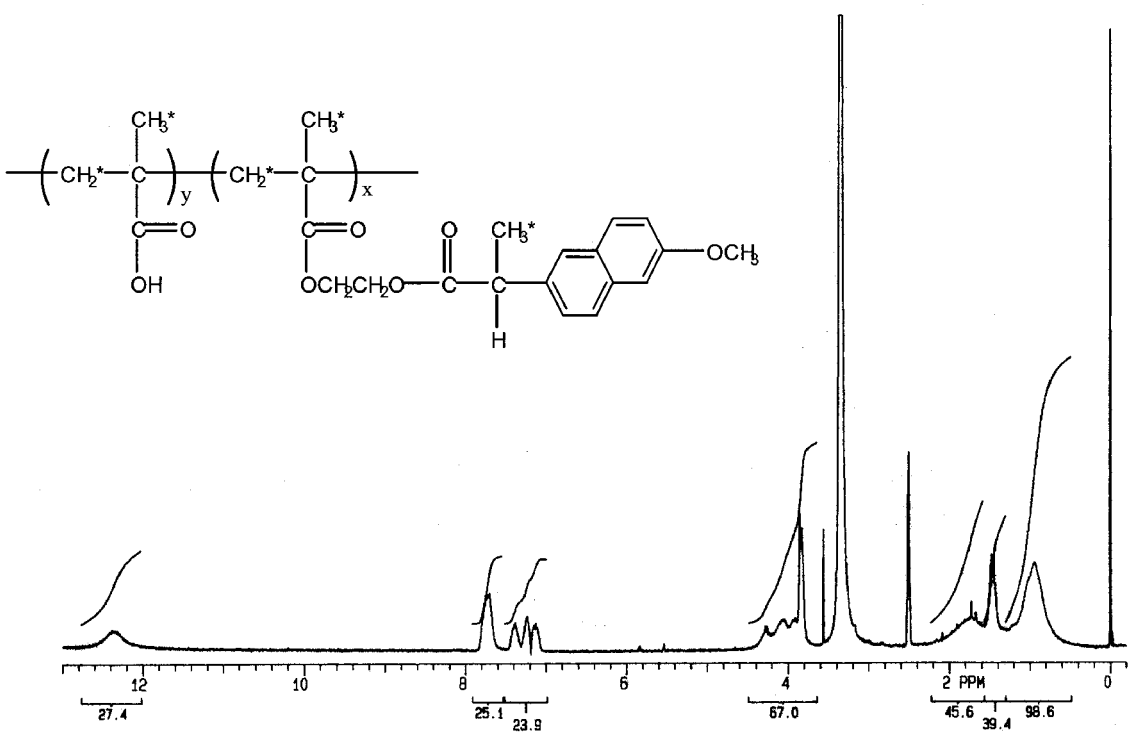

Figure 1 ^{1}H NMR spectra of the polymeric prodrug of naproxen.

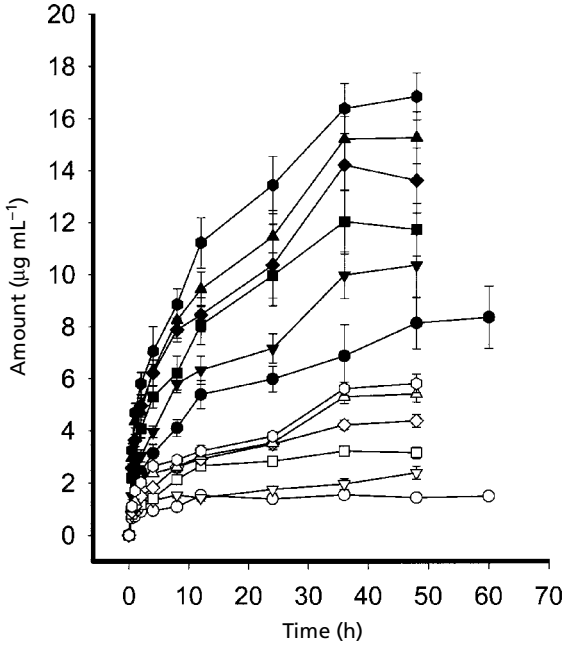

Figure 2 Hydrolysis profiles of various amounts of the polymeric prodrug in 10 mL phosphate buffer, pH 7.4. ●, 5 mg; ▼, 10 mg; ■, 15 mg; ◆, 20 mg; ▲, 25 mg; ⬢, 30 mg. The open and filled legends represent Nap-EtOH and naproxen, respectively.

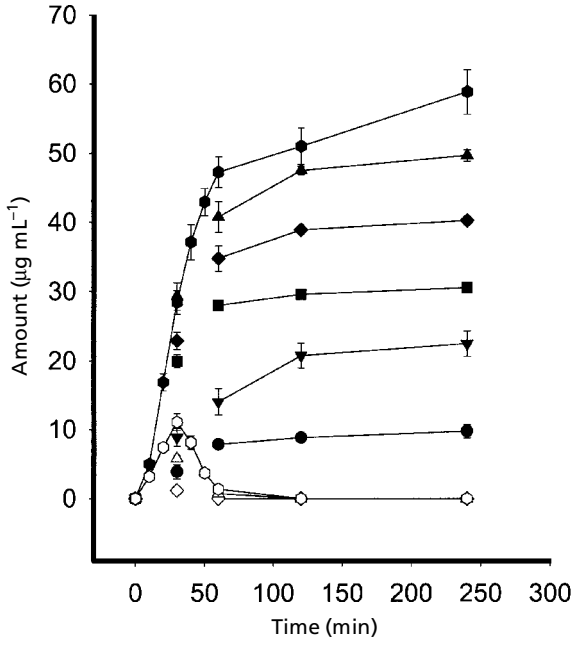

Figure 3 Hydrolysis profiles of various amounts of the polymeric prodrug in 10 mL phosphate buffer, pH 7.4 with esterase. ●, 5 mg; ▼, 10 mg; ■, 15 mg; ◆, 20 mg; ▲, 25 mg; ⬢, 30 mg. The open and filled legends represent Nap-EtOH and naproxen, respectively.

the molar fraction of HN and y is that of methacrylic acid. The HN molar percentage was found to be 26%, which was equivalent to 37.3% (m/m) of free naproxen in the polymeric prodrug. The number-average molecular weight of the polymeric prodrug was 13000, its polydispersity was 4.4 and the water solubility was 0.128 g L^{-1} at 37°C.

Polymeric prodrug hydrolysis in the pH 7.4 phosphate buffer solution

A typical example using carboxylic acid groups in the drug-controlled release was Eudragit S-100, which needs to contain 27.6～30.7 mol% of methacrylic acid for it to be dissolved in the intestinal tract. Drugs that produce side-effects in the stomach (e.g. by irritating the gastric mucosa or by inducing nausea and vomiting) could be coated with, or embedded in, this acrylic resin so that they could pass through the acidic gastric region without undergoing any change in the drug. The same strategy was adapted for our polymeric prodrug to prevent ulcers induced by NSAIDs. To pursue this characteristic property, the dissolution of this polymeric prodrug in different pH buffer solutions was conducted. The absorbance of a compound in the completely dissolved condition was taken as being 100% dissolution. The dissolution percentage at a certain pH value was calculated from the relative ratio of absorbance to that at 100%. The polymeric prodrug was stable at pH values below 6 and completely dissolved at pH 7 (100% dissolution was observed). This implied that the polymeric prodrug was potentially resistant to gastric juices.

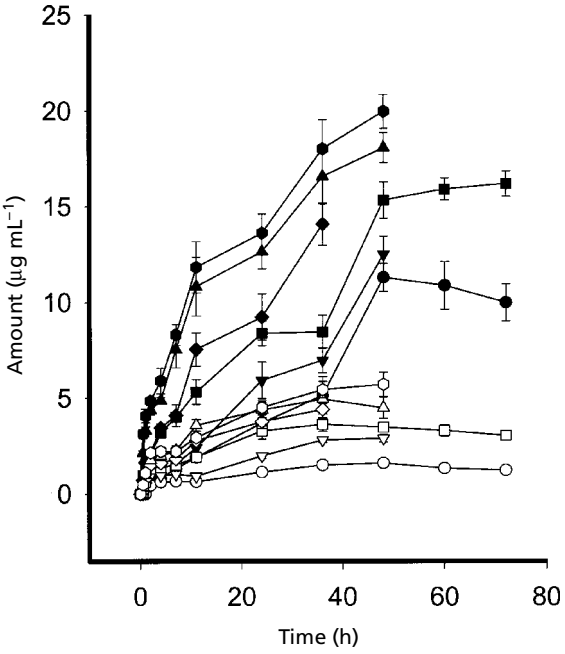

Figure 4 Hydrolysis profiles of various amounts of the polymeric prodrug in 10 mL phosphate buffer, pH 7.4 with lipase. ●, 5 mg; ▼, 10 mg; ■, 15 mg; ◆, 20 mg; ▲, 25 mg; ⬢, 30 mg. The open and filled legends represent Nap-EtOH and naproxen, respectively.

Figures 2, 3 and 4, respectively, illustrate the accumulated amount of naproxen and Nap-EtOH with respect to various substrate amounts in 10 mL of pH 7.4 phosphate buffer solution without enzyme, with esterase, and with lipase at 37°C. The homomethacrylate-type polymer containing naproxen was very stable toward acids and bases. In addition, the hydrophilic polymer was obtained after introduction of methacrylic acid as a counter monomer and degraded in aqueous solution under mild conditions to produce free naproxen and its derivative. The confirmation of the elution time and the UV absorbance, using a photodiode array detector in HPLC analysis, with those of the synthesized Nap-EtOH standard were used to identify Nap-EtOH. An analogous example has been reported (Larsen 1989) wherein two esters were hydrolysed to release the free drug and the drug-containing glycolic acid, when the hydrolytic degradation of naproxen linked to dextran through a glycolic acid spacer was studied. It is assumed that the two ester bonds could be cleaved to release naproxen and the Nap-EtOH, as depicted in Figure 5.

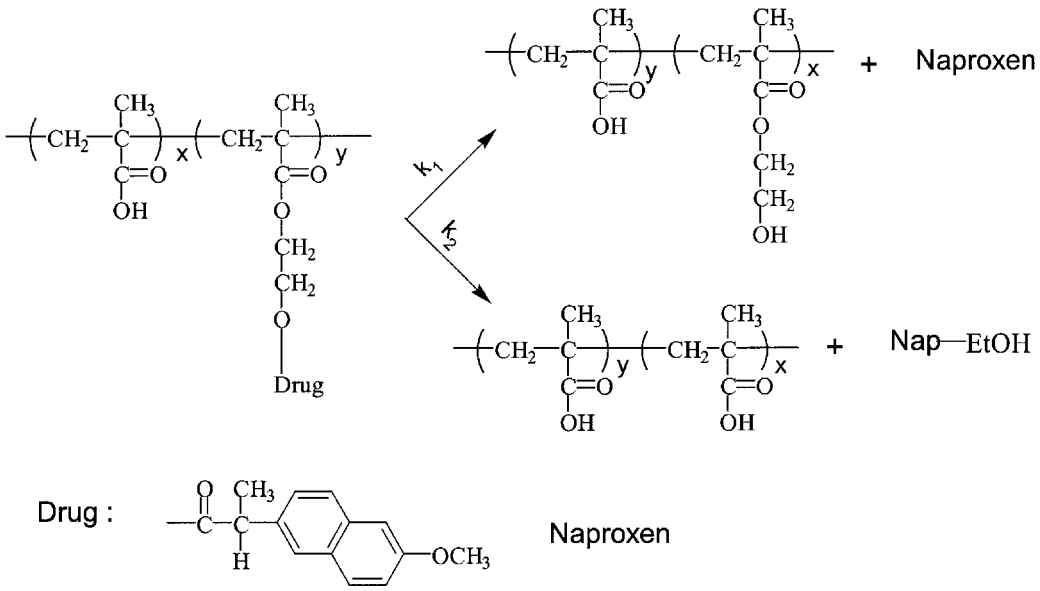

Figure 5 Cleavage of the ester bonds in a polymeric prodrug.

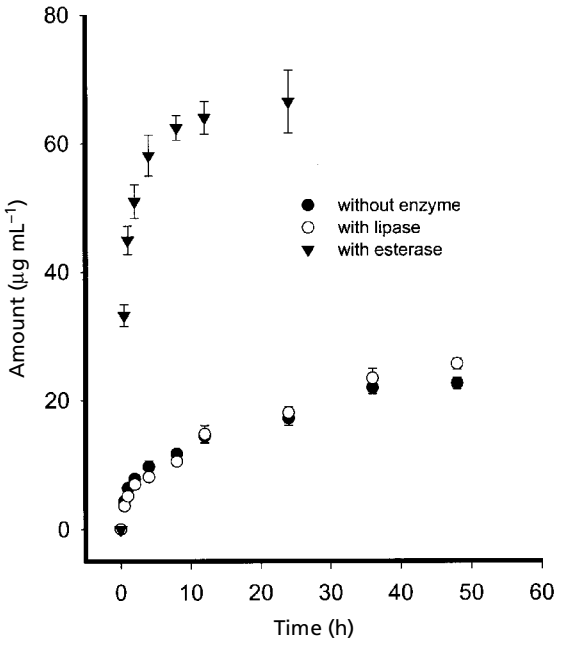

Figure 6 The total concentration of drug released using 30 mg polymeric prodrug without enzyme or in the presence of lipase or esterase.

To evaluate the enzyme activity of esterase and lipase, the accumulated total drug release (naproxen + Nap-EtOH), with or without enzymes, using 30 mg substrate, are plotted in Figure 6. The hydrolysis rate was efficiently increased by esterase but remained intact with lipase in comparison with that without using enzymes. Therefore, the enzymatic catalysis on hydrolysis was only calculated in the presence of esterase. The commonly used Michaelis–Menten equation was adapted. The initial rate of the released naproxen from the polymeric prodrug in the presence of esterase was calculated from the subtraction of data between Figure 3 and Figure 2 after the initial 2 h. The K_m (Michaelis constant) and V_{max} were calculated using the Lineweaver–Burk equation (Lineweaver et al 1934). The calculated K_m value was 5.11×10^{-2} equiv. mol L^{-1} and V_{max} was 2.16×10^{-5} equiv. mol L^{-1} min^{-1}, which implied that k_{cat} was 1.35×10^{-2} min^{-1}.

Anti-inflammatory activity

The inhibition of swelling in carrageenan-induced rat paw oedema, brought about by intraperitoneal administration of the drugs, is shown in Table 1. The percentage of swelling and inhibition was calculated using equations 3 and 4.

$$\text{Swelling } (\%) = [(V_t - V_0)/V_0] \times 100 \quad (3)$$

$$\text{Inhibition } (\%) = \{[(V_t - V_0)_{control} - (V_t - V_0)_{treat}]/(V_t - V_0)_{control}\} \times 100 \quad (4)$$

V_0 and V_t relate to the average volume in the hind paws of rats (n = 8) before any treatment and after anti-inflammatory agent injection, respectively. Pretreatment with anti-inflammatory agents (free naproxen as well as the polymeric prodrug) 0.5 h after carrageenan injection reduced the induced oedema significantly, reaching a maximum inhibition at 2 h. The maximum anti-inflammatory activity of the polymeric prodrug was observed at 2 h and remained practically constant up to 10 h. The anti-inflammatory activity of free naproxen, however, decreased with time. Statistical significance testing using one-way analysis of variance showed that the anti-inflammatory activity of naproxen or the polymeric prodrug were effective in comparison with the control group. However, differences in the anti-inflammatory potency of the polymeric prodrug compared with free naproxen were observed over long periods. Thus, the polymeric prodrug was proved to be a suitable pro-moiety for naproxen.

Conclusion

A polymeric prodrug containing naproxen was successfully synthesized. This polymeric prodrug was pH sensitive, and two ester bonds could be cleaved to release free naproxen and the derivate, Nap-EtOH. The hydrolysis rate was accelerated markedly in the presence of esterase, suggesting the utility of this polymeric prodrug. The anti-inflamma-

Table 1 Percentage of swelling, and inhibition caused by naproxen and the polymeric prodrug, in carrageenan-induced oedema in rats.

	Time (h)							
	0.5	1	2	3	4	8	10	12
Control	46.08±12.57	80.92±14.12	95.52±18.90	80.93±18.34	80.28±18.20	88.07±15.86	85.24±6.01	84.34±14.03
Naproxen	31.93±13.01** (30.7)	42.31±13.52* (47.7)	43.41±17.46* (54.5)	41.23±16.92* (49.0)	40.57±16.60* (49.4)	50.77±13.55* (42.3)	51.73±12.35* (39.3)	59.21±11.06* (29.8)
Polymeric prodrug	37.78±8.46** (18.0)	46.24±13.04* (42.8)	39.94±17.64* (58.2)	33.81±14.18* (58.2)	35.51±17.74* (56.3)	38.37±14.17* (56.4)	42.32±13.32*# (50.3)	44.98±14.98*# (46.5)

Data are presented as means±s.d., n = 8, of swelling (%) calculated from Equation 3, with inhibition (%) calculated from Equation 4 given in parentheses. $^{**}P < 0.05$, $^{*}P < 0.01$, compared with control group. $\#P < 0.01$, compared with naproxen.

tory activity of the polymeric prodrug was also better than that of free naproxen over long periods. The chemical structure of this polymeric prodrug is similar to that of Eudragit, a commonly used enteric coating material in the pharmaceutical industry. Thus, it may be of great interest to use this polymeric prodrug either as a coating or as a matrix material for naproxen. Both diffusion and hydrolytic mechanisms are combined to control the release of naproxen. The synergistic effect is predictable. The side-effects of naproxen are reduced since the dose of naproxen is decreased. Moreover, a sustained release of naproxen may occur with this polymeric prodrug.

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