Preparation of Naproxen-Loaded Poly(ethylene oxide-*b*-methacrylic acid) Micelle and Its pH-dependent Drug Release Behavior

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To achieve effective targeted delivery and satisfactory therapeutic applications, various synthetic polymers have been used for the delivery of therapeutic agents such as drugs and genes.[1-8] Among synthetic polymers, amphiphilic polymers are self-assembled in aqueous solution to minimize contact between water and hydrophobic blocks, and they can contain hydrophobic drugs in the inner hydrophobic core. Recently, the biological signals-responsive smart micelles are reported for the selective release of drug at target organs.[9-12] Some pH- or glutathione-responsive micelle systems constructed with degradable polymers[13-18] have been invented to target cells in the body under specific conditions, such as acidic pH or different concentrations of glutathione.[19-21]

Drugs given by oral administration can be absorbed through a mucous membrane of small intestine and be transported to liver through the hepatic portal vein. After being metabolized in liver, drugs are circulated in the bloodstream. Some of the encapsulated drugs are released from the carriers in stomach due to its acidic environment.[22] After passing through the stomach, the pH of the digestive tract increases by the secretion of the pancreatic juice containing sodium bicarbonate.[23] So, in this study, we designed pH-sensitive polymeric micelle system that could encapsulate drugs at acidic pH and release drugs at basic pH conditions. PMMA is used for this purpose because it has low solubility at neutral and acidic pHs, but is soluble at basic conditions due to the carboxylic acid groups.[24] We synthesized PMMA block-containing diblock copolymer, PEO-*b*-PMAA using PEO macroinitiator. The polymer formed PMMA-core micelle at neutral and acidic conditions, but the micelle will collapse due to the dissolution of PMMA in basic solution. As expected, naproxen[25] encapsulated in the micelle was released up to about 100% at pH 8.0, whereas its release was restricted at pH 1.2.

Experimental Section

Materials. Poly(ethylene glycol) monomethyl ether (M_n = 5,000), methylene chloride (MC), triethylamine (TEA), 2-bromoisobutyryl bromide, *tert*-butyl methacrylate (*t*BMA), tetrahydrofuran (THF), Cu(I)Br, 1,1,4,7,10,10-hexamethyl-triethylenetetramine (HMTETA), naproxen, sodium hydroxide (NaOH), 1,4-dioxane and diethyl ether were purchased from Sigma-Aldrich. Hydrochloride (HCl, 37%) and dialysis mem-

brane (MWCO = 1,000) was purchased from Daejung Chemicals & Metals Co., Ltd., and Hankook Spectrum Company, respectively. Phosphate buffered saline (PBS, pH 7.4) was purchased from Biowhittaker. The *t*BMA was passed through a short column of basic alumina and vacuum distilled prior to use in order to remove the inhibitor. THF was purified by distillation and all other chemicals were used as received.

Synthesis of PEO Macroinitiator. Poly(ethylene glycol) monomethyl ether (25.0 g, 5.0 mmol) was dissolved in 30 mL of MC. TEA (1.01 g, 10.0 mmol). 2-bromoisobutyryl bromide (3.45 g, 15.0 mmol) was added and stirred at 4 °C for overnight. The reaction mixture was filtered to remove triethylammonium bromide. The product in the filtrate was precipitated in 100 mL of diethyl ether three times and dried in vacuum.

Synthesis of PEO-*b*-P*t*BMA. PEO macroinitiator (1.15 g, 0.23 mmol), *t*BMA (16.37 g, 115.0 mmol), and THF (50 mL) were charged into a 250-mL flask. The reaction mixture was degassed by three freeze-pump-thaw cycles and back-filled with N_2. After the reaction mixture was freezed by liquid nitrogen, Cu(I)Br (66.06 mg, 0.46 mmol) and HMTETA (106.09 mg, 0.46 mmol) was introduced into the reaction flask then back-filled with N_2. The reaction mixture immediately became green and progressively more viscous indicating the onset of polymerization. The reaction mixture was maintained under N_2 atmosphere at 60 °C. After 24 h, the reaction solution turned blue on exposure to air, indicating aerial oxidation of the Cu(I) catalyst. The resulting copolymer solution was passed through a silica column to remove the ATRP catalyst.

Synthesis of PEO-*b*-PMAA. PEO-*b*-P*t*BMA was dissolved in 1,4-dioxane (90 mL) for the hydrolysis of *tert*-butyl ester. We added concentrated hydrochloric acid (37 wt %, 6 g, 60.6 mmol), and we heated the mixture under refluxing for 12 h. After dialysis for 24 h, the solution was lyophilized.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra of the polymers were obtained using a Bruker DPX-300 NMR spectrometer (300 MHz).

Micelle Formation and Drug Encapsulation. PEO-*b*-PMAA (100 mg) and naproxen (20 mg) were dissolved in 10 mL of methanol and the solution was added into 60 mL of distilled water. After removal of methanol by vacuum, micelle-dispersed solution was prepared. Non-encapsulated naproxen was removed by centrifuge. The empty micelle without drug was prepared by the same method as mentioned above.

FE-SEM. Micelle solution was placed on an aluminum foil and dried to get a uniform layer of particles, then observed using FE-SEM.

DLS. Micelle sizes were measured using a Zetasizer 3000HAs system (Malvern Instruments, Ltd. Worcestershire, U. K.) at 25 °C. The laser used was a 10 mW HeNe laser and the scattered light was detected at a 90° angle. Zetasizer 3000 (Advanced) Size mode v1.61 software was used for data acquisition. The data were represented as the average value of five runs.

Drug Release Test. In vitro release of naproxen from the micelle was determined using the dialysis membrane diffusion technique. Two mL of drug-loaded micelle solution was transferred into a dialysis tube (MWCO = 1,000) and immersed into 10 mL of release media (pH 1.2 or pH 8.0 buffer solution) in 37 °C and stirred at 250 rpm. Aliquots (0.2 mL) of the release media were withdrawn at time intervals and mixed with methanol (0.2 mL). The mixed solution was monitored by UV-visible spectroscopy at 330 nm to determine the amount of drugs released. After sampling, the release media was supplemented with 0.2 mL of fresh buffer solution to maintain the final volume. UV-visible absorption spectra were measured using the Shimadzu UV-1601PC spectrophotometer. The buffer solutions were prepared as follows. For the preparation of pH 1.2 buffer, 2.0 g of sodium chloride and 7.0 mL of hydrochloride was dissolved in water to make a final volume of 1 L. And, pH 8.0 buffer was prepared with PBS (pH 7.4) by titration with 1.0 M of NaOH.

Results and Discussion

Polymer Synthesis. PEO macroinitiator (PEO-Br) was synthesized by the esterification reaction of the hydroxyl end group of PEO monomethyl ether using 2-bromoisobutyryl bromide in the presence of TEA.[26]

^{1}H NMR (D₂O): δ 3.65 (m, OCH₂CH₂ in PEO chain), 3.38 (s, OCH₃ at PEO chain end), 1.90 (s, CH₃ in 2-bromoisobutyryl bromide)

The diblock copolymer was synthesized by ATRP and hydrolysis. First, *t*BMA monomer was polymerized using PEO-Br as a macroinitiator in THF. After 24 h, the vinyl double bonds at δ 5.5-6.0 were no longer detected by ^{1}H NMR, indicating very high conversion of the *t*BMA to P*t*BMA (> 99%).

^{1}H NMR (CDCl₃): δ 3.65 (m, OCH₂CH₂ in PEO chain), 3.38 (s, OCH₃ at PEO chain end), 2.26-1.82 (br, CH₂ in P*t*BMA block), 1.43 (s, C(CH₃)₃ in *t*BMA), 1.25-0.99 (br, CH₃ groups in P*t*BMA block)

The obtained PEO-*b*-P*t*BMA precursor was converted into PEO-*b*-PMAA by hydrolysis in acidic solution. After 12 h, no signals of *tert*-butyl groups were observed from ^{1}H-NMR in MeOD, indicating complete hydrolysis of *tert*-butyl ester groups. The degree of polymerization was estimated to be 217 based on ^{1}H NMR spectrum (10.7g, yield: 65%).[27,28]

^{1}H NMR (MeOD): δ 3.65 (m, OCH₂CH₂ in PEO chain), 3.38(s, OCH₃ at PEO chain end), 2.30-1.47 (br, CH₂ groups in PMAA block), 1.47-1.01 (br, CH₃ groups in PMAA block)

Micelle Formation. PEO-*b*-PMAA block copolymer form PMAA core-micelle in an aqueous solution. Naproxen was encapsulated in hydrophobic micelle core by co-solvent evaporation method. We could calculate the percentage of naproxen encapsulated in micelle from lyophilized micelle sample. The amount of naproxen in micelle was determined by UV-visible spectroscopy. The encapsulation efficiency was calculated as follows (1.15%).
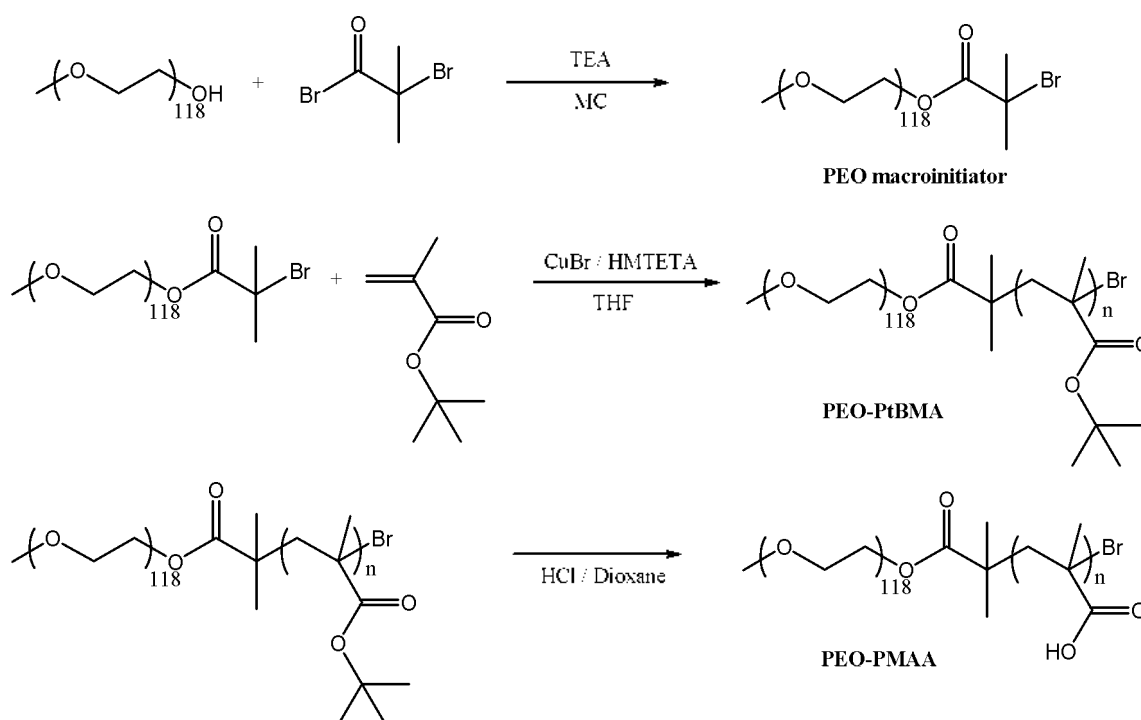
Scheme 1. Synthetic scheme of PEO-*b*-PMAA diblock copolymer.

Encapsulation efficiency =

$$\frac{\text{Weight of encapsulated drug}}{\text{Total weight of drug-loaded micelle}} \times 100$$

The mean size of free micelles was 198.3nm, and the size of drug-loaded micelle was increased slightly to 234.9 nm due to drug encapsulation (Figure 1). And we further observed the
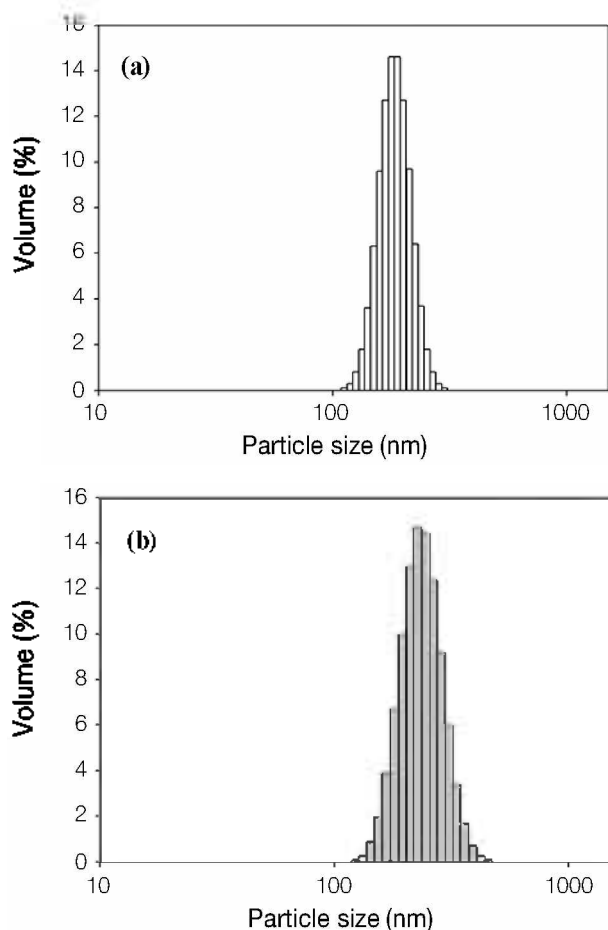
appearance of the naproxen-loaded PEO-*b*-PMAA micelles by FE-SEM. The micelle displayed spherical shapes, and the size distribution was around 100-150 nm (Figure 2). The size observed by FE-SEM was smaller compared to the size detected by DLS, because the micelle experiences the dehydration step during the sample preparation for FE-SEM measurements.

pH-Sensitivity of the Micelle and Drug Release. PEO-*b*-PMAA micelle dispersed in water (neutral pH) displayed a cloudy appearance due to the water-insoluble PMAA core. The photo images when aliquots (0.2 mL) of the opaque micelle solution were added to 1.0 mL of two different buffers (pH 1.2, and pH 8.0, respectively) are presented in Figure 3. The micelle solution at pH 8.0 became transparent compared to the other micelle at low pH, which means that the PMAA block of the polymer is soluble in the basic solution. Because the micelle core has changed from hydrophobic to hydrophilic state under basic conditions, hydrophobic substances encapsulated can be released at pH 8.0 buffer. Figure 4 shows the different release patterns of naproxen at pH 1.2, and pH 8.0. Until 0.5 h, about 30% of naproxen was released rapidly in both conditions. However, the different pH effect on drug release profiles was evident between the two conditions from 1 h. The encapsulated naproxen was further released reaching about 100% at pH 8.0 within 2 h, whereas more than half of the drug was maintained at pH 1.2, and could not be further

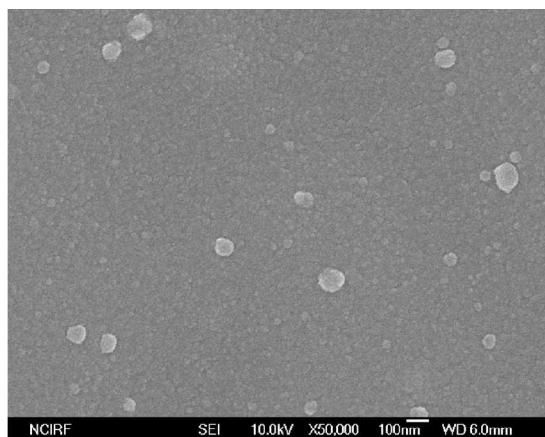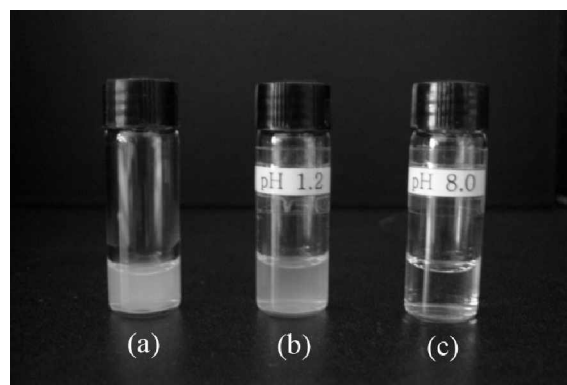**Figure 1.** Size distribution of (a) PEO-*b*-PMAA micelle, and (b) naproxen-loaded PEO-*b*-PMAA micelle.

Figure 3. Photo images of PEO-*b*-PMAA micelle in water (a), in pH 1.2 buffer (b), and in pH 8.0 buffer (c).

Figure 2. FE-SEM image of naproxen-loaded PEO-*b*-PMAA micelle.
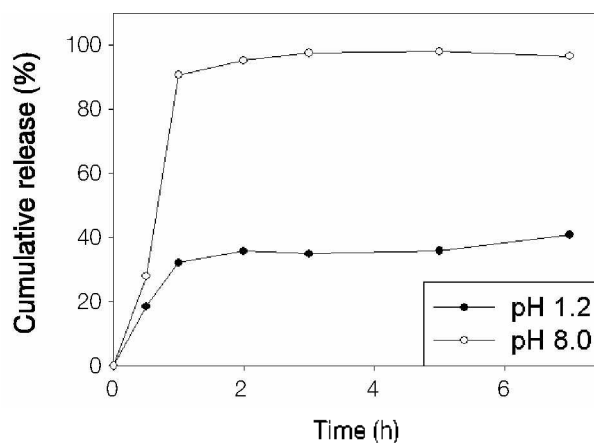

Figure 4. Cumulative release of naproxen from PEO-*b*-PMAA micelle at pH 1.2 and pH 8.0.

released to the media until 7 h.

Conclusion

PEO-*b*-PMAA diblock copolymer was synthesized by ATRP. PMMA block was insoluble at neutral pH, and the micelle was formed by co-solvent evaporation method in an aqueous solution.[29] The micelle can encapsulate naproxen in the micelle core (PMMA block). The micelle contained more than half of naproxen at pH 1.2 until 7h but released up to 100% of naproxen at pH 8.0 within 2 h. As a result, most hydrophobic drug was maintained in PEO-*b*-PMAA at low pH, and the drug was released rapidly at basic pH. The results imply the potential use of PEO-*b*-PMAA micelle system for the small intestine-targeted delivery of various hydrophobic drugs.

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