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MPEG-*b*-Poly(amino urethane) Amphiphilic Block Copolymers and Their pH-Dependent Micellization Behavior

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Introduction

Over the past few decades, increasing attention has been paid to stimuli-responsive polymeric micelles, due to their potential in biomedical applications.[1] An important issue in making self-assembled system for special applications is their capability to respond to external stimuli such as pH, temperature, ionic strength, electric field and biochemical agents.[1,2] They usually exhibit low critical micelle concentration (CMC), stabilizing the drug-loaded micelles in the bloodstream to achieve the long circulation time. The micelles (<200 nm) reduce non-selective uptake by the reticuloendothelial system (RES), and show enhanced permeability and retention effects (EPR) at solid tumor tissue sites.[3-5] Most importantly, the micellar drug carrier can be stimuli-responsive by introducing stimuli-sensitive building blocks into the polymer structure. The stimuli-sensitive mechanism is based on the chemical structure of the polymer. For example, polymers bearing weakly acidic or basic groups exhibit a response to the solution pH. To develop a pH-sensitive system, acidic moieties such as carboxylic acids[6] and sulfonamide derivatives,[7-11] or basic moieties such as tertiary amines[12,13] were introduced into the copolymers. However, the number of systems that respond to the physiologically accessible pH range of 4.5-7.4 is quite limited. In the recent work of our group, a pH-dependent polymeric micelle based on methoxy poly(ethylene glycol)-*b*-poly(*β*-amino ester)s (MPEG-*b*-PAE) was reported.[14-17] It shows excellent pH-dependant

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micellization behavior in aqueous solution.

In the present study, a kind of novel pH-sensitive block copolymer composed of MPEG and poly(amino urethane) (PAU) is reported. Polyurethanes are an important class of polymers that have found many applications as biomaterials due to their excellent physical properties and relatively good biocompatibility.[18] The MPEG based pH-sensitive poly(amino urethane)s were synthesized by the condensation polymerization of bis-1,4-(hydroxyethyl)piperazine (HEP) and 1,6-diisocynato hexamethylene (HDI) in the presence of dibutyltin dilaurate as a catalyst. The resulting copolymers were characterized by FT-IR, nuclear magnetic resonance (NMR) spectroscopies and gel permeation chromatography (GPC). The pH-dependent micellization behavior, critical micelle concentration (CMC) and the pH-dependent particle size were investigated in detail.

Experimental

Materials. Methoxy poly(ethylene glycol) (MPEG) (M_n= 2,000, 5,000), anhydrous 1,2-dichloroethane, toluene, dibutyltin(II) dilaurate, bis-1,4-(hydroxyethyl)piperazine (HEP), 1,6-diisocyanato hexamethylene (HDI), pyrene, and phosphate buffer silane tablets were purchased from Sigma-Aldrich. Diethyl ether, chloroform and dimethylformamide (DMF) were obtained from Samchun Chemical Co. (Korea) and were used as received.

Synthesis of MEPG-*b*-PU. The MPEG based poly(urethane)s were synthesized by the condensation reaction between MPEG-OH, HEP and HDI. The molar ratio of hydroxyl group to isocyanate group (OH/NCO) was controlled to be 1.05:1. The typical reaction procedure is as fellows: To a dry 250 mL round bottom flask equipped with a magnetic stir bar, MPEG was added and the flask was placed in an oil bath at 100 °C under vacuum, maintained at this temperature for 2 h and then cooled to 80 °C. HEP and catalytic amount of dibutyltin dilaurate catalyst was added and again vacuum was applied for 30 min. After that the flask was kept under nitrogen atmosphere and 50 mL of anhydrous toluene/1,2-dichloroethane (50:50) mixed solvent was added. After the solid had completely dissolved, HDI was added and the mixture was allowed to react at 80 °C. After 1 h, the flask was cooled to room temperature and the mixture was diluted with chloroform. The resulting polymer solution was precipitated in 10 fold excess of diethyl ether. The precipitated polymer was dried under vacuum at room temperature for 48 h.

Characterization. The resulting polymers were characterized by ^{1}H and ^{13}C NMR (500 MHz JNM-LA FT-NMR), FT-IR (NICOLET, IR 200). The molecular weight and polydispersity index (PDI) were measured by gel permeation chromatography (GPC, Shodex-KF 802.5, KF 803L), with

DMF as the eluent at a flow rate of 1 mL/min. The molecular weight was calibrated against low polydispersity PEG standards.

pH-Dependent Micellization. The micellization-demicellization behavior of the pH-sensitive block copolymers were investigated by fluorescence spectroscopy using pyrene as a probe. A buffer solution containing 0.01 M phosphate saline buffer was prepared. A stock solution of pyrene in THF was added to the buffer solution and the THF was removed by heating at 60 °C for a few hours. The final pyrene concentration was 1×10^{-6} M. The excitation spectrum of pyrene was recorded at 392 nm. The pK_a value of the polymer was measured by the titration method. In this case, 50 mg of the polymer was dissolved in 50 mL distilled water, after which the pH was adjusted to be less than 5.0. With the addition of 0.1 N NaOH solution, the pH was recorded to obtain a titration profile. The pK_a values of the polymers were calculated from the derivative values of the titration curves, which correspond to the inflection point. The micelle size was characterized by DLS (dynamic light scattering, Malvern Instrument Ltd. Series 4700) with a helium laser at 633 nm and a digital correlator. When the difference between the measured and calculated baselines was less than 0.1%, the correlation function was deemed acceptable. The scattering angle was fixed at 90° and the temperature was adjusted to 25.8 °C. The concentration of the solution was kept at 1 mg.mL^{-1} and the micelle size was checked at various pH values.

Results and Discussion

Synthesis and Characterization of MPEG-*b*-PAU. The synthesis route of MPEG-*b*-PAU is shown in Scheme I. To avoid the produce of triblock copolymer with MPEG at both ends, slightly excessive -OH groups (OH/NCO = 1.05:1 (mol/mol)) were used in the synthesis. The MW and PDI were determined by GPC.

Even though slightly excessive OH groups were used, the product synthesized by the coupling reaction using HDI probably contained a small amount of triblock copolymer with MPEG at both ends. This was confirmed by the GPC measurement. As shown in Figure 1, a slight shoulder at a higher MW region is observed in the GPC trace of the block
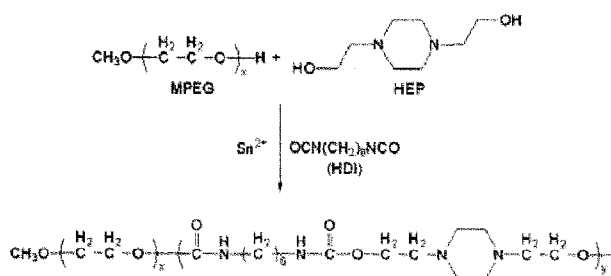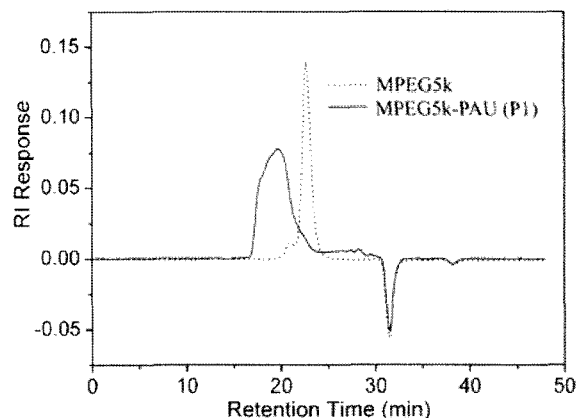

Scheme I. Synthesis route of MPEG-PAU.

Figure 1. GPC traces of MPEG5k and MPEG-PAU block copolymers.

Table I. Composition and Molecular Weight of MPEG-*b*-PAU

No.	M_n (MPEG)	Feed ratio: HEP/HDI/ MPEG (mol/mol/mol)	PAU Content (wt%)^a	M_n^b	PDI^b
P1	5,000	10/10/1	59.4	17,600	1.70
P2	2,000	10/10/1	64.8	8,700	1.91
P3	2,000	6/6/1	51.9	7,300	1.73

^{a}Calculated by ^{1}H NMR. ^{b}Determined by GPC.

copolymer, indicating the presence of a small amount of triblock copolymer with MPEG at both ends. The coexistence of a small amount of triblock copolymer should not affect markedly the pH-sensitivity of the amphiphilic copolymer because of the definite pK_a. The composition and MW of the copolymer are shown in Table I.

The chemical structure of synthesized copolymer was examined by FT-IR spectroscopy. The characteristic C-O-C stretching vibration of PEG is observed at 1102 cm^{-1}. An intensive band at 1720 cm^{-1} is assigned to free carbonyl stretching band (C=O$_{free}$), and a shoulder at a lower wavenumber is attributed to hydrogen bonded carbonyl stretching region (C=O$_{H-bond}$). In addition, the absorbance at 3340 cm^{-1} is attributed to N-H stretching band, which confirms the presence of poly(amino urethane) segments. Furthermore it can be seen that the peak corresponding to the NCO stretching is not observed in the region of 2200 cm^{-1}. This indicates that the isocyanate groups have been completely reacted.

The block copolymers were further characterized by ^{1}H NMR and ^{13}C NMR spectroscopy. Figure 2(A) shows the proton NMR spectrum of polymer P1 in CDCl$_3$. The typical peaks corresponding to the protons of MPEG and poly(amino urethane) segments indicate clearly the formation of the PEG-*b*-PAU block copolymer. Figure 2(B) shows the ^{13}C NMR spectrum of polymer P1. The typical ^{13}C peak of carbonyl carbon of urethane groups is observed at 156.7 ppm, and the ^{13}C peak of isocyanate carbonyl carbon at 122.9 ppm
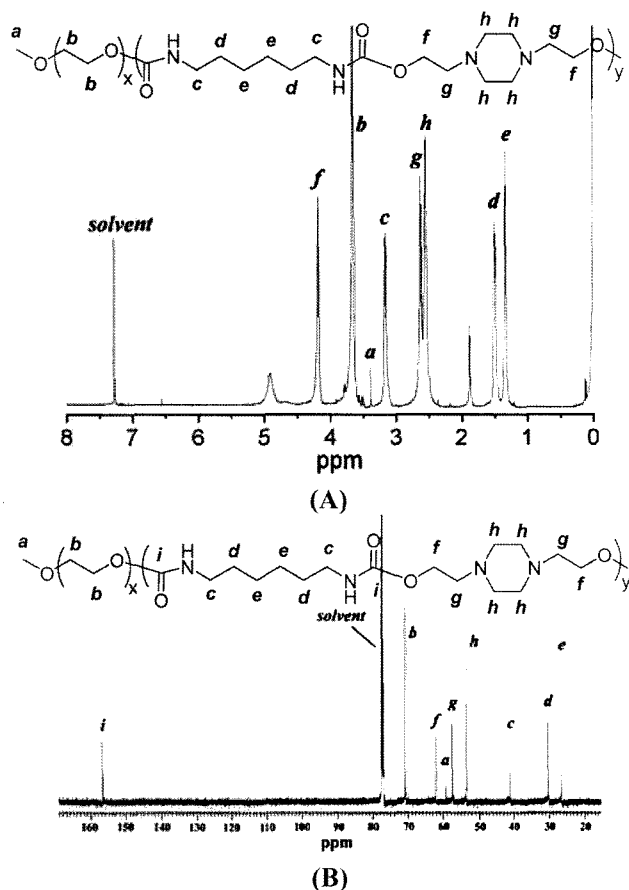
Figure 2. ^{1}H NMR (A) and ^{13}C NMR (B) of spectra of MPEG-*b*-poly(amino urethane) (P1).

Figure 3. Titration curve of the block copolymer P1.

Figure 4. (a) I_{337}/I_{334} intensity ratio (from pyrene excitation spectra) as a function of pH for the block copolymer (P1, P2 and P3) solutions; (b) Plots of the I_{337}/I_{334} intensity ratio (from pyrene excitation spectra) vs. log (concentration) at various pH values.

is not observed. This indicates that the isocyanate groups are completely consumed and not present in the polymer. The ^{1}H and ^{13}C NMR results confirm the successful synthesis of the MPEG-*b*-PAU block copolymers.

Acid-Base Titration Curve. The solution property of the block copolymer was investigated as a function of the solution pH. Figure 3 shows the acid-base titration curve of the block copolymer (P1). The titration curve indicates that the pK_a of the copolymer is around 6.6, which is close to reported value of other piperazine-based systems.[1]

Micellization-Demicellization Behavior. The micellization-demicellization was observed by fluorescence spectroscopy using pyrene as a probe. Figure 4(a) shows the pH-dependence of the I_{337}/I_{334} ratio. As the solution pH is increased, the tertiary amine moiety becomes deprotonated. The micellization-demicellization transition pH was estimated from the increase in the I_{337}/I_{334} ratio, which indicates a more hydrophobic (micellar) environment for the pyrene probe. From Figure 4(a), the micellization of the block copolymer P1 occurs in the pH range of 6.0-6.6, which agrees well with the result of acid-base titration experiment.

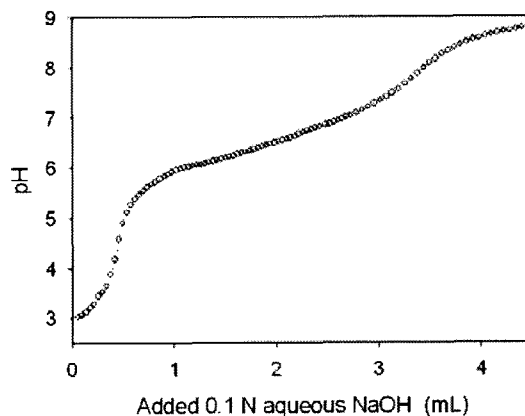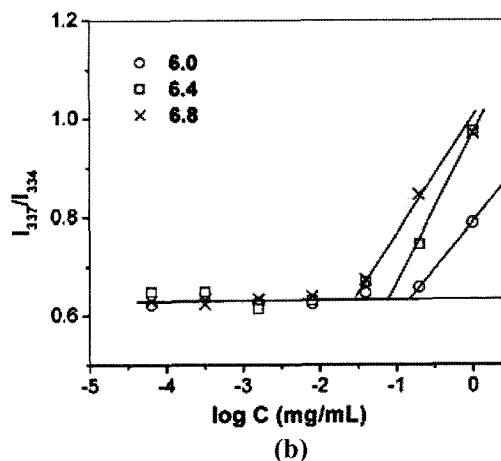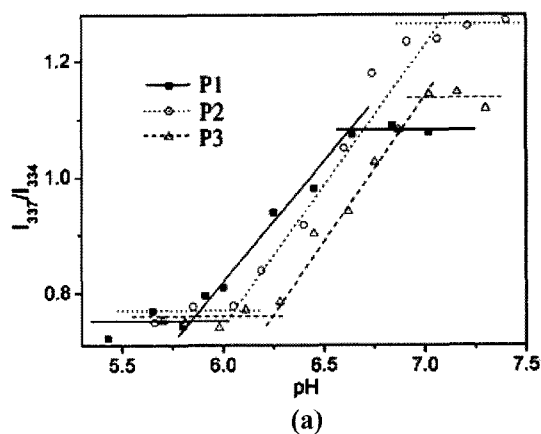Additionally, the effect of block length on the pH-dependent

micellization of the block copolymer was investigated. The pH-dependent I_{337}/I_{334} ratios of the solutions of P2 and P3 are evaluated. As shown in Figure 4(a), P2 shows a micellization-demicellization transition at a higher pH region than P1. This may be attributed that the hydrophobicity of
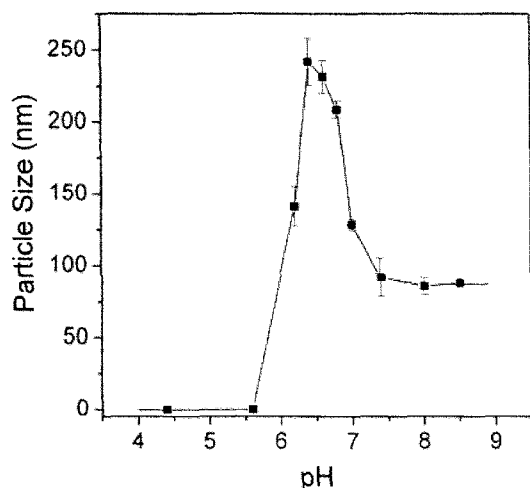
Figure 5. The size of the particles formed in the copolymer P1 solution as a function of pH.

the deionized PAU block of P1 is stronger than that of P2 due to the higher PAU block length of P1, even though the relative PAU content in P2 is higher than that in P1 (Table I). This indicates that the hydrophobicity of the PAU block is highly affected by its block length. Similarly, P3 shows a transition region at a higher pH region than P2, due to the lower PAU block length of P3.

pH-Dependent Critical Micellization Concentration (CMC). The fluorescence spectroscopy was performed to investigate the CMCs of the block copolymer at various pH. A small intensity change in the intensity ratio of the first and third vibrational band occurs in micellar media when using a pyrene probe. Figure 4(b) shows the I_{337}/I_{334} ratios as a function of the copolymer concentration at various pH values. The CMC values were determined at pH 6.0, 6.4 and 6.8 for polymer P1. The CMC values of P1 at pH 6.0, 6.4 and 6.8 are around 0.15, 0.08 and 0.03 mg.mL^{-1}, respectively.

Dynamic Light Scattering (DLS). The size and size distribution of the micelles were measured by means of the dynamic light scattering (DLS) method. The effect of pH on the particle size of the polymeric micelles is shown in Figure 5. At a low pH (below 6.0), no particle is detected, caused by the ionization of the PAU block. In contrast, in the micellization-demicellization transition region (pH 6.2-6.8), larger particles are detected, due to the formation of micelles with loose-packed structure. As the pH is increased to above 7.0, the particle size decreases markedly and maintains at around 85 nm, attributed to the formation of micelles with close-packed structure.

Conclusions

The MPEG-PAU block coplymers were successfully synthesized and their chemical structures were well character-

ized by ^{1}H, ^{13}C NMR and FT-IR spectroscopies. The aqueous solution properties of the copolymers were evaluated by acid-base titration, fluorescence spectroscopy and dynamic light scattering. The block copolymers exhibited a pH-dependent micellization-demicellization transition in aqueous solution. The fluorescence spectroscopy and DLS measurements indicate that the micellization-demicellization transition occurs at pH 6.0-6.6. The amphiphilic block copolymers are expected to find applications in biomedical field.

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