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pH-Sensitivity Control of PEG-Poly(β-amino ester) Block Copolymer Micelle

Su Jong Hwang, Min Sang Kim, Jong Kwon Han, and Doo Sung Lee*

Department of Polymer Science & Engineering, Sungkyunkwan University, Suwon 440-746, Korea

Bong Sup Kim

Polymer Technology Institute, Sungkyunkwan University, Suwon 440-746, Korea

Eun Kyung Choi

Department of Radiation Oncology, University of Ulsan, Ulsan 680-749, Korea

Heon Joo Park

Department of Microbiology, College of Medicine, Inha University, Incheon 402-751, Korea

Jin Seok Kim

College of Pharmacy, Sookmyung Women's University, Seoul 140-732, Korea

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Abstract: Poly(ethylene glycol) methyl ether (PEG)-poly(β-amino ester) (PAE) block copolymers were synthesized using a Michael-type step polymerization, and the construction of pH-sensitive polymeric micelles (PM) investigated. The β-amino ester block of the block copolymers functioned as a pH-sensitive moiety as well as a hydrophobic block in relation to the ionization of PAE, while PEG acted as a hydrophilic block, regardless of ionization. The synthesized polymers were characterized using ^{1}H-NMR, with their molecular weights measured using gel permeation chromatography. The pK_b values of the pH-sensitive polymers were measured using a titration method. The pH-sensitivity and critical micelle concentration (CMC) of the block copolymers in PBS solution were estimated using fluorescence spectroscopy. The pH dependent micellization behaviors with various bisacrylate esters varied within a narrow pH range. The critical micelle concentration at pH 7.4 decreased from 0.032 to 0.004 mg/mL on increasing the number of methyl group in the bisacrylate from 4 to 10. Also, the particle size of the block copolymer micelles was determined using dynamic light scattering (DLS). The DLS results revealed the micelles had an average size below 100 nm. These pH-sensitive polymeric micelles may be good carriers for the delivery of an anticancer drug.

Keywords: block copolymers, pH-sensitive, polymeric micelle, β-amino ester.

Introduction

In recent years, there has been a rapid growth in the field of drug delivery system (DDS). The continuous development of DDS is driven by the need to maximize therapeutic activity while minimizing negative side effects. Polymeric micelles (PM) composed of amphiphilic block copolymers have been shown to possess significant potential as delivery systems providing several advantages over conventional colloidal drug carriers.[1-4] Their generally small size (<100 nm) allows them to minimize scavenging by the mononuclear phagocyte system, and water insoluble drugs can be loaded in their hydrophobic-hydrophilic core-shell structure. Thus core-shell micelle structure in aqueous solution is formed by the balance of hydrophilic and hydrophobic parts, and a tight shell around the micellar core is formed by the driving force of micelle formation. Here, the major driving force is commonly referred to be the entropy-driven hydrophobic interaction to form tight core. This enables them to attain prolonged circulation and altered biodistribution of drug encapsulated within micelles due to the reduced reticuloendothelial systems (RES) clearance and renal filtration. PM loaded with anticancer drugs can thus be used to passively target the tumor via the enhanced permeation and retention (EPR) effect which permits the accumulation of drug carrier conjugates at certain tumor sites.[5-7] This passive targeting for can-

*Corresponding Author. E-mail: dslee@skku.edu

cerous tumors may be enhanced by modifying the micelle so as to induce a response to external stimuli. The micelles composed of stimuli responsive block copolymers can be designed to respond to various stimuli in temperature,[8,9] pH,[10,11] and electric field,[12,13] which make possible controlled release on a target site. Among them, pH sensitivity is one of the most interesting properties in both scientific and technological fields.[14] There is speculation that pH sensitive PM could serve for the delivery of drugs to tumors, inflamed tissues or endosomal compartments, since they are all associated with a lower pH than normal tissue. pH-Sensitive PM could enhance the antitumor efficiency of a drug by accumulating in the target area, destabilizing cellular membranes and/or releasing their contents as the pH of the surrounding environment decreases.[15] Generally, in order to endow pH-sensitivity on the micelle, the amphiphilic block copolymer forming the micelle should be incorporated with ionizable group, for example carboxylic acid[16,17] or amine group.[18,19] Ionizable groups act as a hydrophilic or hydrophobic part of the polymer, causing a reversible soluble-insoluble transition to appear as the hydrophobicity of the polymer changes. An acidic group such as carboxylic acid is ionized at pHs above pK_a and deionized at pHs below pK_a, whereas a basic group such as an amine is deionized at pHs above pK_b and ionized at pHs below pK_b.[14]

In our previous study,[20] we prepared a pH-sensitive block copolymer consist of poly(ethylene glycol) methyl ether (PEG) and poly(β-amino ester) (PAE) and evaluated the effect of molecular weight of PAE block on the pH-sensitive micellization behavior.

In this study, we prepared pH-sensitive block copolymers with various bisacrylate esters of PAE to control the pH-sensitivity of the PM. Bisacrylate ester groups in PAE were expected to serve as a pH-sensitive controllable moiety in the physiological pH condition.

Experimental

Materials. Poly(ethylene glycol) methyl ether (PEG; M_n= 5,000) was supplied by ID Biochem, Inc (Korea), and was dried for 2 h at 110 °C in vacuum. 1,4-Butanediol diacrylate, 1,6-hexanediol diacrylate, 1,8-octanediol, 1,10-decanediol, triethyl amine, acryloyl chloride, 4,4'-trimethylenedipiperidine, anhydrous methylene chloride and pyrene were purchased from Aldrich, Korea. Chloroform, *n*-hexane, hydrochloric acid (35-37%) were purchased from Samchun Chemical, Korea.

Synthesis of Acrylated PEG. For the synthesis of acrylated PEG (PEG-A), the following experimental procedure was applied: 100 mL of anhydrous methylene chloride, 10.0 g of PEG and 0.3 g of triethyl amine were added into a 250 mL two neck round bottom flask equipped with a stirrer and a dropping funnel. The solution was cooled to 0 °C. 0.3 mL of acryloyl chloride was dissolved in anhydrous methylene chloride and then added dropwise to the stirred solution. The reaction temperature was maintained at 0 °C for 2 h and then at room temperature for 20 h. After filtration, the mixture was extracted with 1 N HCl solution several times. The organic phase was concentrated using a rotary evaporator. After being precipitated in *n*-hexane, PEG-A was obtained (yield 90%).

Synthesis of 1,8-Octanedioldiacrylate and 1,10-Decanediol Diacrylate. In a dried flask was placed 5 g of two kins of diols (1,8-octanediol and 1,10-decanediol) dissolved in 100 mL of anhydrous methylene chloride and 3 eq mol of triethyl amine. The mixture was cooled to 0 °C and 3 eq mol of acryloyl chloride was added slowly into this solution, and then this solution was stirred magnetically at 0 °C for 2 h and then at room temperature for 20 h. After filtration, the reaction mixture was washed thoroughly with water, and concentrated using a rotary evaporator. Further the mixture then was extracted with *n*-hexane. The oil phase was concentrated using a rotary evaporator and then product was obtained (yield 35%).

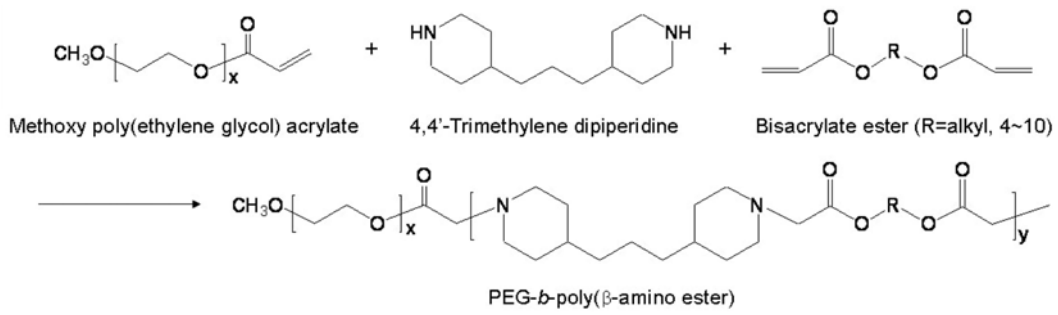
Synthesis of PEG-PAE Block Copolymers. The PEG-PAE block copolymers with different contents and compositions of β-amino ester were synthesized according to the method reported in our previous paper.[20]

Briefly, PEG-A (0.1 mol), bisacrylate ester (1.0 mol) and 4,4'-trimethylenedipiperidine (1.1 mol) were weighed into three separate vials and dissolved in chloroform. The solution containing bisacrylate ester and 4,4'-trimethylenedipiperidine were added into the PEG-A solution dropwise. The resulting reaction mixture was kept in oil bath at 50 °C with continuous stirring for 48 h. After cooling to room temperature, the solution was concentrated using a rotary evaporator and dripped slowly into vigorously stirring *n*-hexane (Scheme I).

Characterization. The synthesized block copolymers were characterized by ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra were recorded by means of a 500 MHz FT-NMR (Utility Inova 500 NB, Varian) spectrometer. $CDCl_3$ was used as a solvent.

The molecular weights and molecular weight distribution were determined with a gel permeation chromatography (GPC) with two stryragel columns (Shodex KF-802.5, KF-803 L) and a differential refractive index (Shodex, RI-101). The molecular weights of the polymers were calibrated against PEG standards (Waters Co.) with the molecular weight ranging from 420 to 22,100 using tetrahydrofuran as a mobile phase.

Fluorescence Measurement. The pH sensitivity and critical micelle concentration (CMC) of the block copolymers in phosphate buffered saline (PBS) solution were estimated by fluorescence spectroscopy using pyrene as a probe. Fluorescence spectra were recorded by an AMINCOBOWMAN® Series 2 luminescence spectrometer (SLM-AMINCO, USA) at room temperature. The pyrene solution in THF was poured into the PBS buffer solution, and the THF was eliminated by stirring at 60 °C for 2 h. The final concentration of pyrene in buffer solution was 1.0×10^{-6} M. The excitation spectra were



Scheme I. Michael reaction of PEG-*b*-poly(*β*-amino ester).

recorded from 310 to 350 nm with an emission wavelength of 392 nm. The pH sensitivity was taken from the intensity ratios of I_{337} to I_{334} at various pH. In order to investigate the CMC, the intensity ratios of I_{337} to I_{334} were plotted as a function of logarithm of polymer concentration.

***pK_b* Value Determination.** The base dissociation constant (pK_b) value of the various copolymers was measured by the acid-base titration method. In each case, 50 mg of the polymer was dispersed in 50 mL distilled water and the pH adjusted so as to be less than 5.0, and then the polymer was dissolved. 50 μL of 0.1 N NaOH solution was added and measured the pH, and then the pK_b was determined at an inflection point of a titration curve.

Dynamic Light Scattering (DLS). The micelle size was determined by DLS using Malvern PCS100 spectrogoniometer and Brookhaven BI-9000AT digital autocorrelator with a helium laser at 633 nm. The scattering angle was kept at 90° and the temperature was adjusted to 25 °C. The concentration of the polymer solution was kept at 1 mg/mL.

Results and Discussion

Synthesis and Characterization of PEG-PAE Block Copolymer. To investigate the effect of the alkyl chain length on the formation of pH-sensitive polymeric micelles in the aqueous solution, four kinds of bisacrylate esters were used such as 1,4-butanedioldiacrylate, 1,6-hexanedioldiacrylate, 1,8-octandedioldiacrylate and 1,10-decanedioldiacrylate. By using these bisacrylate esters, the corresponding PEG-PAE was synthesized, which resulted in the PEG-A, as white powder with yields over 95% and the structure was confirmed by ^{1}H-NMR spectroscopy (Figures 1(a) and 1(b)). Figure 1(a) shows ^{1}H-NMR spectra of methoxy PEG in $CDCl_3$. In the ^{1}H-NMR spectra of PEG-A in $CDCl_3$ (Figure 1(b)) the signal of c at 4.0 ppm is shifted to c' at 4.3 ppm and the signals of d, e and f appeared at 6.2, 5.9 and 6.4 ppm, respectively.

The 1,8-octandedioldiacrylate and 1,10-decanedioldiacrylate were obtained as yellow clear crude with yields 35% around and the structures were confirmed by ^{1}H-NMR (Figure 2 and Figure 3). Figure 2 and Figure 3 shows that, after acrylation the signals of a and b are shifted to a' and b' and the signals d, e and f appeared. From these results we confirmed the successful acrylation.

The PEG-PAE copolymers were synthesized by the Michael-type step polymerization of a bisacrylate ester, a diamine compound and PEG-A. The molecular weight and molecular weight distribution were determined by GPC. Table I shows the average molecular weight and molecular weight distribution of the various block copolymers.

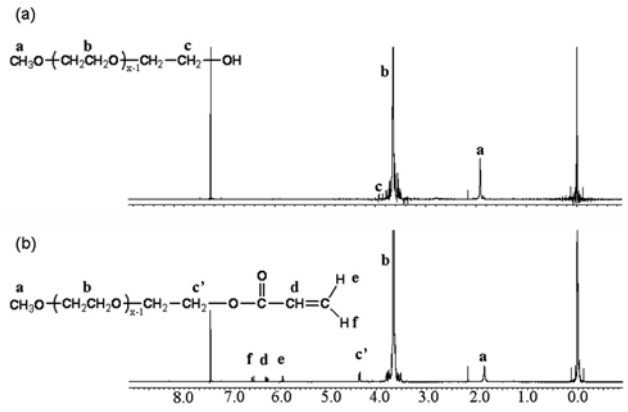


Figure 1. ^{1}H-NMR spectra of (a) MPEG and (b) acrylated MPEG.

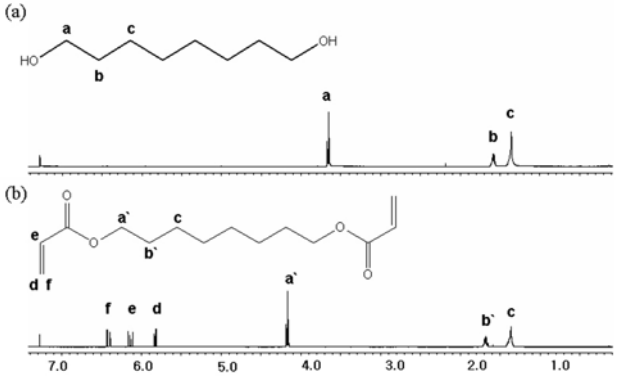


Figure 2. ^{1}H-NMR spectra of (a) 1,8-octanediol and (b) 1,8-octanedioldiacrylate.

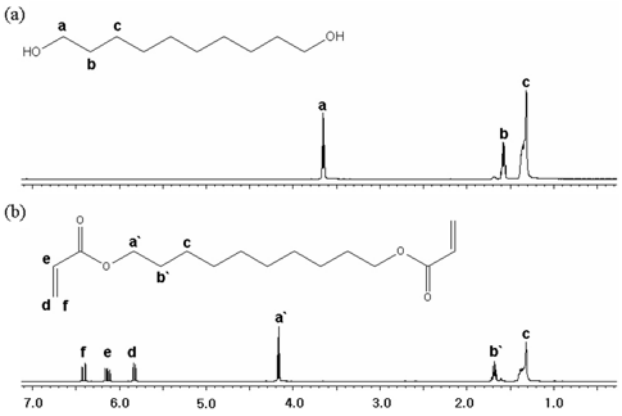


Figure 3. ^{1}H-NMR spectra of (a)1,10-decanediol and (b) 1,10-decanedioldiacrylate.

Table I. The Molecular Weight of the Synthesized Polymers

Sample Name	M_p	M_n	M_w	PDI
PEG-TDP-B	17,035	13,729	16,750	1.2
PEG-TDP-H	14,702	17,433	22,560	1.3
PEG-TDP-O	15,182	11,182	14,742	1.3
PEG-TDP-D	14,837	12,594	15,604	1.2

M_p: Molecular weight at peak point.

All of the polymers are named using the bisacrylate ester, e.g. PEG-TDP-B (1,4-butanedioldiacrylate), PEG-TDP-H (1,6-hexanedioldiacrylate), PEG-TDP-O (1,8-octanedioldiacrylate), PEG-TDP-D (1,10-decanedioldiacrylate).

***pK*$_b$ Value Determination.** The acid-base titration profiles of copolymers are presented in Figure 4. All of the polymer solutions exhibited a buffering pH region at pH 5 to 8. The pK_b value of PEG-TDP-B was 7.38, PEG-TDP-H was 6.85, PEG-TDP-O was 6.68 and that of PEG-TDP-D was 6.50.

Fluorescence Spectroscopy. Fluorescence spectroscopy was employed to investigate the pH induced micellization behavior of the block copolymers using pyrene as a probe. The intensity ratios of pyrene (I_{337}/I_{334}) were used to obtain information on the CMC of the block copolymers as a measure of polarity in the local environment, where I_{334} and I_{337} are the intensities of the first (334 nm) and the third (337 nm) vibronic peaks of pyrene fluorescence. When the block copolymers form micelles, the pyrene is arrested in the hydrophobic core of the micelle and the intensity ratio increases. In each case, the polymers were dissolved in PBS pyrene buffer solutions at constant concentration (1 mg/mL) and the pH was adjusted at pH 5.0, and then the pH increased to 8.0. Polymer solutions with various pHs were obtained by lowering the pH through the addition of 1 N HCl solution. Figure 5 shows the ratios I_{337}/I_{334} for pyrene of PEG-TDP-H solution as a function of the pH. In this figure, it can be seen that the PEG-TDP-H shows a sharp transition behavior.

As the methyl group number in bisacrylate is increased, the pH change region is shifted to lower as shown in Figure 5. In the case of the PEG-TDP-B block copolymer, the micelle begins to be disrupted below pH 7.40 and is fully ionized at pH 7.20. In the case of PEG-TDP-H, the ionized region is shifted to lower from pH 7.15 to 6.90. And PEG-TDP-O and PEG-TDP-D show a continuous pattern. According to the result, we could control the pH change region by various bisacrylate esters. When pH of solution was increased from pH 5.0 to 8.0, the block copolymer experienced structure transition from unimer to self-assembled micelle. With the increase of pH, deionized β-amino ester group enabled the polymer chains to associate themselves and form micelle structure. It is remarkable that the synthesized block copolymers showed reversible micellization-

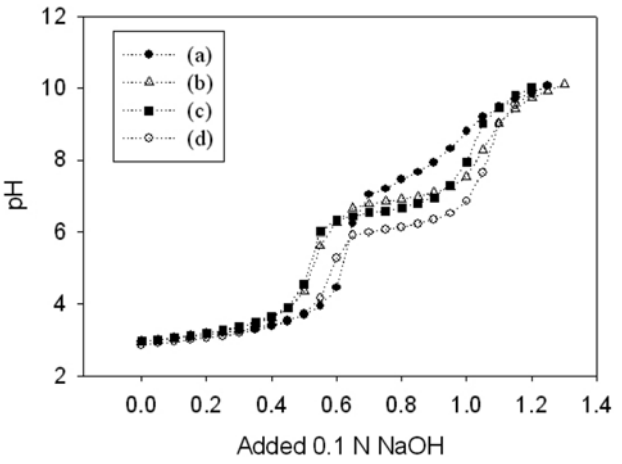


Figure 4. Titration curves of various pH-sensitive block copolymers; (a) PEG-TDP-B, (b) PEG-TDP-H, (c) PEG-TDP-O, and (d) PEG-TDP-D.

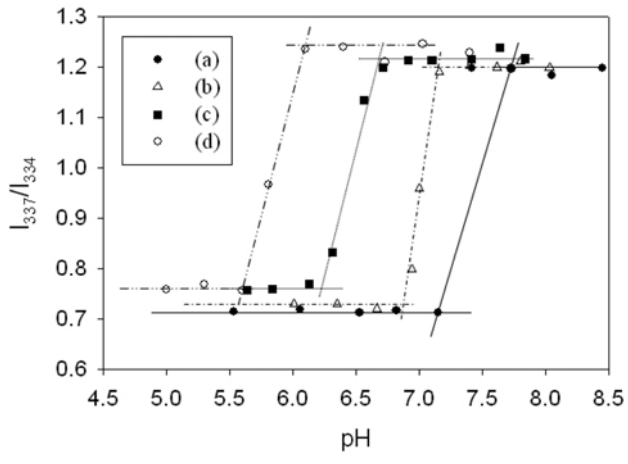


Figure 5. Fluorescence intensity ratio I_{337}/I_{334} of the micelle from pyrene excitation spectra as a function of pH; (a) PEG-TDP-B, (b) PEG-TDP-H, (c) PEG-TDP-O, and (d) PEG-TDP-D.

demicellization behavior within narrow pH region and we can control the pH change region.

The CMC determinations were also carried out by fluorescence spectroscopic studies. For more accurate CMC determination, the intensity ratio (I_{337}/I_{334}) is shown as a function of log concentration. Figure 6 shows the CMC curves of the polymers. As shown in Figure 7 the CMC values decreases with the number of methyl group increasing from 4 to 10. The CMC values in pH 7.4 solutions are about 0.032 mg/mL of PEG-TDP-B, 0.016 mg/mL of PEG-TDP-H, 0.006 mg/mL of PEG-TDP-O, and 0.004 mg/mL of PEG-TDP-D. More methyl group number of bisacrylate ester would increase its hydrophobicity and thus enhance the ability of the polymer to form micelles.

DLS. Dynamic light scattering was employed to determine the micelle diameter and to obtain information on the geometric shape of the micelle. The CONTIN algorithms were used in the Laplace inversion of the autocorrelation function to obtain micelle size. The mean diameter was calculated from the Stokes-Einstein equation. Figure 8 shows the typical size distribution of the block copolymeric micelles. DLS results showed that the micelle size is 50 nm around regardless of the kind of bisacrylate ester. Figure 8 also shows that the micelle of the block copolymers is extremely pH-sensitive and controllable by various bisacrylate ester.

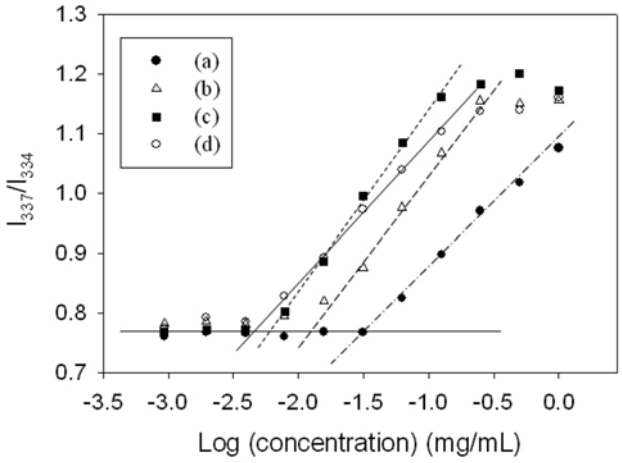


Figure 6. Fluorescence intensity ratio I_{337}/I_{334} from pyrene excitation spectra as a function of polymer concentration at pH 7.4; (a) PEG-TDP-B, (b) PEG-TDP-H, (c) PEG-TDP-O, and (d) PEG-TDP-D.

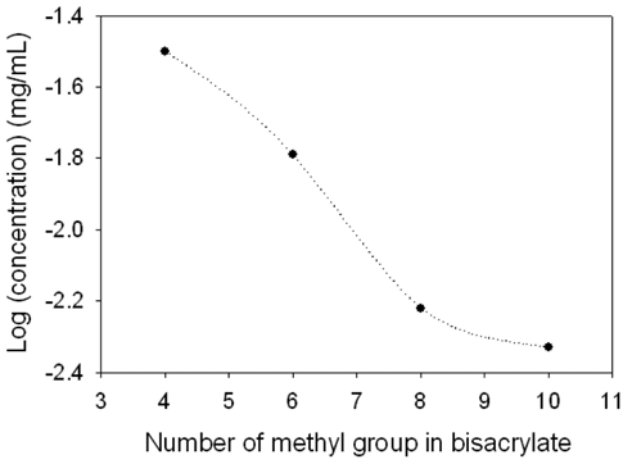


Figure 7. CMC dependence as a function of number of methyl group in bisacrylate.

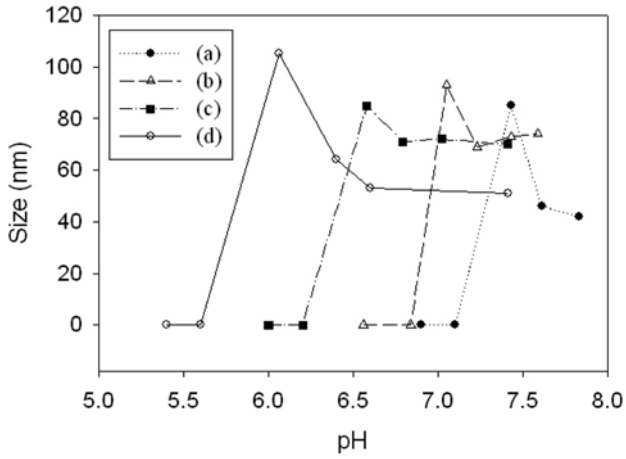


Figure 8. Size change of micelles as a function of pH at 25 °C as measured by DLS; (a) PEG-TDP-B, (b) PEG-TDP-H, (c) PEG-TDP-O, and (d) PEG-TDP-D.

pH-Sensitive micellization behavior was confirmed by titration, fluorescence and DLS. As the number of methyl group of bisacrylate ester increased, a destabilized pH was shifted to lower value. It may be due to the relationship between hydrophobicity and ionization. In our previous study,[21,22] higher molecular weight PAE was more difficult to be ionized due to the entropic effect, and then ionized at lower pH. The methyl group effect is similar to it. Higher hydrophobicity, more number of methyl group, reduces the solubility of polymer and this affects the degree of ionization.

Conclusions

We successfully synthesized the amphiphilic PEG-PAE pH-sensitive block copolymers with an wide range of pH-sensitivity window and evaluated their self-assembling behaviors in aqueous media. These block copolymers yielded micelles properties depending on the pH variations. PEG-PAE block copolymers were shown to dissociate upon a decrease of pH. It can be applicable to intracellular delivery when the polymer shows pH sensitivity at pH 5-6, indicating the endosomal pH.[23] Also, when it shows pH sensitivity above pH 6, the polymer can be used for extracellular delivery at low pH of cancer environment. We suggest that these block copolymers would be suitable micellar carriers of

hydrophobic drug in field of intracellular delivery and anticancer drug delivery.

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