Polyion Complex (PIC) Flower-shaped Nano-micelles formed from Anionic Triblock and Cationic Diblock Copolymers

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Abstract

Poly (sodium 2-(acrylamido)-2-methylpropanesulfonate)-block-poly(ethylene glycol)-block-poly(sodium 2-(acrylamido)-2-methylpropanesulfonate) (PAMPS27,53-PEG227-PAMPS27) and poly(ethylene glycol)-block-poly(3-(methacryloylamino)propyl) trimethylammonium chloride (PAMPS27-PMAPTAC27, m = 27,53, and 106) with different PMAPTAC chain lengths were prepared via reversible addition-fragmentation chain transfer controlled living radical polymerization using PEG-based macro chain transfer agents. The subscript numbers in abbreviation represent the degree of polymerization of the block. Mixing of aqueous solutions of the oppositely charged anionic triblock copolymer, PAMPS27,53-PAMPS27 and cationic diblock copolymers, PEG27-PMAPTAC27 led to the spontaneous formation of Polyion Complex (PIC) micelles. The characterization data of NMR, light scattering, ζ-potential, and transmission electron microscopic measurements indicated that the PIC flower micelles were formed comprising the segregated PIC core and surrounded hydrophilic linear and loop PEG shell chains. Aggregation number and hydrodynamic radius achieved maximum values when anionic and cationic block chain lengths of the block copolymers were approximately the same.

Keywords: Controlled living radical polymerization; Polyion complex micelle; Water-soluble polymer

Introduction

If “A” and “B” blocks are hydrophilic and hydrophobic blocks, respectively, amphiphilic AB diblock copolymers form core-shell type polymer micelles in water. The hydrophobic “B” blocks associate between interpolymer chains to form the hydrophobic core, and the hydrophilic “A” blocks surround the core as hydrated shells. This kind of core-shell polymer micelles can be applied to a hydrophobic drug carrier, because the drug can be incorporated into the hydrophobic “B” core. In the case of ABA triblock copolymers in water, the polymers form polymer micelles composed of the hydrophobic “B” block core and hydrophilic “A” block shells. Structure of the core-shell polymer micelles formed from ABA triblock copolymers are similar to that formed from AB diblock copolymers. On the other hand, in the case of BAB triblock copolymers in water at the low concentration, the polymers form flower micelles composed of the hydrophobic “B” core and hydrophilic loop “A” shells such as petals [1]. Furthermore, at high concentration of the BAB triblock copolymers, the aqueous solution leads to gelation or precipitation, because network structures are formed due to interpolymer hydrophobic interactions between “B” blocks.

Kadam et al., [2] have reported the synthesis of flower micelles composed of poly (2-methacryloyloxyethyl acrylate)-block-poly(ethylene oxide)-block-poly(2-methylacryloyloxyethyl acrylate) triblock copolymer bearing polymerizable groups on the hydrophobic blocks. The transient flower micelles structures in water were permanently fixed by cross-linking the methacrylate moieties in the micelles cores under UV light. Graaf et al., [3] have reported that amphiphilic BAB triblock copolymers consisting of Poly (Ethylene Glycol) (PEG) as hydrophilic A block and thermo-responsive poly (N-isopropylacrylamide) (pNIPAm) B blocks from flower micelles below the Lower Critical Solution Temperature (LCST) for pNIPAm blocks in water. These reported examples of flower micelles are based on hydrophobic interactions.

In general, polymer-based nano-aggregates in water are formed due to various driving forces such as interpolymer hydrophobic interactions, hydrogen bonding, Van der Waals, and electrostatic interactions [4-8]. The driving forces of polymer micelle core formation are not only hydrophobic interactions but also electrostatic interactions, which have attracted attention. Kataoka et al., [9-11] reported preparation of oppositely charged double hydrophilic diblock copolymers, poly (ethylene glycol)-block-poly(L-lysine) (PEG-P(Lys) and poly(ethylene glycol)-block-poly(ε,ω-aspartic acid) (PEG-P(Asp)). When these oppositely charged diblock copolymers are neutralized in water, water-soluble Polymer Complex (PIC) micelles are formed due to the electrostatic interactions. The PIC micelles are composed of the segregated PIC core formed by charged blocks of cationic P (Lys) and anionic P (Asp), which are surrounded by electrically neutral hydrophilic PEG shells.

We prepared oppositely charged double hydrophilic diblock copolymers (PEG-PMAAPTAC and PEG-PAMPS) via Reversible Addition-Fragmentation Chain Transfer (RAFT) controlled living radical polymerization of (3-(Methacryloylamino) Propyl) trimethylammonium chloride (PAMPS)-block-poly(2-methacryloyloxyethyl acrylate) triblock copolymer bearing polymerizable groups on the hydrophobic blocks. The transient flower micelles structures in water were permanently fixed by cross-linking the methacrylate moieties in the micelles cores under UV light. Graaf et al., [3] have reported that amphiphilic BAB triblock copolymers consisting of Poly (Ethylene Glycol) (PEG) as hydrophilic A block and thermo-responsive poly (N-isopropylacrylamide) (pNIPAm) B blocks from flower micelles below the Lower Critical Solution Temperature (LCST) for pNIPAm blocks in water. These reported examples of flower micelles are based on hydrophobic interactions.
Trimethylammonium Chloride (MAPTAC) and sodium 2-(Acrylamido)-2-Methylpropanesulfonate (AMPS) using PEG-based monofunctional chain transfer agent [12-14]. When these oppositely charged PEG-PMAPTAC and PEG-AMPS are mixed with stoichiometrically charge neutralization in water, water-soluble PIC micelles are formed, which composed of segregated PIC core composed of cationic PMAPTAC and anionic PAMPS blocks and the outer hydrophilic nonionic PEG shells [15].

In this study, we prepared PIC flower micelles in water (Figure 1). An anionic ABA triblock copolymer (PAMPS₄₈-PEG₂₂₇-PAMPS₄₈, AEA) composed of PAMPS and PEG blocks was prepared via RAFT radical polymerization. Cationic diblock copolymers (PEG-PMAPTAC, EMₙ) with different chain lengths of the PMAPTAC block were also prepared via RAFT. When AEA and EMₙ were mixed in water, PIC flower micelles were formed through electrostatic interactions between the PAMPS and PMAPTAC blocks (Figure 1). The resulting structures were analyzed using light scattering, NMR, ζ-potential, and Transmission Electron Microscopy (TEM) measurement techniques.

Figure 1: (a) Chemical structures of PAMPS₄₈-PEG₂₂₇-PAMPS₄₈ (AEA) and PEG₄₇-PMAPTACₙ(EMₙ, n = 27,53, and 106). (b) Schematic representation of a flower micelle composed of AEA and EMₙ.

### Materials and Methods

#### Chemicals and materials

4-Cyanopentanoic acid Dithiobenzozate (CDP) was synthesized according to the method reported by McCormick and coworkers [16]. Poly (ethylene glycol)-based chain transfer agent (PEG-CPD), number-average molecular weight (Mₐ) = 2.26 × 10³, number-average Degree of Polymerization (DP) = 47, molecular weight distribution (Mₙ/Mₐ = 1.02) was synthesized as previously reported [15]. 3-(Methacryloylamino)Propyl) Trimethylammonium Chloride (MAPTAC) (50 w% in water) from Aldrich was passed through an inhibitor-remover column. 2-(Acrylamido)-2-Methylpropanesulfonate (AMPS) using HO-PEG-OH (Mₐ = 9.40 × 10³, 50.1 g, 5.01 mmol), CPD (3.38 g, 12.1 mmol), and a trace of DMAP over a period of 30 min. After the reaction mixture was stirred for 20 h at 40°C, it was filtrated to remove dicyclohexylurea. The solvent was removed, and the crude product was purified by silica-gel chromatography using a mixture of chloroform and methanol (9/1, v/v) as eluent, affording CDP-PEG-CPD as a red powder (42.2 g, 79.0%). Mₐ and Mₙ/Mₐ values determined by Gel-Permeation Chromatography (GPC) to be 1.00 × 10⁴ and 1.18, respectively.

### Synthesis of poly (ethylene glycol)-based bifunctional chain transfer agent (CDP-PEG-CPD)

A predetermined amount of AMPS (3.13 g, 15.1 mmol) was neutralized with NaOH (0.60 g, 15.1 mmol) in 30.6 mL of water. To this solution the mixture was added predetermined amounts of V-501 (15.5 mg, 0.0552 mmol). The solution was deoxygenated by purging with Ar gas for 30 min. Polymerization was carried out at 70°C for 16 h. After polymerization, the mixture was poured into a large excess of THF to precipitate the resulting polymer which was dialyzed against pure water for one day. The cationic diblock copolymer (CDP₄₇-PEG₂₂₇-PAMPS₄₈, AEA) was recovered by a freeze-drying technique (4.05 g, 85.8%). The Mₐ and Mₙ/Mₐ values determined by GPC were 2.32 × 10⁴ and 1.42, respectively. DP for the one PAMPS block was 48, as estimated by ¹H NMR.

### Preparation of cationic diblock copolymers (PEG₄₇-PMAPTACₙ) [15]

A representative example for the preparation of the cationic diblock copolymer is as follows: MAPTAC (5.52 g, 25.0 mmol), V-501 (70.1 mg, 0.25 mmol), and PEG-CPD (1.13 g, 0.50 mmol) were dissolved in water (41.0 mL). The mixture was deoxygenated by purging with Ar gas for 30 min. Polymerization was carried out at 70°C for 5 h. The polymerization mixture was poured into a large excess of acetone to precipitate the resulting polymer. The polymer was purified by re-precipitating from methanol into a large excess of acetone twice. The cationic diblock copolymer (PEG₄₇-PMAPTACₙ) obtained was dried in a vacuum oven at 60°C for 24 h (5.81 g, 87.4 %). The Mₐ and Mₙ/Mₐ values were estimated by GPC to be 1.11 × 10⁴ and 1.02, respectively. DP for the one PAMPS block was 53 as estimated by ¹H NMR.

### Preparation of Polyion Complex (PIC) micelles

AE and EMₙ were separately dissolved in 0.1 M NaCl aqueous solutions, which were allowed to stand overnight at room temperature to achieve complete dissolution. For the preparation of PIC micelles, an EMₙ solution was added drop wise to an AEA solution over a period of 5 min at room temperature with stirring, and the mixture was allowed to equilibrate for at least one day prior to measurement. The mixing ratio of the two oppositely charged block copolymers was adjusted based on the mole fraction of AMPS units.
(f_{\text{AMPs}} = [\text{AMPs}]/([\text{AMPs}] + [\text{MAPTAC}]), where [\text{AMPs}] and [\text{MAPTAC}] are the mole concentrations of AMPs and MAPTAC units, respectively. The total polymer Concentration (C_p) for PIC micelles of AEA/EM_{27}, AEA/EM_{53}, and AEA/EM_{106} were kept constant at 1 g/L.

**Measurements**

GPC measurements for cationic polymer samples were performed using a Shodex Nanoscale SI-1 pump and a Tosoh RI-8012 Refractive Index (RI) detector equipped with a Shodex 10.0 μm bead size Ohpak SB-804 HQ column (exclusion limit ~10<sup>7</sup>) working at 40 °C with a flow rate of 0.6 mL/min. A 0.5 M NaSO<sub>4</sub> aqueous solution containing 0.5 M acetic acid was used as eluent. The values of M<sub>n</sub> and M<sub>r</sub>/M<sub>n</sub> for cationic polymer samples were calibrated using standard poly (2-vinylpyridine) samples of 6 different molecular weights ranging from 5.70 × 10<sup>3</sup> to 2.16 × 10<sup>6</sup>. GPC measurements for anionic polymer samples were performed using a Tosoh DP-8020 pump and a Tosoh RI-8020 RI detector equipped with a Shodex Asahipak 7.0 μm bead size GF-7H MQ column (exclusion limit ~10<sup>6</sup>) working at 40 °C with a flow rate of 0.6 mL/min. A phosphate buffer at pH 9 containing 10 vol % acetonitrile was used as eluent. The values of M<sub>n</sub> and M<sub>r</sub>/M<sub>n</sub> for anionic polymers were calibrated using standard sodium polystyrene sulfonate samples of 11 different molecular weights ranging from 1.37 × 10<sup>3</sup> to 2.16 × 10<sup>6</sup>.

<sup>1</sup>H NMR spectra were obtained using a Bruker DRX-500 spectrometer operating at 500 MHz using deuterium lock at a constant temperature of 20 °C during the whole run. Sample solutions of the polymer for <sup>1</sup>H NMR measurements were prepared in D<sub>2</sub>O containing 0.1 M NaCl at C<sub>p</sub> = 1 g/L.

Light scattering measurements were performed using an Otsuka Electronics Photal DLS-7000 light scattering equipment with a multi-r digital time correlator (ALV-5000/EPP). A He-Ne laser (10.0 mW at 632.8 nm) was used as a light source. Sample solutions for light scattering measurements were filtered using a 0.2 μm pore size polytetrafluoroethylene filter. In Static Light Scattering (SLS) measurements, the weight-average molecular weight (M<sub>n</sub>), z-average Radius of gyration (R<sub>Z</sub>), and second virial coefficient (A<sub>v</sub>) values were estimated from the relation,

\[
\frac{K C_p}{R_g} = \frac{1}{M_n} \left( 1 + \frac{4}{3} \frac{R_g}{q}^2 \right) + 2 A_v C_p
\]

where R<sub>g</sub> is the difference between the Rayleigh ratio of the solution and that of the solvent, \( K = 4\pi n^2 (dn/dC_p)^2 / N A^2 \) with dn/dC<sub>p</sub> representing the refractive index increment against C<sub>p</sub>, NA is Avogadro’s number, and q is the magnitude of scattering vector. The q value is calculated from q = (4πn/λ)sin(θ/2), where n is the refractive index of the solvent, λ is the wavelength of light source (= 632.8 nm), and θ is the scattering angle. The known Rayleigh ratio of toluene was used to calibrate the instrument. Values of dn/dC<sub>p</sub> at 633 nm were determined using an Otsuka Electronics Photot DRM-3000 differential refractometer. In the Dynamic Light Scattering (DLS) measurements, to obtain the relaxation time distribution τ<sub>A</sub>(τ), an inverse Laplace Transform (ILT) analysis was performed using the REPES algorithm [18,19]. The relaxation time (τ = t - 1) is a function of θ [20]. The Diffusion coefficient (D) is calculated from D = (τ/q<sup>2</sup>) q<sup>2</sup>. The hydrodynamic radius (R<sub>H</sub>) is given by the Stokes-Einstein equation, R<sub>H</sub> = k<sub>B</sub>T/(6πηd), where k<sub>B</sub> is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity. The details of DLS instrumentation and theory are described in the literature [21].

ζ-potential measurements were performed using a Malvern Zetasizer Nano-ZS equipped with a He-Ne laser light source (4 mW at 632.8 nm) at 20 °C. ζ-potential was calculated from the electrophoretic mobility (μ) using the Smoluchowski relationship, ζ = qμε (κa >> 1) where η is the viscosity, ε is the dielectric constant of the medium, and κ and a are the Debye-Hückel parameter and the particle radius, respectively [22].

Transmission Electron Microscopy (TEM) observations were carried out with a JEOL JEM-2100 microscope at an accelerating voltage of 200 kV. Samples for TEM observations were prepared by placing one drop of aqueous solution on a copper grid coated with thin films of Formvar. Excess water was blotted using a filter paper. The samples were stained with sodium phosphotungstate and dried under vacuum for one day.

**Results and Discussion**

PEG<sub>n</sub>-PMAPTAC<sub>m</sub> was prepared via RAFT of cationic MAPTAC using PEG-CPD in our laboratory previously, which was used in this study [15]. In Figure 2, a time-conversion relationship is depicted along with the first-order kinetic plot for polymerization of AMPs. There was an induction period of 238 min, which may be due to the slow rate of the formation of the 4-cyanopentanoic acid radical fragment, as reported by McCormic and coworkers [23]. The kinetic plot for RAFT polymerization of AMPs, shown in Figure 2, indicates that the concentration of the propagating radical remained constant during the polymerization.

![Figure 2: Time-conversion (○) and the first-order kinetic plots (□) for the polymerization of AMPs in the presence of CPD-PEG-CPD in water at 70°C.](image)

![Figure 3](image) Comparing GPC elution curves (RI response) for HO-PEG-OH and PAMPS<sub>48</sub>-PEG<sub>227</sub>-PAMPS<sub>48</sub> showing the initial and final time points for polymerization.

Figure 4 shows <sup>1</sup>H NMR spectra of EM<sub>n</sub>, AEA, and AEA/EM<sub>m</sub> in D<sub>2</sub>O containing 0.1 M NaCl. DP (= m) and M (NMR) of the PMAPTAC block in EM<sub>n</sub> were determined from the integral intensity ratio of the resonance bands due to the pendant methyl and methylene protons in the PMAPTAC block around 3.1 to 3.4 ppm and the PEG main chain protons at 3.8 ppm.

![Figure 4c](image) shows the <sup>1</sup>H NMR spectrum of a stoichiometrically charged...
neutralized mixture of EM₈ and AEA in D₂O containing 0.1 M NaCl. The intensities of the resonance bands associated with the PMAPTAC pendent methyl protons at 3.1 ppm and PAMPS pendent methyl protons at 1.5 ppm were extremely weak compared with those associated with the PEG main chain protons at 3.8 ppm. This observation suggested that motions of the PMAPTAC and PAMPS blocks were restricted as a result of the formation of PIC by these oppositely charged block chains. On the other hand, the motion of the PEG blocks was not restricted due to the formation of PEG shells.

If the polymerization is assumed to be ideally living in nature, then the theoretical $M_n$ ($M_n$(theo)) can be calculated as

$$M_n$(theo) = [$M_1$]₀ × $\frac{\chi_m}{[CTA]_0}$ $M_m$ + $M_{CTA}$ (2)

where [$M_1$]₀ is the initial molar concentration of monomer, [CTA]₀ is the initial molar concentration of Chain Transfer Agent (CTA), $\chi_m$ is the percentage conversion of the monomer, $M_m$ is the molecular weight of the monomer, and $M_{CTA}$ is the molecular weight of CTA. The $M_n$(NMR) values for EM₈ and AEA were calculated from ¹H NMR data. As shown in Table 1, the $M_n$(NMR) values for EM₈ and AEA were in reasonable agreement with $M_n$(theo). However, the $M_n$(theo), $M_n$(GPC), and $M_n$(theo)/$M_n$(GPC) values for EM₈ and AEA were slightly different, because poly(2-vinylpyridine) or poly(sodium styrenesulfonate) were used as a standard polymer to calibrate $M_n$(GPC), respectively, and its volume-to-mass ratio may be different from that of EM₈ and AEA [24].

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Table 1: Number-average Molecular weight ($M_n$), Molecular weight distribution ($M_w/M_n$), hydrodynamic radius ($R_h$), and ζ-potential for the polymers.

<table>
<thead>
<tr>
<th>Samples</th>
<th>$M_n$(theo) *10⁴</th>
<th>$M_w$(NMR) *10⁴</th>
<th>$M_w$(GPC) *10⁴</th>
<th>$M_w/M_n$</th>
<th>$R_h$(nm)</th>
<th>ζ-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM₈</td>
<td>0.78</td>
<td>0.83</td>
<td>0.82</td>
<td>1.03</td>
<td>4.5</td>
<td>18.2</td>
</tr>
<tr>
<td>EM₅₃</td>
<td>1.36</td>
<td>1.41</td>
<td>1.11</td>
<td>1.02</td>
<td>4.3</td>
<td>24.3</td>
</tr>
<tr>
<td>EM₁₀₆</td>
<td>2.52</td>
<td>2.58</td>
<td>1.51</td>
<td>1.02</td>
<td>6.1</td>
<td>25.4</td>
</tr>
<tr>
<td>AEA</td>
<td>3.21</td>
<td>3.26</td>
<td>3.22</td>
<td>1.42</td>
<td>6.1</td>
<td>-14.4</td>
</tr>
</tbody>
</table>

Figure 3: GPC elution curves for a sample of HO-PEG-OH ($M_n = 9.40 \times 10^3$; $M_w/M_n = 1.06$) (-----) and triblock copolymer of PAMPS₄-PEG₂₂₇-PAMPS₄₈ (AEA, $M_n = 2.32 \times 10^4$; $M_w/M_n = 1.42$) (----).

Figure 4: ¹H NMR spectra for (a) EM₈, (b) AEA, and (c) AEA/EM₈ micelle in D₂O containing 0.1 M NaCl at 20°C. Assignments are indicated for the resonance peaks.

Figure 5: (a) Light scattering intensities and (b) $R_h$ for PIC micelles of AEA/EM₁₀₆ (○), AEA/EM₅₃ (□), and AEA/EM₅₇ (▲) as a function of $f_{AMPS} (= [AMPS]/( [AMPS] + [MAPTAC]))$ in 0.1 M NaCl aqueous solutions. [AMPS] and [MAPTAC] represent the concentrations of the AMPS and MAPTAC units, respectively. The total polymer concentration was kept constant at 1 g/L.

[Table 1 and Figures 3, 4, 5 are not included in the text.]
increasing DP of the cationic PMAPTAC block. The ζ-potential value for AEA was negative due to pendant sulfonate anions in the PAMPS blocks. When EM₅₃ and AEA were mixed to prepare PIC micelles, the ζ-potential values for PIC micelle of AEA/EM₅₃ were close to zero (Table 2). This observation suggested that AEA and EM₅₃ were almost stoichiometric charge neutralization of PAMPS and PMAPTAC segments.

The Rₓ and scattering intensity values of AEA/EM₅₃ micelle were the largest compared to those of AEA/EM₁₀₆ and AEA/EM₂₇ micelles. Kataoka et al., reported that when the anionic and cationic chain length of block copolymers are approximately the same, the aggregation number (N₄ₓ), defined as the total number of polymer chains forming one micelle, is the largest of the PIC micelles [11]. The values of DP for anionic PAMPS in AEA and cationic PMAPTAC in EM₅₃ are 48 and 53, respectively. AEA and EM₅₃ may form complex easily, because these DP values of charged blocks in AEA/EM₅₃ are closer than those of AEA/EM₁₀₆ and AEA/EM₂₇. Therefore, a pair of AEA/EM₅₃ micelles may have the largest aggregation number (N₄ₓ).

Values of Rₓ for the block copolymers were determined by DLS at C_p = 1 g/L in 0.1 M NaCl, as listed in Table 1. The Rₓ values ranging from 4.5 to 6.1 nm appear to be reasonable for unimers of these block copolymers. Figure 6a shows Rₓ distributions for AEA/EM₅₃ micelles. The values of Rₓ estimated from the distributions were summarized in Table 2. The Rₓ values of AEA/EM₁₀₆, AEA/EM₂₇, and AEA/EM₁₅₁ micelles were 32.4, 41.0, and 15.2 nm, respectively. When 1.5 M NaCl was added to the AEA/EM₅₃ aqueous solution, the Rₓ value decreased. This observation suggests that the micelle is dissociated by adding NaCl.

The relaxation rates (Γ) measured at different scattering angles (θ) were plotted as a function of the square of the magnitude of the scattering vector (q²) in Figure 6b. A linear relation passing through the origin indicates that the relaxation modes are virtually diffusive [25]. The Rₓ value estimated from slope of the Γ versus q² plot, was found to be in good agreement with the Rₓ value calculated from Zimm plots, obtained at θ = 90° (Figure 6a). Because the angular dependence was negligible, Rₓ values were estimated at a fixed θ of 90°. In Figure 6c, the Rₓ values are plotted against C_p. The Rₓ values of AEA/EM₁₀₆, AEA/EM₂₇, and AEA/EM₁₅₁ micelles were approximately 32, 41, and 15 nm, respectively, which were practically constant independent of C_p in the range of 0.2 to 1 g/L. From DLS results, the stoichiometrically charge neutralized mixture of AEA and EM₅₃ may form flower micelles without intermicellar aggregates because of the unimodal Rₓ distributions and independence of C_p in the range of 0.2 to 1 g/L.

Figure 7 shows a typical example of Zimm plots for AEA/EM₁₀₆ micelle. Apparent values of Mₓ and Rₓ, determined from Zimm plots, were listed in Table 2. N₄ₓ for the ratio of Mₓ values for PIC micelle and unimer. N₄ₓ for AEA/EM₁₀₆, AEA/EM₂₇, and AEA/EM₁₅₁ micelles were 50,735 and 302, respectively. N₄ₓ for AEA/EM₁₅₁ micelle shows maximum number compared with those of AEA/EM₁₀₆ and AEA/EM₁₀₆ micelles. The Rₓ/Rₚ value is useful for characterizing the shape of molecular assemblies. The theoretical value of Rₓ/Rₚ for a homogeneous hard sphere is 0.778, however the ratio increases substantially for less dense structures and polydisperse mixtures; for example, Rₓ/Rₚ = 1.5 to 1.7 for flexible linear chains in good solvents, whereas Rₓ/Rₚ ≥ 2 for a rigid rod [26-28]. As shown in Table 2, the Rₓ/Rₚ ratios for the micelle were found to be 0.88-0.99, which suggested that the shape of PIC micelles was fairly close to spherical shape.

The density of PIC micelles (dPIC) can be calculated by

\[ d_{\text{PIC}} = \frac{M_w}{N_A V_{\text{PIC}}} \]  

where NA is Avogadro’s number and VPIC is the volume of a PIC micelle. Vₚ can be calculated to be \( V_{\text{PIC}} = 4\pi R_{\text{PIC}}^3 / 3 \). Values of dPIC for AEA/EM₁₀₆, AEA/EM₁₅₁, and AEA/EM₁₅₁ micelles were calculated to be 0.096, 0.109 and 0.129 g/cm³, respectively. These values are close to the density (dPIC) of 0.050 - 0.148 g/cm³ of PIC micelles formed from the mixture of PEG-P (Lys) with PEG-P(Asp) [9]. The dPIC value for AEA/EM₁₀₆ micelle with long cationic PMAPTAC block was larger than that for AEA/EM₁₀₆ micelle with short cationic PMAPTAC block. This observation suggested that PIC micelle of AEA/EM₁₀₆ with short cationic block length may be more hydrated, i.e., the content of water
molecules in AEA/EM_{27} micelle may be larger than that in AEA/EM_{26} micelle, because the volume of PEG chains in one AEA/EM_{27} micelle was larger than those in AEA/EM_{26} and AEA/EM_{28} micelles.

Figure 7: A typical example of Zimm plots for AEA/EM_{26} micelle in 0.1 M NaCl aqueous solution.

Figure 8 shows TEM images for PIC micelles composed of AEA/EM_{27}, AEA/EM_{53}, and AEA/EM_{106}. The average diameters of PIC flower micelles composed of AEA/EM_{27}, AEA/EM_{53}, and AEA/EM_{106} estimated from TEM were 14 ± 4, 53 ± 2 and 49 ± 5 nm, respectively, which were smaller than the 2R_h values, estimated from DLS (Table 2). This implied that the PIC micelles shrink after the removal of water in dry state to measure TEM [29]. Spherical shape for PIC micelles can be observed, suggesting that the aggregates are individual flower micelles without intermicellar aggregation.

Figure 8: TEM images for (a) AEA/EM_{27}, (b) AEA/EM_{53}, and (c) AEA/EM_{106} micelles.

Conclusions

Cationic diblock copolymers, EM_h with different cationic PMAAPTAC block lengths and anionic triblock copolymer, AEA were prepared via RAFT radical polymerization in water using a PEG-based mono- and bifunctional CTA. The oppositely charged EM_h and AEA were mixed in aqueous solutions with stoichiometrically charge neutralized to form PIC micelles. The charge neutralization of the PIC micelles was confirmed with ζ-potential. The cationic PMAPTAC block in EM_h and anionic PAMPS block in AEA formed segregated PIC core, which was confirmed with restricted motion of the ionic blocks by 1H NMR. The PEG blocks formed linear and looped shell chains surrounding the PIC segregated core to form water-soluble PIC flower micelles. Light scattering and TEM data supported formation of the individual PIC flower micelles. The oppositely charged block copolymer combination with similar ionic block chain lengths formed the PIC micelle with maximum R_h and N_m. It is expected that the PIC flower micelles can be applied for the carrier of the charged drugs, because the PIC flower micelles can incorporate charged guest molecules in the PIC core. When the concentration of the PIC flower micelles increase above more than 1 g/L, the AEA and EM_h may form network structure to be gel. Currently, polymer gel formation at high polymer concentrations is studied in our laboratory.

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References


