

Fabrication of Nanoaggregates of a Triple Hydrophilic Block Copolymer by Cetyltrimethylammonium Chloride Binding

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A triple hydrophilic block copolymer composed of poly(ethylene oxide), poly(sodium 2-acrylamido-2-methylpropanesulfonate), and poly(methacrylic acid) (PEO–PAMPS–PMAA) does not form a micelle by itself when it is dissolved in water. However, if the anionic PAMPS and/or PMAA blocks are electrically neutralized with a cationic surfactant, such as cetyltrimethylammonium chloride (CTAC), micelle-like nanoaggregates are obtained, where the core is formed by the insolubilized PAMPS and/or PMAA blocks. Formation of the nanoaggregates was confirmed by dynamic light scattering (DLS) measurements and scanning electron microscopy (SEM), while the binding of CTAC to PEO–PAMPS–PMAA was monitored by electrophoresis measurements. The aggregates were characterized by fluorescence spectroscopy as well as DLS and SEM. It was found that the nanoaggregates have a spherical structure, and the hydrodynamic diameter ranges from 125 to 193 nm depending on the concentrations of the PEO–PAMPS–PMAA and CTAC. The critical aggregate concentration is on the order of 10^{-4} g L⁻¹ when the ionic blocks of PEO–PAMPS–PMAA are fully neutralized with CTAC.

Introduction

Micro- and nanosized materials with unique morphology and functions have become quite important in a wide range of fields such as drug delivery systems, water-miscible carriers, contaminant removers, and so forth.^{1–3} In this respect, significant attention has been paid to the preparation of nanosized particles from amphiphilic AB diblock and ABA triblock copolymers in aqueous solutions.^{4–13} Upon dissolving the amphiphilic block copolymer into water, its self-assembly occurs, resulting in the formation of polymeric micelles having a hydrophobic core and a hydrophilic corona. This core–shell structure provides the polymeric micelle with the function of a vehicle for drug delivery, for example, since the hydrophobic micelle core creates a microenvironment for the incorporation of lipophilic drugs, while the hydrophilic shell provides a stabilizing interface between the hydrophobic core and the aqueous medium.^{14,15} Typical studies

were found for poly(styrene-*b*-ethylene oxide),^{16–18} poly(styrene-*b*-acrylic acid),¹⁹ poly(styrene-*b*-methacrylic acid),²⁰ and so forth. Although the micelles of amphiphilic block copolymers have various advantages compared to the micelles of low-molecular-weight surfactants, there is a drawback to the amphiphilic block copolymer micelles in that they cannot incorporate ionic species into the core because the core is hydrophobic in nature.

Recently, steadily increasing attention has been paid to the double hydrophilic block copolymers (DHBCs), such as poly(ethylene oxide-*b*-methacrylic acid) (PEO-*b*-PMAA), because they self-assemble in a unique manner.²¹ The DHBC does not form a micelle by itself when it is dissolved in water. However, when one of its blocks is made insoluble with a suitable counterion, such as a surfactant, metal ion, or polyion, it forms a micelle having a core consisting of an insolubilized block. The electrostatic interaction (or coordinate bond in some cases) between the added counterion and one block of the DHBC plays a primary role in the micellization process. For example, PEO-*b*-PMAA forms micelles when the anionic PMAA block is insolubilized with a counterion, such as cetyltrimethylammonium chloride (CTAC).²² These nanoaggregates have proven to be stable, monodisperse, and able to incorporate ionic species into the core block because of their ionic nature. Other typical examples of the nanoaggregate formation of DHBCs were reported by Kataoka et al.^{23–26} and

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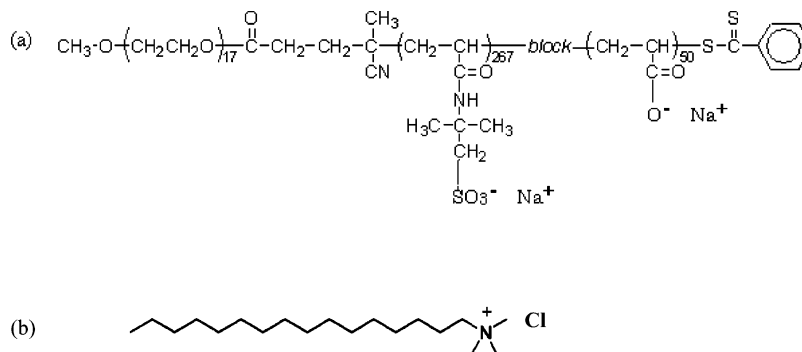


Figure 1. Structures of (a) PEO-PAMPS-PMAA and (b) CTAC.

Kavanov et al.,^{27,28} and various aspects of the micelles of DHBCs have been extensively reviewed by Cölfen.²¹

The polymeric micelles so far reported are mostly concerned with the AB diblock or ABA *symmetric* triblock copolymers. As for the micelles of the ABC *asymmetric* triblock copolymers, only a few groups have investigated them, although they are expected to have a variety of structures and functionalities.^{29–32} One of the advantages of the ABC triblock copolymers is that they provide the micelle with a core-shell-corona structure, in which the core, shell, and corona play different roles. For example, we have fabricated hollow silica nanoparticles templated by the micelles of ABC triblock copolymers.³³ In this case, the core is a template of the hollow, the shell is a nanoreactor for the sol-gel reaction, and the corona is a stabilizer of the polymer-silica nanocomposite.³³ In this context, it seems interesting to study the micelles of the ABC-type *triple hydrophilic block copolymers* (i.e., all three of the blocks are water-soluble). It is expected that the ABC-type triple hydrophilic block copolymers form micelle-like aggregates when their ionic blocks are made insoluble with counterions, and that the aggregates have much more complex nanostructures and functionalities compared to those of the DHBCs.

On the basis of our earlier studies^{22,34–37} and those of others,^{38–46} we tried to fabricate the nanoaggregates using

alkyltrimethylammonium chloride (RTAC) and a triple hydrophilic block copolymer composed of poly(ethylene oxide), poly-(sodium 2-acrylamido-2-methylpropanesulfonate), and poly-(methacrylic acid) (PEO-PAMPS-PMAA) (Figure 1a). The RTAC employed is CTAC (Figure 1b). CTAC is a cationic surfactant and thus may bind to the anionic PAMPS and PMAA blocks of PEO-PAMPS-PMAA by an electrostatic interaction, resulting in insolubilization of both anionic blocks. As expected, we obtained the nanoaggregates of PEO-PAMPS-PMAA. The properties of the nanoaggregates have been studied by various techniques, including dynamic light scattering (DLS), scanning electron microscopy (SEM), turbidimetry, electrophoresis measurements, and fluorescence spectroscopy. To the best of our knowledge, there has been no report on the micelles of triple hydrophilic copolymers.

Experimental Section

Materials. CTAC was purchased from Tokyo Chemical Industry (TCI) and used without further purification. Pyrene (Py) was supplied from Aldrich and purified by vacuum sublimation. The homopolymer, PMAA, having a molecular weight of 150 000, was purchased from Scientific Polymer Products, Inc. 4-Cyanopentanoic acid dithiobenzoate (CPD) was synthesized according to the method reported by McCormick and co-workers.⁴⁷ A homopolymer of sodium 2-acrylamido-2-methylpropanesulfonate (PAMPS, $M_n = 1.48 \times 10^4$, $M_w/M_n = 1.20$) was prepared by reversible addition-fragmentation chain transfer (RAFT) radical polymerization, as previously reported.⁴⁸ CH_2Cl_2 was dried over 4 Å molecular sieves and distilled. Poly(ethylene glycol) methyl ether (MeOPEO, $M_n = 750$, M_w/M_n (estimated from gel permeation chromatography (GPC)) = 1.06) and 4,4'-azobis(4-cyanopentanoic acid) from Aldrich were used as received without further purification. The water was purified by a Millipore Mill-Q system.

Synthesis of PEO-Based Chain Transfer Agent (PEO-CTA). A CH_2Cl_2 solution (100 mL) of *N,N'*-dicyclohexylcarbodiimide (5.67 g, 27.4 mmol) (Kishida Chemical) was added dropwise to a CH_2Cl_2 solution (100 mL) of MeOPEO (10.3 g, 13.7 mmol), CPD (4.60 g, 16.5 mmol), and a trace of 4-(dimethylamino)pyridine (Kanto Chemical) over a period of 30 min. After the reaction mixture was stirred for 24 h at 40 °C, it was filtered to remove the dicyclohexylurea. The solvent was removed, and the crude product was purified by silica gel column chromatography using a mixture of CHCl_3 and methanol (95/5, v/v) as the eluent, which afforded 11.0 g of PEO-CTA as a red oil (78.1% yield). ^1H NMR (CDCl_3) δ (ppm): 2.17

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Preparation of Triblock Copolymers (PEO–PAMPS–PMAA). The AMPS (2.10 g, 10.1 mmol) (TCI) was neutralized with NaOH (0.38 mg, 9.53 mmol) in 7.9 mL of water, and 4,4'-azobis(4-cyanopentanoic acid) (1.91 mg, 0.0068 mmol) and PEO-CTA (33.1 mg, 0.033 mmol) were added to the solution. The mixture was degassed by purging with Ar gas for 30 min in a 25 mL three-necked round-bottom flask with a rubber septum. The polymerization was carried out at 70 °C for 4 h. The reaction mixture containing the PEO–PAMPS diblock copolymer was extracted with a syringe for the ^1H NMR measurement to determine the conversion (84.5%). The deoxygenated second monomer, sodium methacrylate (Wako Pure Chemical Industries) (394 mg, 3.64 mmol) dissolved in 3.0 mL of water was then introduced into the reaction mixture by a syringe. The reaction mixture was maintained at 70 °C for 29 h. The ^1H NMR data for the polymerization system is shown in the Supporting Information (Figure S1). The polymer was purified twice by reprecipitating from water into a large excess of acetone. The polymer was dissolved in pure water and was recovered by freeze-drying. The values of the number-average degree of polymerization (DP_n) for PEO, PAMPS, and PMAA are 17, 267, and 50, respectively, estimated by ^1H NMR (Figure S2, Supporting Information). The M_n and M_w/M_n values determined by GPC are 5.65×10^4 and 1.27, respectively (Figure S3, Supporting Information).

The ^1H NMR spectrum was obtained by a Bruker DRX-500 spectrometer operating at 500 MHz. The GPC measurements were performed using a Tosoh DP-8020 pump equipped with a Tosoh RI-8021 refractive index detector and a Shodex 7.0 μm bead size GF-7M HQ column using a phosphate buffer (pH 9.0) containing 20 vol % acetonitrile as the eluent at a flow rate of 0.6 mL/min. The sample solutions were filtered by a 0.2- μm pore size membrane filter before the measurements. The molecular weights of the sample polymers were calibrated with standard sodium poly(styrene sulfonate) samples.

Preparation of Nanoaggregates. A known amount of PEO–PAMPS–PMAA was dissolved in water by gently stirring for several days. The aqueous solution of PEO–PAMPS–PMAA was then transferred into a volumetric flask to obtain the stock solution (1 g L^{-1}). A known amount of the PEO–PAMPS–PMAA stock solution was mixed with the required amount of CTAC. Hydrochloric acid or sodium hydroxide was added to the solution of CTAC/PEO–PAMPS–PMAA to obtain the desired pH.

The amount of CTAC added to the PEO–PAMPS–PMAA solution is expressed by the *apparent degree of neutralization* (DN), which is defined as

$$\text{DN}(\%) = \frac{(\text{amount of surfactant in molar unit}) \times 100}{(\text{amount of ionic groups in the polymer in base molar unit})} \quad (1)$$

It should be noted here that all of the experiments were carried out at pH 9, at which the carboxylic groups in the PEO–PAMPS–PMAA are completely ionized as well as the sulfonic groups.⁴⁹ Hence, the base molar concentrations of both PAMPS and PMAA are taken into account in the DN calculation.

Turbidimetry. The turbidity was measured by a Jasco Ubest-50 UV/vis spectrophotometer. The turbidity was calculated as $(100 - T)/100$, where T is the transmittance (%) at 400 nm for the solution in the cell with an optical path length of 1 cm.

DLS Measurements. The DLS measurements were carried out by an Otsuka ELS-800 apparatus at a fixed 90° scattering angle. The correlation functions were analyzed by a histogram method and used to determine the diffusion coefficient (D) of the particles. The hydrodynamic radius (R_h) was calculated from D using the Stokes–Einstein equation:

$$R_h = k_B T / (6\pi\eta D) \quad (2)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity.

Zeta-Potential Measurements. The measurements of the electrophoretic mobility (EPM) were performed at 25 °C by an Otsuka ELS-8000 apparatus. The zeta-potential of the particles was calculated from the EPM using the Smoluchowski equation:

$$\mu_E = \zeta\epsilon/\eta \quad (3)$$

where μ_E is the EPM, ζ is the zeta-potential, ϵ is the permittivity of the solvent, and η is the viscosity.

Fluorescence Spectroscopy. The fluorescence spectra of the samples were recorded by a Jasco FP-6500 fluorescence spectrophotometer (right angle geometry, 1 cm \times 1 cm quartz cell). First, a known volume of the Py stock solution (6×10^{-5} M in methanol) was transferred to a 10-mL volumetric flask. The solvent was gently evaporated under a nitrogen gas stream. A known volume of the PEO–PAMPS–PMAA stock solution was then added to the volumetric flask containing Py, followed by the addition of the surfactant solutions. Finally, water was added to the level of the marked line after the pH was adjusted to the desired value with a hydrochloric acid or sodium hydroxide solution. The final concentration of Py was 0.6 μM , which is close to the saturation concentration of Py in the water at 22 °C. The excitation and emission wavelengths for the measurements of the fluorescence and excitation spectra, respectively, are given in the figure caption for each spectrum together with the bandwidth.

SEM Measurements. The SEM observations were obtained by an SEM, JSM-5300E, from JEOL, Japan. For the sample preparation, a drop in the aggregate solution having a polymer concentration of 0.04 g L^{-1} was placed on a copper grid and dried at room temperature for several hours.

Results and Discussion

Turbidity Measurements. It is interesting to examine which of the two blocks (PAMPS or PMAA) of the PEO–PAMPS–PMAA is more strongly bound to the CTAC. Binding of CTAC to the PAMPS or PMAA block can be monitored by the turbidity measurements because the binding of CTAC to these blocks makes them insoluble in water due to charge neutralization. Figure 2 represents the turbidity change in the solutions of the PAMPS and PMAA homopolymers at pH 9 as a function of the DN. The concentrations of the sulfonic group in the PAMPS homopolymer solution is equal to that of the carboxylic group in the PMAA homopolymer solution. In the PAMPS homopolymer systems, the turbidity began to increase at about 10% DN, while, in the PMAA homopolymer systems, the turbidity began to increase at about 30% DN. This indicates that the binding of CTAC to the PAMPS homopolymer is stronger than that to the PMAA homopolymer.

SEM Characterization. From Figure 2, it is clear that the binding of CTAC to PAMPS or PMAA makes these polymers insoluble in water, so that it is expected that the addition of CTAC to the aqueous solution of PEO–PAMPS–PMAA leads to the formation of aggregates with a core of CTAC-bound PAMPS and/or CTAC-bound PMAA and a corona of free PAMPS, free PMAA, and PEO. An SEM measurement was performed to confirm the formation of the nanoaggregates of CTAC/PEO–PAMPS–PMAA. Figure 3 represents an SEM image of the CTAC/PEO–PAMPS–PMAA nanoaggregates prepared in solution with a polymer concentration of 0.04 g L^{-1} . We can clearly see the particles of the aggregates in the SEM image, although the micelles are not well separated from each other.

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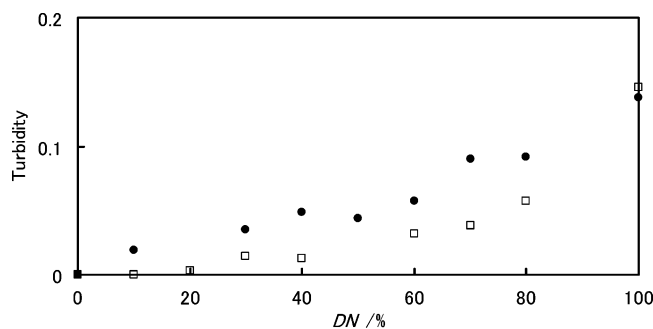


Figure 2. Turbidity of the solutions of CTAC/PAMPS (●) and CTAC/PMAA (□) as a function of the DN at pH 9. The base-molar concentration of the sulfonate group (0.19 mM) in the PAMPS homopolymer is equal to that of the carboxylate group (0.19 mM) in the PMAA homopolymer.

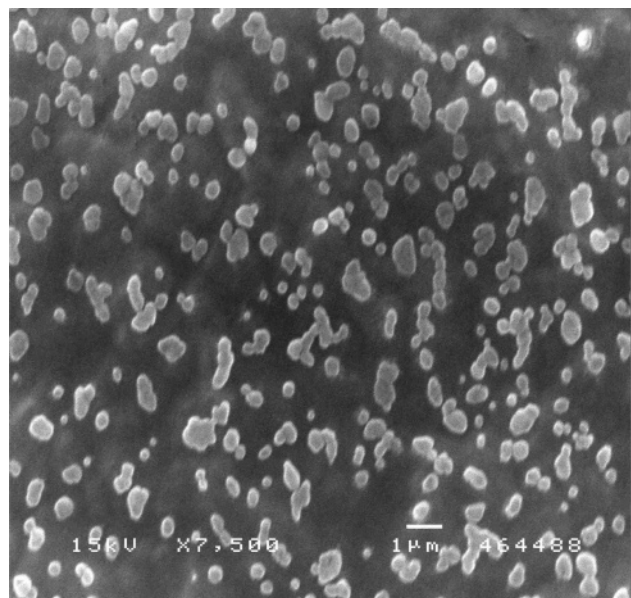


Figure 3. SEM image of CTAC/PEO-PAMPS-PMAA nanoaggregates at pH 9. The concentration of PEO-PAMPS-PMAA and CTAC is 0.04 g L⁻¹ and 0.19 mM, respectively.

DLS Measurements. Figure 4a shows a plot of the hydrodynamic diameters ($2R_h$) of the nanoaggregates of CTAC/PEO-PAMPS-PMAA as a function of DN at a constant polymer concentration (0.06 g L⁻¹). Since the concentration of the CTAC in the present system is much lower than its critical micelle concentration (cmc) (1.40 mM),⁵⁰ even for the sample with the highest DN, CTAC does not form its own micelles. From Figure 4a, the aggregate formation seems to begin at about 5% DN. The average hydrodynamic diameter of the CTAC/PEO-PAMPS-PMAA nanoaggregates is 280 nm at 5% DN, but it decreases from 280 to 164 nm upon increasing the DN from 5 to 80%. This seems to be due to the fact that, at a lower DN, the PAMPS or PMAA core of the micelles is swollen with water because of the repulsion between the unneutralized sulfonate or carboxylate groups of PEO-PAMPS-PMAA. However, at a higher DN, the repulsive force between the anionic groups of PEO-PAMPS-PMAA is electrically cancelled by the additional CTAC, which led to a more compact and hydrophobic micelle core. The hydrodynamic diameter of the nanoaggregates shows a minimum at 80% DN, and starts to increase after 100% DN. The reason for this phenomenon is currently unclear. One of the possibilities is that CTAC is bound to the CTAC/PEO-PAMPS-PMAA

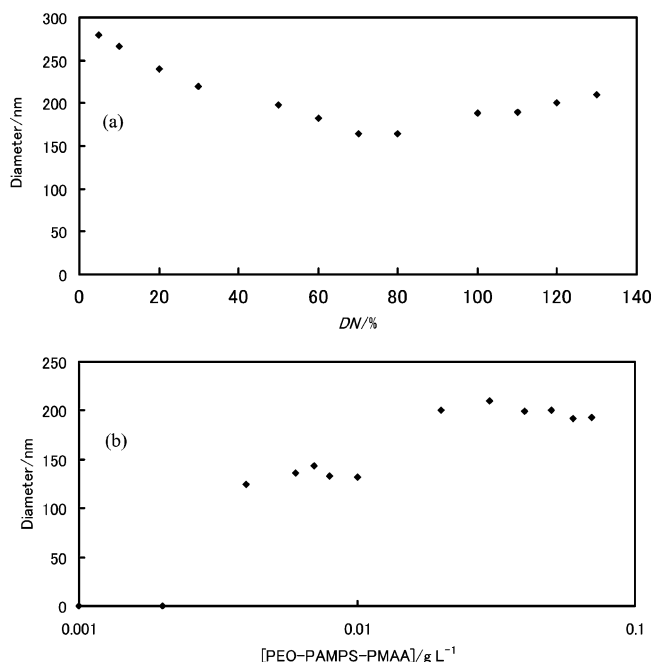


Figure 4. Dependence of hydrodynamic diameter of CTAC/PEO-PAMPS-PMAA nanoaggregates at pH 9 as a function of (a) the DN at a constant PEO-PAMPS-PMAA concentration of 0.06 g L⁻¹ and (b) the polymer concentration at a constant DN of 100%.

nanoaggregates by a hydrophobic interaction after 80% DN, and CTAC forms bridges between the aggregate particles to produce the secondary aggregates. The distribution plots of the hydrodynamic diameter for the aggregates of CTAC/PEO-PAMPS-PMAA at 100% DN when the concentration of the polymer is 0.06 g L⁻¹ are shown in the Supporting Information (Figure S4).

The dependence of the size of the aggregates on the PEO-PAMPS-PMAA concentration at a fixed DN (100%) is presented in Figure 4b. The size of the nanoaggregates was unchanged for several weeks. The formation of the CTAC/PEO-PAMPS-PMAA nanoaggregates seems to start when the concentration of the polymer is 0.004 g L⁻¹. However, this concentration cannot be regarded as the critical aggregate concentration (cac) because DLS does not have a sufficient sensitivity to be employed for the cac determination. The cac is estimated later by fluorescence spectroscopy.

The diameter of the aggregates is almost constant (125 nm) in the concentration range from 0.004 to 0.01 g L⁻¹, but increases from 125 to 193 nm thereafter. This appears to be due to the secondary aggregate formation in the higher concentration region.

Zeta-Potential Measurements. In order to confirm the binding of the CTAC to the sulfonate or carboxylate group of the polymer, an electrophoresis measurement was carried out. In Figure 5, we plotted the dependence of the zeta-potential of the CTAC/PEO-PAMPS-PMAA nanoaggregates on the DN when the concentration of PEO-PAMPS-PMAA is fixed at 0.06 g L⁻¹. The zeta-potential increases from -42 to 5 mV when the DN increases from 5 to 100%. This gives us concrete evidence that the cationic CTAC is bound to the anionic blocks of the PEO-PAMPS-PMAA.

It is surprising, however, that the zeta-potential does not monotonously increase with an increase in the DN. It suddenly increases from -33 to 5 mV during the increase in the DN from 80 to 100%, although it shows a slow increase in the lower DN range (5–80%). This fact implies cooperative binding of the CTAC to the PEO-PAMPS-PMAA. Therefore, not only electrostatic, but also hydrophobic interactions between the CTAC

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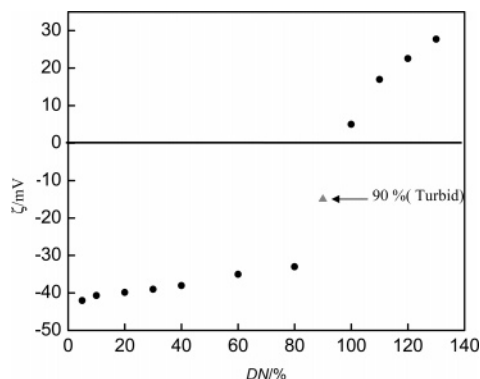


Figure 5. Dependence of the zeta-potential of CTAC/PEO-PAMPS-PMAA nanoaggregates on DN at pH 9. The concentration of PEO-PAMPS-PMAA = 0.06 g L⁻¹.

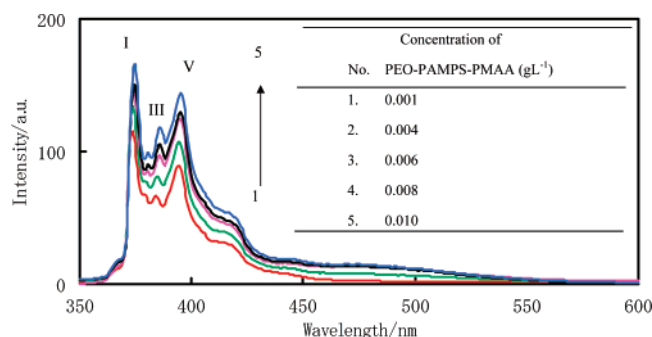


Figure 6. Dependence of the fluorescence intensity of Py (0.6 μM) on the concentration of CTAC/PEO-PAMPS-PMAA nanoaggregates at pH 9. DN = 100%. Py is excited at 334 nm. The bandwidths are 5 and 1 nm on the excitation and emission sides, respectively.

and the polymer seem to play an important role in the aggregate formation. The zeta-potential increases from 5 to 28 mV after a 100% DN. This indicates the incorporation of an excess amount of the cationic CTAC into the CTAC/PEO-PAMPS-PMAA complexes. The binding of an excess amount of CTAC is also explained by the hydrophobic interaction between the CTAC and the polymer.

Fluorescence Spectroscopy. Fluorescence measurements were carried out to determine the cac of the aggregate of CTAC/PEO-PAMPS-PMAA. Figure 6 represents the fluorescence spectra of Py (0.6 μM) in the CTAC/PEO-PAMPS-PMAA solutions with various concentrations of PEO-PAMPS-PMAA at a fixed DN (100%). Band I is a 0-0 band of the S₁ → S₀ transition, and bands III and V are the vibronic bands. From Figure 6, it was found that the fluorescence intensity of Py increases upon increasing the concentration of the polymer from 0.001 to 0.01 g L⁻¹. This shows that the Py molecules were transferred from the aqueous phase to the PEO-PAMPS-PMAA nanoaggregates. When Py is located in the nanoaggregates, deactivation of the fluorescent state is suppressed, resulting in an increase in the fluorescence quantum yield. The increase in the fluorescence intensity is also ascribed to the increase in the absorbance at the excitation wavelength.

One of the useful fluorescence parameters of Py is the intensity ratio between bands I and III (the so-called I_1/I_3 ratio).^{51,52} This parameter has been widely used to determine the cmc of conventional detergent micelles^{51,52} and polymeric micelles^{17,53,54}

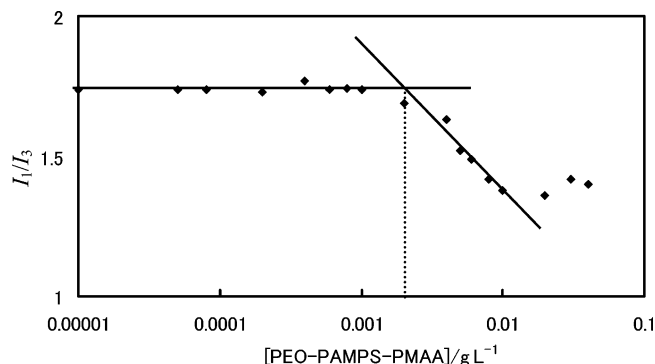


Figure 7. Dependence of I_1/I_3 of Py (0.6 μM) on the concentration of CTAC/PEO-PAMPS-PMAA nanoaggregates at pH 9. DN = 100%.

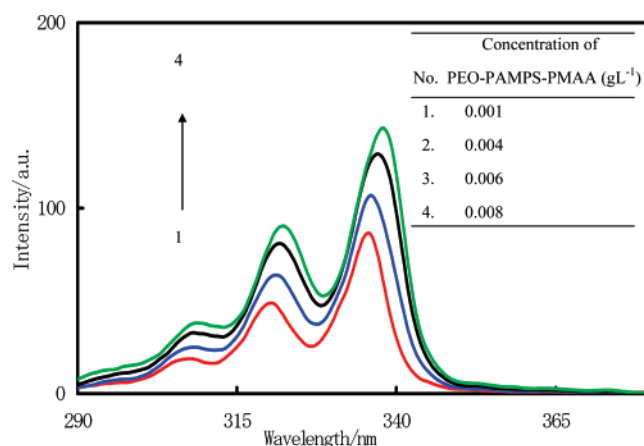


Figure 8. Dependence of excitation spectra of Py (0.6 μM) on the concentration of CTAC/PEO-PAMPS-PMAA nanoaggregates at pH 9. DN = 100%. λ_{em} = 390 nm. The bandwidths are 5 and 1 nm on the excitation and emission sides, respectively.

because the I_1/I_3 ratio is sensitive to the solvent polarity; e.g., it is about 1.87 in water, but 0.61 in hexane.⁵² When Py is added to micellar solutions, it is incorporated into the hydrophobic domain of the micelles. The incorporation of Py into the hydrophobic domain of the micelles produces a lower I_1/I_3 value than that in the aqueous bulk phase. Thus, we can observe a significant change in the I_1/I_3 ratio at the point of the cmc if we plot this parameter versus the surfactant (or polymer) concentration. The dependence of I_1/I_3 on the polymer concentration at DN = 100% is presented in Figure 7. At lower concentrations below 0.001 g L⁻¹, the I_1/I_3 ratio is almost the same as that in water. However, the I_1/I_3 ratio starts to decrease when the concentration of the polymer is 0.002 g L⁻¹. According to Winnik et al.,¹⁷ the intersection of the two straight lines in Figure 7 (0.002 g L⁻¹) determines the upper limit of the cac. It should be noted here that the intersection does not give the cac (or cmc) but gives only the upper bound of the cac for the polymeric micelles.¹⁷ In order to obtain the cac, we have to measure the excitation spectra. This is in contrast to the case of low molecular weight surfactants. We will later show the treatment based on the excitation spectra.

It is interesting to compare the I_1/I_3 ratio of Py in the CTAC/PEO-PAMPS-PMAA nanoaggregate solutions with that in neat solvents or a surfactant. The I_1/I_3 value is 1.40 in the nanoaggregate solution at the higher polymer concentrations. This value (1.40) is close to that observed for acetic acid (1.37), ethyl acetate

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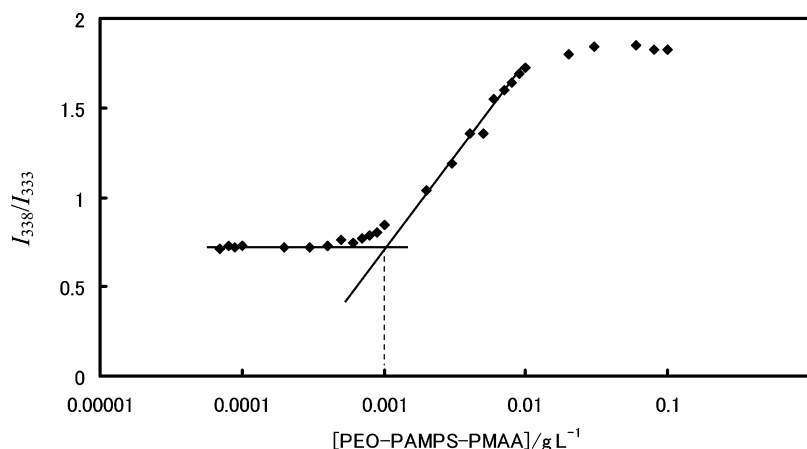


Figure 9. Plots of I_{338}/I_{333} versus the concentration of the CTAC/PEO-PAMPS-PMAA nanoaggregates at a fixed DN of 100%.

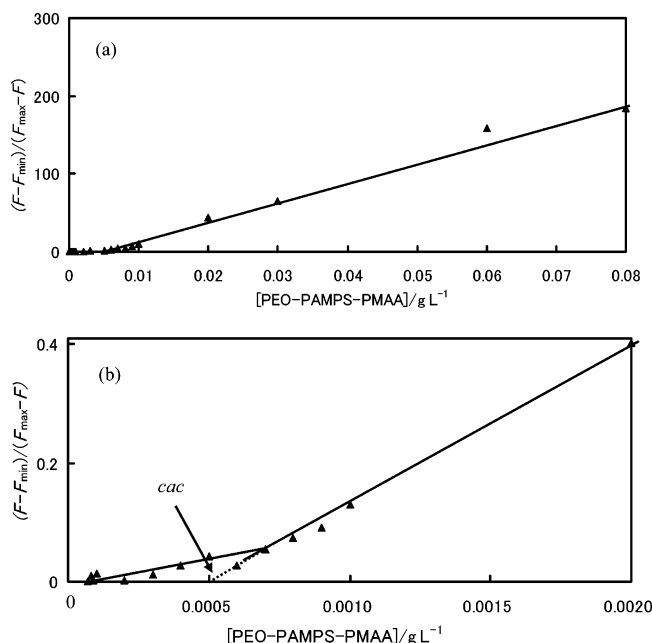


Figure 10. (a) Plots of $(F - F_{\min})/(F_{\max} - F)$ versus the CTAC/PEO-PAMPS-PMAA nanoaggregates at a fixed DN of 100% and (b) plots of panel a at low polymer concentrations.

(1.37), and CTAC micelles (1.35).^{50,51} Therefore, the polarity of the microenvironment of the Py molecules in the CTAC/PEO-PAMPS-PMAA nanoaggregates seems to be close to that in acetic acid, ethyl acetate, or CTAC micelles under the assumption that the fraction of Py in the aqueous bulk phase is negligible.

According to Winnik et al., an accurate cmc of polymeric micelles can be obtained from the excitation spectra of Py.¹⁷ Figure 8 represents the excitation spectra of Py in the solutions of the CTAC/PEO-PAMPS-PMAA nanoaggregates. We note the significant red shift of the excitation spectra upon increasing the polymer concentration. This fact indicates that the absorption maximum of Py at 333 nm in water shifts to a longer wavelength (338 nm) when Py is solubilized in the hydrophobic domains of the nanoaggregates.

First, we are concerned with a plot of the fluorescence intensity ratio (I_{338}/I_{333}) of the excitation spectra versus the logarithm of the polymer concentration, because this plot also provides the upper bound of the cac.¹⁷ Here, I_{338} and I_{333} denote the peak intensity of the bands at 338 and 333 nm, respectively. An example of the plots is presented in Figure 9 when the DN is fixed at 100%. From Figure 9, it is found that, at a low polymer concentration, the ratio of I_{338}/I_{333} takes a value characteristic of

Py in water, while, at high polymer concentrations, it takes a value of Py entirely in the hydrophobic environment. The intersection of the lower horizontal tangent and the slope tangent in Figure 9 corresponds to the upper bound of the cac of the CTAC/PEO-PAMPS-PMAA system when DN = 100%. The obtained value (0.001 g L⁻¹) is close to that obtained from the I_1/I_3 ratio in Figure 7.

On the basis of the treatment by Winnik et al.,¹⁷ we plotted the polymer concentration (c) versus $(F - F_{\min})/(F_{\max} - F)$ in order to determine the exact cac of the CTAC/PEO-PAMPS-PMAA aggregates, where F_{\max} and F_{\min} are the intensity ratios (I_{338}/I_{333}) in the high and low polymer concentration ranges, respectively, and F is the intensity ratio in the intermediate concentration range (Figure 9). According to Winnik et al.,¹⁷ we can obtain the cac as the extrapolated intercept with the c -axis of the line in the plot of $(F - F_{\min})/(F_{\max} - F)$ versus c . The exact cac is thus determined to be 0.0005 g L⁻¹ from Figure 10. It should be noted here that the cac value of 0.0005 g L⁻¹ is one order lower than the value at which the particle formation is detected in the DLS measurements (see Figure 4). This fact demonstrates the sensitivity of fluorescence techniques in determining the cac of the polymer aggregates.

Conclusion

We have fabricated nanoaggregates of a *triple hydrophilic block copolymer* PEO-PAMPS-PMAA by insolubilizing the anionic block PAMPS or PMAA with a cationic surfactant CTAC. The nanoaggregates were characterized by various techniques, such as turbidimetry, SEM, DLS, zeta-potential measurements, and fluorescence spectroscopy.

Turbidity measurements revealed that the CTAC is more strongly bound to PAMPS than to PMAA, indicating that the core of the aggregates is first formed by the PAMPS. SEM images provided evidence for the formation of almost spherical nanoaggregates. DLS measurements clarified the changes in the hydrodynamic diameter of the nanoaggregates in aqueous solutions as a function of the DN at a fixed polymer concentration, or as a function of the polymer concentration at a fixed DN. When DN = 100%, the hydrodynamic diameter of the aggregates ranges from 125 to 193 nm, corresponding to the change in the polymer concentration from 0.004 to 0.07 g L⁻¹. The zeta-potential measurements were used to monitor the binding of CTAC to the polymer. The increase in the zeta-potential from -42 to 28 mV upon increasing the DN from 5 to 130% indicates the effective incorporation of CTAC into the polymer. However, the zeta-potential does not monotonously increase with the increased DN, suggesting that, in addition to an electrostatic

interaction, a hydrophobic interaction plays an important role in binding the CTAC to the polymer.

Fluorescence spectroscopy was used to determine the cac of the CTAC/PEO–PAMPS–PMAA system. Py was used as a fluorescence probe, because it has been widely employed for characterizing polymeric micelles.^{17,53,54} Among the various fluorescence parameters of Py, the I_1/I_3 ratio in the fluorescence spectrum and the I_{338}/I_{333} ratio in the excitation spectrum were used to determine the cac. From the I_1/I_3 ratio, the upper bound of the cac was estimated to be 0.002 g L^{-1} . The I_1/I_3 ratio also provided information that the polarity around the Py molecules in the CTAC/PEO–PAMPS–PMAA nanoaggregates is close to that in a neat solvent of acetic acid or CTAC micelles. From the excitation spectra, the cac of the nanoaggregate of CTAC/PEO–PAMPS–PMAA is determined to be 0.0005 g L^{-1} when DN is 100%.

The significance of this kind of aggregate is their ability to incorporate ionic species into the core part and to give the incorporated species various microenvironments, leading to potential applications for the aggregates, such as drug carriers, nanoreactors, and nanocontainers.

Finally, it seems interesting to study the effects of pH and salt on the fabrication of the PEO–PAMPS–PMAA nanoaggregates.

If we decrease the pH to 5 or lower, the ionization of the carboxylic group in the PMAA block will be suppressed, leading to the situation in which CTAC only binds to the PAMPS block. This will result in the formation of a micelle with a PAMPS/CTAC core and a PEO+PMAA mixed corona, because the PMAA block is water-soluble even after the carboxylic group is protonated. On the other hand, the addition of a salt (e.g., NaCl) will significantly affect the binding of the CTAC to the PAMPS and PMAA blocks, giving different cac values and other properties. Such studies will be done in the future.

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Supporting Information Available: Figures showing the ^1H NMR spectra of the polymerization system and PEO–PAMPS–PMAA, the GPC elution profile, and the distribution plots of the hydrodynamic diameter for the aggregates. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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