

Physicochemical Properties of Micelles of Poly(styrene-*b*-2-vinylpyridine-*b*-ethylene Oxide) in Aqueous Solutions

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Micelles of poly(styrene-*b*-2-vinylpyridine-*b*-ethylene oxide) (PS-*b*-P2VP-*b*-PEO) triblock copolymer were prepared in aqueous solutions. The physicochemical properties of the micelles were investigated by dynamic light scattering, zeta-potential measurements, atomic-force microscopy, and fluorescence spectroscopy. The micelles had an average diameter of about 200 nm under acidic conditions. The addition of dextran sulfate to a micellar solution under acidic conditions resulted in a significant decrease in the micelle size. This was due to a conformational change in the P2VP block form extended to shrunken forms after the cationic P2VP block was electrically neutralized with negative dextran sulfate. The release of anionic dye (Eosin Y) from the micelle particles was studied by a dialysis method, and proved to take tens of hours.

Recently, polymeric micelles from double hydrophilic block copolymers in aqueous solutions have attracted much attention.[1–5] In these micelles, complexation with a substrate through an electrostatic interaction or a coordination reaction can reverse the hydrophilicity of one block into hydrophobicity, resulting in forming the micelle core. For example, poly(ethylene oxide-*b*-methacrylate) (PEO-*b*-PMA) forms micelles if the PMA block is insolubilized in water by complexation with cationic surfactants or metal ions.[4,5] One of the advantages of double hydrophilic block copolymer micelles, compared with conventional polymeric micelles formed from amphiphilic block copolymers,[6] is that they can incorporate *ionic* species into the core domain. This property is especially important when polymeric micelles are employed as nano-reactors for ionic reactions, or as carriers for ionic drugs.[1]

From the viewpoint of delivery systems for ionic drugs, another property is required in vivo: i.e., the micelle should not collapse after being highly diluted in the blood stream. For such a requirement, there seem to be two hopeful ways. One is to employ polymeric micelles with a cross-linked core,[7–9] and the other is to use *frozen* micelles,[10,11] in which the exchange of unimers between the micelle and the aqueous bulk phase takes a long time scale of hours or more. In this respect, polymeric micelles having both *ionic* and *frozen* inner layers are anticipated. This attempt seemed to be achieved by the use of ABC type triblock copolymers, in which the A and B blocks formed a frozen core and an ionic shell, respectively, and the C block formed a corona.

Very recently, three groups investigated core-shell-corona micelles of the poly(styrene-*b*-2-vinylpyridine-*b*-ethylene oxide) (PS-*b*-P2VP-*b*-PEO) triblock copolymer, which seems to fulfill the requirements described above.[12–15] Gohy et al. reported on a pH-sensitive morphological change in the core-shell-corona aqueous micelles of the PS-*b*-P2VP-*b*-PEO triblock copolymer.[12,13] According to them, the volume of the P2VP shell is significantly increased at a pH lower than 5, because this block is protonated at a low pH, resulting in an extension of the P2VP chain due to a repulsion between protonated pyridyl groups. Stepanek et al. studied the micellization behavior of the PS-*b*-P2VP-*b*-PEO triblock copolymer, mainly using fluorescence correlation spectroscopy (FCS), and demonstrated that the micelle was easily transformed to the secondary aggregates of the micelle.[14] In their experiments, the secondary aggregation was provoked by stirring, shaking, and filtration of micellar solutions. They stressed the advantage of FCS compared to light-scattering techniques because the former does not need a filtration process. Khanal et al. found that the PS-P2VP-PEO micelle undergoes a significant size change by adding sodium dodecyl sulfate (SDS) under acidic conditions.[15] The diameter of the micelle decreases with increasing SDS concentration. They ascribed the decrease in the diameter to a conformational change in the P2VP block from extended to shrunken structures. The binding of anionic SDS to the protonated P2VP block decreases the repulsion between the P2VP chains, resulting in a shrinkage of the P2VP domain.

In the present work, we investigated the physicochemical properties of the PS-*b*-P2VP-*b*-PEO micelle, while focusing on the ionic interactions in the P2VP shell. We found by dynamic light scattering (DLS) measurements that the PS-*b*-P2VP-*b*-PEO micelle showed a significant size change by incorporating dextran sulfate into the P2VP shell. From dialysis, followed by vis-absorption spectroscopy, we found that the retention of an anionic dye (Eosin Y) in the micelle continued for at least tens of hours.

Experimental

Materials. PS-*b*-P2VP-*b*-PEO (Polymer Source Inc.) was used as supplied. The molecular weights was $\bar{M}_n$(PS) = 14100, $\bar{M}_n$(PVP) = 12300, and $\bar{M}_n$(PEO) = 35000. The distribution of the molecular weights was: $\bar{M}_w/\bar{M}_n = 1.04$ for PS, 1.06 for PS-*b*-P2VP, and 1.08 for PS-*b*-P2VP-*b*-PEO. Pyrene (Py, Aldrich) was purified by vacuum sublimation. Naphthalene (N, zone-refined) from TCI was used as supplied. Sodium dextran sulfate (DS, Wako, MW = 5000) and Eosin Y (EY, Wako) were used

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without further purification.

Sample Preparation. A weighed polymer sample was dissolved in an acidic aqueous solution, and then heated at 65 °C for ca. 2 h. After being cooled, the solution was transferred to a volumetric flask to give a final concentration of 0.1 g L^{-1}. The solution was agitated with a magnetic stirrer at room temperature for several days until a clear solution was obtained (stock solution). The pH of the thus obtained stock solution was ca. 3. A known amount of the stock solution was transferred to a 10 mL volumetric flask. Then, a fluorescence probe and/or other chemicals were added to the flask as needed, followed by dilution with neutral, acidic or alkaline water to give a desired concentration. The sample solutions were kept at room temperature for one night before the measurements.

Characterization of the Micelle. DLS was measured with an Otsuka ELS-800 at a fixed 90° scattering angle. The hydrodynamic diameter (D_h) was obtained by a cumulant method. The zeta-potential of the particles was calculated based on the electrophoresis mobility, which was measured with an Otsuka ELS-8000 apparatus. Fluorescence spectra were recorded on a Jasco FP-6500 spectrofluorometer. Absorption spectra were recorded on a Jasco Ubest-50 spectrophotometer. Atomic force microscopy (AFM) images were obtained in dynamic force mode (corresponding to the tapping mode) with a SPA 300 unit together with a SPI 3700 control station (Seiko Instruments Industry Co. Japan) in air. The samples for AFM observations were prepared by evaporating a drop of the micellar solutions on freshly cleaved mica in air.

Results and Discussion

Detection of Micelle Formation. The micelle formation of the same polymer has already been described in the literature.[12–14] However, we tried to detect micelle formation in our own sample, because the sample preparation procedure was different from that in the literature. Figure 1 shows D_h of the micelles as a function of the polymer concentration at pH 3. A constant diameter of about 185 nm was obtained when the polymer concentration was above 0.005 g L^{-1}. The polydispersity of the micelle diameter was about 0.1 in the analysis by the cumulant method. It should be noted that the diameter in a low polymer concentration region ($c < 0.005$ g L^{-1}) was somewhat smaller than that in a high-concentration region. The reason is currently unclear. One of the possible reasons is that the reliability of the measurements is comparatively low in this region, due to low scattering intensity.

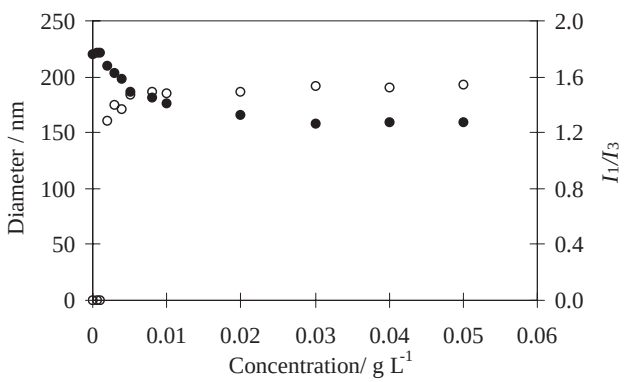

Fig. 1. Hydrodynamic diameter of the micelles (○) and I_1/I_3 ratio of pyrene (●) as a function of the polymer concentration at pH 3. The zero value in the diameter at low polymer concentrations ($c < 0.002$ g L^{-1}) indicates that the scattering intensity was too weak to give reliable hydrodynamic diameter.

Micelle formation was also confirmed by an analysis of the vibronic fine structure of pyrene fluorescence (Fig. 2). It is well known that the polarity of the microenvironment of pyrene can be probed by the I_1/I_3 ratio in its fluorescence spectrum.[16,17] A decrease in the I_1/I_3 ratio with increasing polymer concentration indicates a preferential solubilization of pyrene into the micelles. The I_1/I_3 ratio at the polymer concentration above 0.02 g/L is 1.3, which is an intermediate value between those in PS film (1.05) and P2VP film (1.49).[18] This fact implies that pyrene is located at the interface between the PS and P2VP layers.

Micelle formation was also confirmed by AFM measurements. Figure 3 represents an AFM image for a sample at pH 3. The micelles were close to spherical particles with average diameters of 130 nm at pH 3. In DLS measurements, we

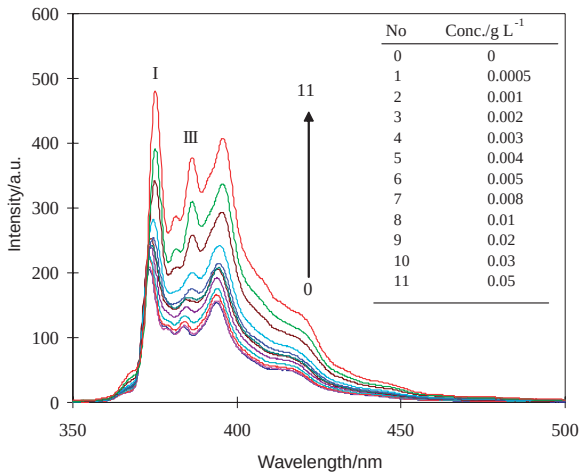

Fig. 2. Fluorescence spectra of Py in micellar solutions of PS-*b*-P2VP-*b*-PEO at pH 3. Concentration of Py is fixed at 0.6 μM. Py is excited at 335 nm. Band widths are 5 nm and 1 nm in excitation and emission sides, respectively.

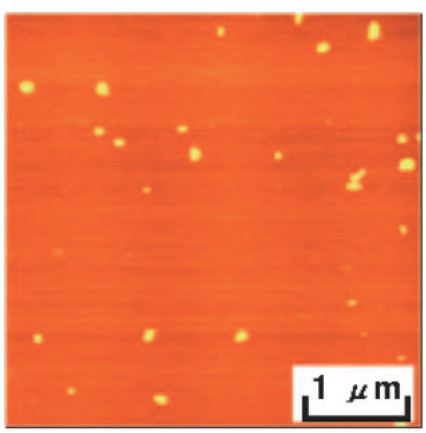

Fig. 3. AFM image of the micelles of PS-*b*-P2VP-*b*-PEO (0.025 g L^{-1}) at pH 3.

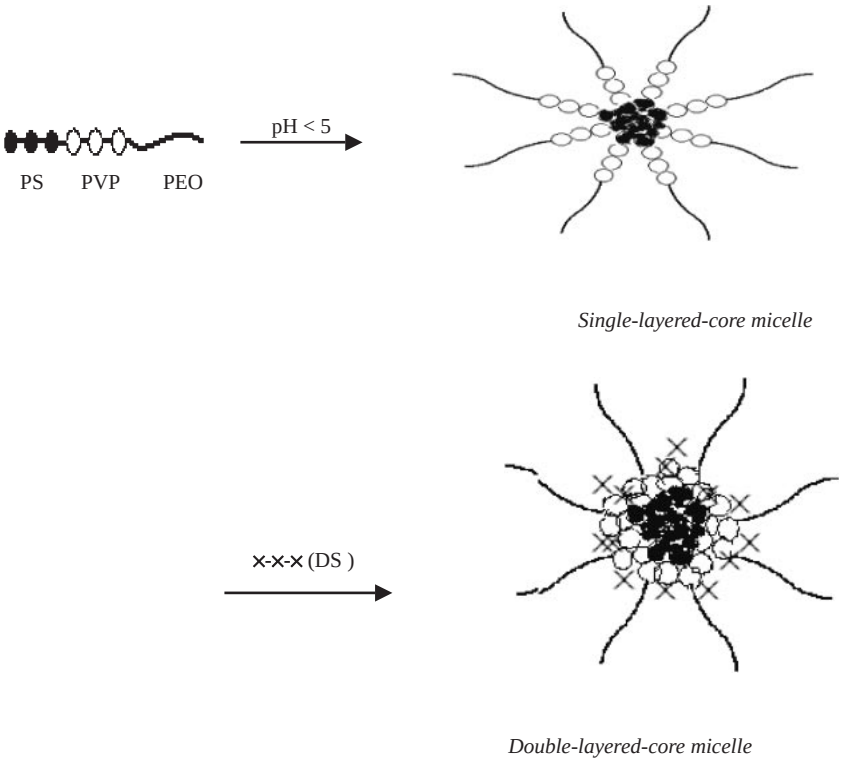

Fig. 4. Schematic illustration of morphological change in the micelle of PS-*b*-P2VP-*b*-PEO induced by addition of DS.

obtained a diameter of 185 nm at pH 3. The average diameter in AFM measurements was smaller than that in DLS. This is reasonable because AFM yields a number-averaged diameter, whereas DLS yields a z-averaged diameter. The AFM data provide strong evidence for micelle formation.

It should be noted here that the micelle size is not well coincident with those reported by Gohy et al.[12,13] and Stepanek et al.[14] For example, Gohy et al. obtained $D_h = 69$ nm at pH > 5 for the same polymer as ours. The difference among the groups seems to be due to the fact that the size of a frozen micelle is strongly dependent on the sample-preparation procedure, because the micelle formation equilibrium was freezed; that is, the micelles were not in the thermodynamical equilibrium states in every case. We dissolved the sample directly into water, while Gohy et al. prepared it by dialysis of the organic solution against water.

Incorporation of DS. We were interested in incorporating of DS into the micelle particles of PS-*b*-P2VP-*b*-PEO, because DS is known to have anticoagulant activity.[19] The incorporation of DS was monitored by the change in the micellar size, since we found in a previous study that the PS-*b*-P2VP-*b*-PEO micelle shows a significant decrease in size at pH < 5 if the protonated P2VP block is electrically neutralized with an anionic surfactant, sodium dodecyl sulfate (SDS).[15] The decrease in the size of the PS-*b*-P2VP-*b*-PEO micelle is explained by a conformational change of the P2VP block from extended to shrunk forms. Therefore, we expected that we could observe a similar change when DS was used instead of SDS. The change in the micellar size is schematically shown in Fig. 4. To express the amount of added DS, we introduced an apparent *degree of neutralization* (DN), which is defined by:

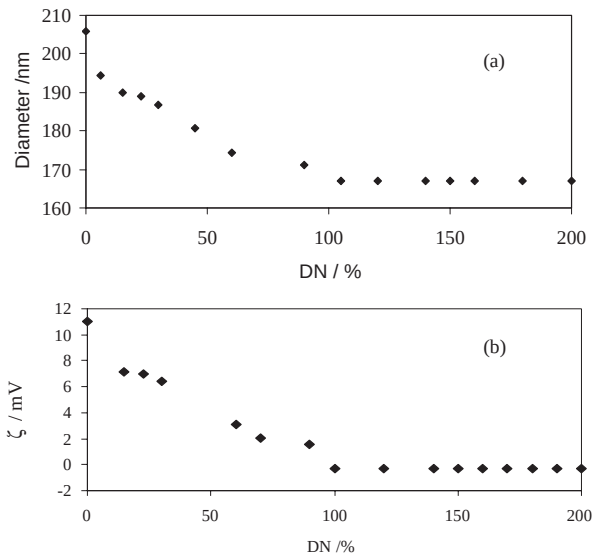

Fig. 5. Dependence of hydrodynamic diameter (a) and zeta-potential (b) of DS/PS-*b*-P2VP-*b*-PEO on DN (i.e., added DS) at pH 3. Concentration of PS-*b*-P2VP-*b*-PEO is fixed at 0.025 g/L.

$$\mathrm{DN}(\%) = \frac{(\text{Amount of sulfate group in mole unit}) \times 100}{(\text{Amount of pyridyl group in mole unit})}. \quad (1)$$

Figure 5a shows the dependence of D_h of the DS/PS-*b*-P2VP-*b*-PEO nanoaggregates on DN at pH 3. When we added DS to the PS-*b*-P2VP-*b*-PEO micelle, the hydrodynamic diameter decreased with increasing amount of DS (i.e., DN), and finally reached a minimum value at 100% DN. Further addition

of DS did not bring about a significant change in D_h. It should be noted here that D_h at DN = 0% is 205 nm, which is significantly larger than that in Fig. 1. The difference seems to originate from the fact the micelle has a frozen nature due to a glassy PS core.[10,11] It is known that frozen micelles are in the states of the local minima of the Gibbs free energy, and the transition from the local minima to the real minimum is kinetically frozen.[20] Therefore, it is not surprising that the micelle size differs from sample to sample if they are prepared in a different batch.

In order to confirm the binding of DS to the micelle of PS-*b*-P2VP-*b*-PEO, we carried out zeta-potential measurements. Figure 5b represents a plot of the zeta-potential of the complexes as a function of DN at pH 3. The successive addition of DS resulted in a continuous decrease in the zeta-potential from 11 to 0 mV. Importantly, at 100% DN, the zeta-potential was 0 mV, indicating that the positive charge of the pyridine unit was completely neutralized by anionic DS. The zeta-potential was not decreased after DN was 100%, indicating that no further binding of DS occurred. These facts indicate that the interaction between the PS-*b*-P2VP-*b*-PEO micelle particle and DS is dominated by an electrostatic interaction, and that one-to-one stoichiometric binding of the sulfate group of DS to the pyridine unit of the P2VP block takes place.

Release of Organic Dye. The release of a small organic molecule from the PS-*b*-P2VP-*b*-PEO micelle is interesting in view of the controlled release of medicine. We carried out an experiment by a dialysis method,[21] using EY as a representative of small organic molecules. Because EY is an organic dye with a carboxylic group, it takes an anionic form under basic conditions. EY was incorporated into the micelles of PS-*b*-P2VP-*b*-PEO in an aqueous solution. The initial concentrations of EY and PS-*b*-P2VP-*b*-PEO in the micellar solution were 15 μmol/L and 0.01 g/L, respectively. These concentrations correspond to 80% DN under the assumption that the carboxylic group of EY and the pyridyl group of P2VP are fully ionized. A dialysis bag containing 10 mL of the micellar solution was put into 300 mL of water, which had a known pH adjusted with HCl or NaOH. Then, the concentration of EY inside the dialysis bag was monitored at an adequate time interval by visible absorption spectroscopy.

Figure 6 shows the change in the absorbance of EY at pH 3 as a function of time. The concentration of EY inside the dialysis bag seems to decrease exponentially. Therefore, we tried to analyze the curve by a kinetic model, as shown in Scheme 1:

$$D_M \xrightarrow{K_1} D_{W1} \xrightarrow{K_2} D_{W2},$$

Scheme 1.

where D_M stands for a dye molecule (i.e., EY) incorporated into the micelle. D_{W1} and D_{W2} denote the dye molecule in aqueous bulk phases inside and outside of the dialysis bag, respectively; k_1 is a rate constant for the dye to exit from the micelle to the aqueous phase inside the dialysis bag, and k_2 is a rate constant for the dye to diffuse from the inside to the outside of the dialysis bag across the membrane. For simplicity, we assume that the first step in Scheme 1 is the rate-determining step (i.e., $k_1 \ll k_2$). Then, we obtain a first-order kinetic equation,

$$d[D_M]_t/dt = k_1[D_M]_t, \tag{2}$$

where $[D_M]_t$ indicates the molar concentration of D_M at time t. The solution of this equation is

$$[D_M]_t = [D_M]_0 \exp(-k_1 t). \tag{3}$$

As we monitored, the absorbance (A) of the dye molecules inside the dialysis bag, the absorbance at time t, is given by

$$A_t = \varepsilon([D_M]_t + [D_{W1}]_t), \tag{4}$$

where ε stands for the molar extinction coefficients of the dye. Under the assumption that the first step in Scheme 1 is the rate-determining step, $[D_{W1}]_t$ in Eq. 4 is negligible (i.e., $[D_{W1}]_t = 0$). From Eqs. 3 and 4, we obtain:

$$A_t = \varepsilon[D_M]_t = \varepsilon[D_M]_0 \exp(-k_1 t), \tag{5}$$

$$\ln(A_t/A_0) = -k_1 t, \tag{6}$$

where A_0 is the absorbance at time 0 (i.e., $A_0 = \varepsilon[D_M]_0$).

In Fig. 7, $\ln(A_t/A_0)$ is plotted against t. The plot gives a rate constant of 0.0387 h^{-1}, although the plot deviates from a straight line to some extent. Here, we define the *release time* (τ) as the reciprocal of k. Then, we obtain $\tau = 25.8$ h. The obtained release time indicates that the retention of EY in the micelle is long enough for the micelle to be employed for a controlled release. Therefore, the results imply potential applications of this type of core-shell-corona micelles to Drug-delivery systems.

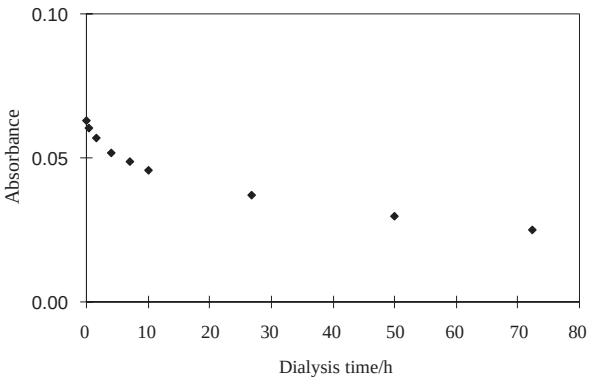

Fig. 6. Dependence of the absorbance of EY on dialysis time at pH 3.

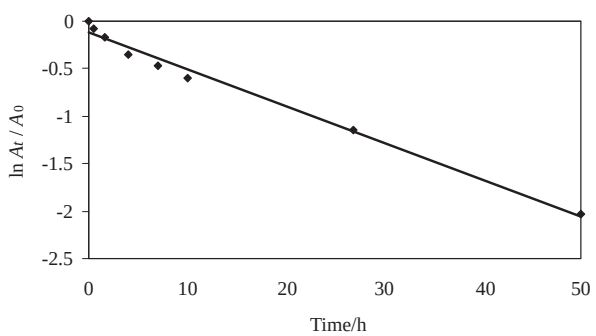

Fig. 7. Plot of $\ln(A_t/A_0)$ against dialysis time. This plot is based on the data in Fig. 6.

Conclusion

We have revealed that the micelle of the PS-*b*-P2VP-*b*-PEO triblock copolymer has an ability to incorporate *ionic* molecules into the inner layers of the micelle particle. It was also shown that the release time of EY from the micelle particles is on the order of 10 h. Although the PS-*b*-P2VP-*b*-PEO micelle may not be suitable for clinical use, because of a possible toxicity of the P2VP block, the strategy of the present study will be applicable to developing practical drug carriers by replacing the P2VP block with a cationic biocompatible polymer.

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