Colloid Polym Sci (2008) 286:1203–1207
DOI 10.1007/s00396-008-1879-x

SHORT COMMUNICATION

Block copolymer micelles with dye loaded on their shells or cores

Eri Yoshida · Tomoko Naito

Received: 26 February 2008 / Revised: 22 April 2008 / Accepted: 26 April 2008 / Published online: 12 June 2008

Abstract We prepared reversible micelles with dye loaded on the shells or cores by ion exchange. A poly(4-styrylmethyl triphenylphosphonium chloride)-block-polystyrene diblock copolymer prepared from poly(4-vinylbenzylchloride)-block-polystyrene and triphenylphosphine was reacted with methyl orange in a mixed solvent of benzene and acetonitrile to produce poly[4-styrylmethyl triphenylphosphonium 4-(4-dimethylamino)phenylazobenzenesulfonate]-block-polystyrene. The loading of the methyl orange on the micellar shells or cores was dependent on the composition of the mixed solvents. Dynamic light scattering demonstrated that the loading of the dye on the cores significantly expanded the micelles when compared to that on the shells. The loading of the dye on the cores shifted the UV wavelength of the methyl orange, whereas that on the shells produced no changes in the UV wavelength. Transmission electron microscopy confirmed the formation of the spherical micelles.

Keywords Poly(4-styrylmethyl triphenylphosphonium chloride)-block-polystyrene · Poly[4-styrylmethyl triphenylphosphonium 4-(4-dimethylamino)phenylazobenzenesulfonate]-block-polystyrene · Methyl orange · Ion exchange · Shell-loading micelles · Core-loading micelles · Micellization

E. Yoshida (✉) · T. Naito
Department of Materials Science,
Toyohashi University of Technology,
1-1 Hibarigaoka, Tempaku-cho,
Toyohashi, Aichi 441-8580, Japan
e-mail: eyoshida@tutms.tut.ac.jp

Introduction

The importance of block copolymer micelles has been increasing in recent years because the micelles can be size-controlled on a nano-scale and encapsulated a variety of substances such as drugs [1, 2], genes [3, 4], enzymes [5, 6], and dyes [7, 8]. The block copolymer micelles usually encapsulated them in the hydrophobic cores through a van der Waals interaction and electrostatic attraction [9]. The core-loading micelles play a role in the transportation of the substances, and a number of publications have reported such results. On the other hand, there are few publications concerning the shell-loading micelles in spite of their utility, although the synthesis of micelles with shell blocks having end-functional groups was reported [10]. The loading of substances on the micellar shells is useful for supporting low molecular weight functional substances on the surface of nanoparticles.

The synthesis of micelles loading a dye on the shell blocks has been already reported through the micellization of a nonamphiphilic block copolymer supporting the dye molecules [11]. The block copolymer was obtained by the copolymerization of the dye monomer having a polymerizable group and a monomer to form the core blocks of the micelles. While this method provides quantitative loading of the dye molecules on the shells, the method was difficult to apply to a variety of commercial dyes because most commercial dyes have no polymerizable groups. A new method of preparing the reversible micelles loading dye on the shells or cores was determined by ion exchange. The loading of dyes by ion exchange is expected for use with a great variety of dyes, since many commercial dyes contain sodium sulfonate and/or carboxylate [12, 13] and ammonium salts [14, 15]. This short communication describes the synthesis

of dye-loaded reversible micelles on the shells or cores by ion exchange.

Experimental

Instrumentation The ^{1}H NMR measurements were conducted using a Varian 300 FT NMR spectrometer. The UV spectra were obtained using a Shimadzu UV-160A UV–Vis recording spectrophotometer. Dynamic light scattering measurements (DLS) were performed with a Photal Otsuka Electronics ELS-8000 electrophoretic light scattering spectrophotometer equipped with a system controller, an ELS controller, and a He–Ne laser operating at λ=632.8 nm. Transmission electron microscopy (TEM) measurements were performed using a JEOL JEM-2010 electron microscope.

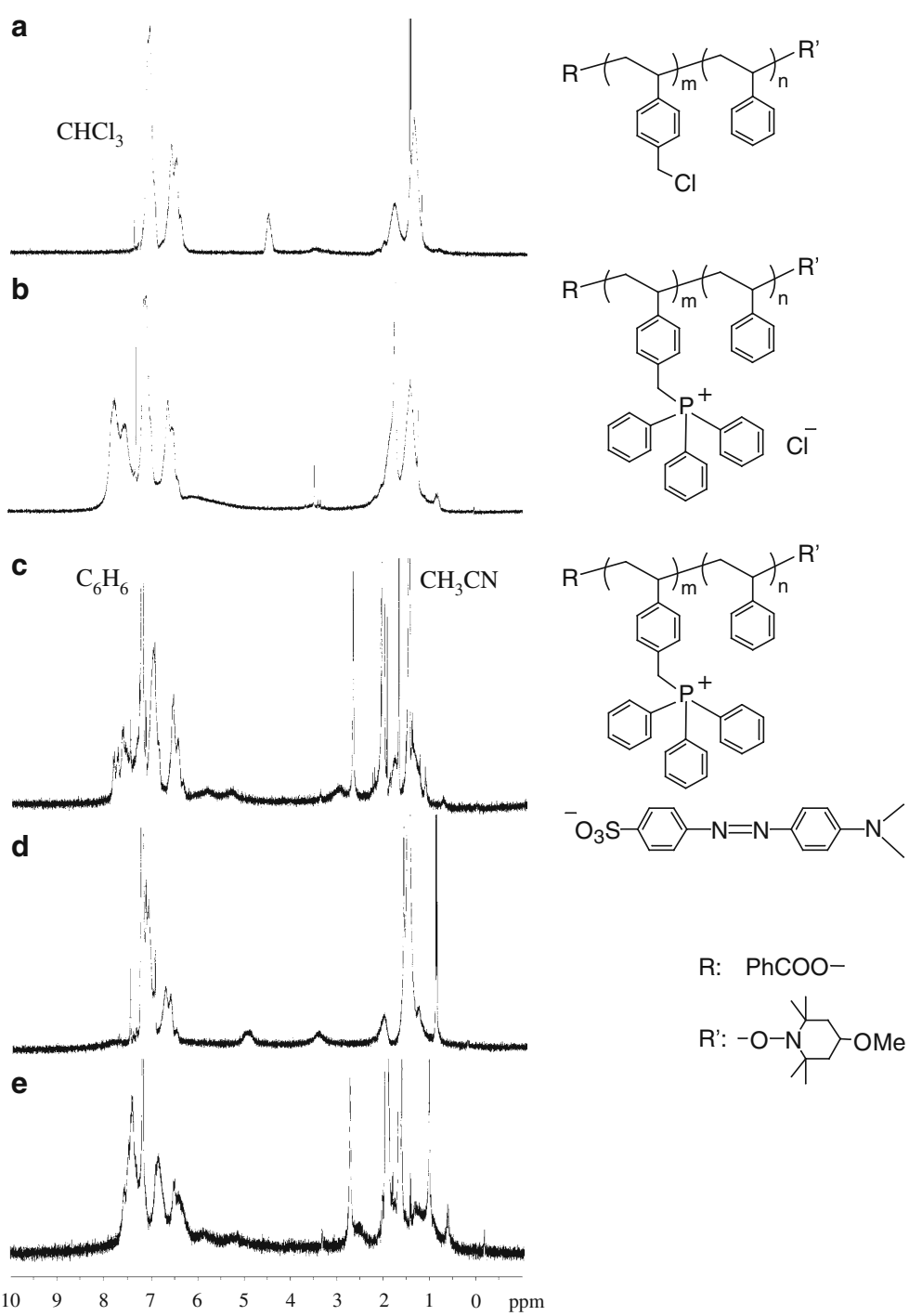

Fig. 1 ^{1}H NMR spectra of PVBC-*b*-PSt (**a**), PP$^+$Cl$^-$-*b*-PSt (**b**), PP$^+$SO$_3^-$-*b*-PSt (**c**–**e**). Solvent: $CDCl_3$ (**a** and **b**) and Bz/An-*d*=5/5 (**c**), 9/1 (**d**), and 1/9 (**e**)

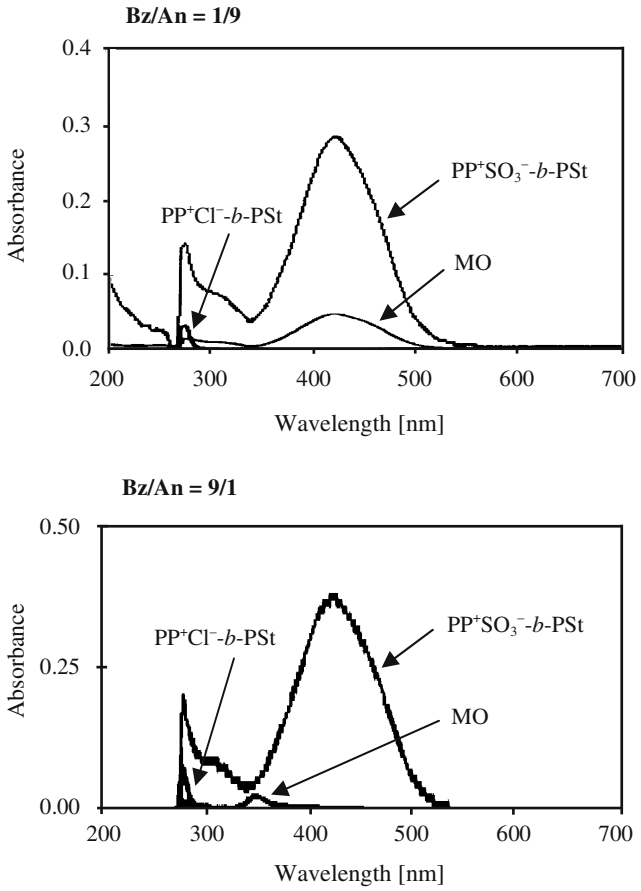

Fig. 2 UV spectra of the $PP^+SO_3^-$-*b*-PSt micelles, methyl orange (MO), and the PP^+Cl^--*b*-PSt micelles in Bz/An=1/9 and 9/1. Bz/An=1/9: $[PP^+SO_3^-$-*b*-PSt$]=2.00\times10^{-2}$, $[MO]=5.71\times10^{-3}$, and $[PP^+Cl^-$-*b*-PSt$]=0.143$ g/L. Bz/An=9/1: $[PP^+SO_3^-$-*b*-PSt$]=2.86\times10^{-2}$, $[MO]=8.16\times10^{-3}$, $[PP^+Cl^-$-*b*-PSt$]=2.04\times10^{-2}$ g/L

Materials Chloroform and acetonitrile were purified by refluxing on calcium hydride for several hours and distilled over calcium hydride. Benzene was purified by distillation over sodium. Extrapure triphenylphosphine and methyl orange were used without further purification.

Synthesis of poly(4-vinylbenzylchloride)-block-polystyrene diblock copolymer (PVBC-b-PSt) PVBC-*b*-PSt was prepared as reported previously [16]. The molar ratio of the VBC unit to the St was VBC/St=0.186:0.814. The absolute molecular weight of the copolymer was determined by ^{1}H NMR to be Mn(PVBC-*b*-PSt)=17,000-*b*-49,000. The molecular weight and the molecular weight distribution by size exclusion chromatography was Mn=37,000 and Mw/Mn=1.90 based on polystyrene standards.

Synthesis of poly(4-styrylmethyl triphenylphosphonium chloride)-block-polystyrene (PP^+Cl^--b-PSt) The PVBC-*b*-PSt copolymer (300 mg, the VBC unit=0.493 mmol) and triphenylphosphine (6.471 g, 24.7 mmol) were dissolved in chloroform (15 mL). The mixture was heated under refluxing for 48 h. The solution was concentrated by an evaporator and was poured into hexane (1 L) to precipitate a polymer. The polymer was purified by repeated reprecipitation from chloroform into hexane. The precipitate was dried in vacuo for several hours to obtain PP^+Cl^--*b*-PSt (412 mg).

Synthesis of poly[4-styrylmethyl triphenylphosphonium 4-(4-dimethylamino)phenylazobenzenesulfonate]-block-polystyrene ($PP^+SO_3^-$-b-PSt) The PP^+Cl^--*b*-PSt copolymer (1 mg, the P^+Cl^- unit=1.15×10^{-3} mmol) was dissolved in acetonitrile (3.5 mL). Methyl orange (0.4 mg, 1.22×10^{-3} mmol) was dissolved in distilled water (0.5 mL), and acetonitrile (3 mL) was added to the methyl orange solution. The methyl orange solution was added to the copolymer solution, and this mixture was heated at 30 °C for 20 h. The mixture was evaporated to remove the acetonitrile and was dried in vacuo for 2 h. The residue was dissolved in a mixed solvent of acetonitrile (3.5 mL) and benzene (3.5 mL). The solution was passed through a glass filter to remove sodium chloride and the methyl orange unreacted. The filtrate was concentrated with an evaporator and was dried in vacuo for several hours to obtain $PP^+SO_3^-$-*b*-PSt (1.4 mg).

Light scattering measurements of $PP^+SO_3^-$-b-PSt micelles $PP^+SO_3^-$-*b*-PSt (1.4 mg,) was dissolved in a mixed solvent of benzene (0.7 mL) and acetonitrile (6.3 mL). The solution was passed through a glass filter and was put into a cell. The solution was subjected to light scattering at $\theta=90°$ at 20 °C. The hydrodynamic diameters and scattering intensity distributions of the micelles were obtained by the Marquadt analysis [17].

TEM observation A drop of the $PP^+SO_3^-$-*b*-PSt micellar solution was allowed to fall on a Cu grid with a carbon substrate, and the solvent was immediately evacuated using a filter paper. The grid was dried in air for a few hours and then subjected to TEM observations.

Results and discussion

A PP^+Cl^--*b*-PSt diblock copolymer was synthesized by the reaction of PVBC-*b*-PSt and triphenylphosphine in order to perform the ion exchange with an anionic dye. The reaction was carried out in chloroform under reflux for 48 h to produce PP^+Cl^--*b*-PSt. The ^{1}H NMR analysis demonstrated that the chlorobenzyl groups were quantitatively converted into the benzyl triphenylphosphonium chloride. Figure 1a and b shows the ^{1}H NMR spectra of the PVBC-*b*-PSt and PP^+Cl^--*b*-PSt copolymers in chloroform-*d*. The PP^+Cl^--*b*-PSt copolymer was confirmed to show no self-assembly

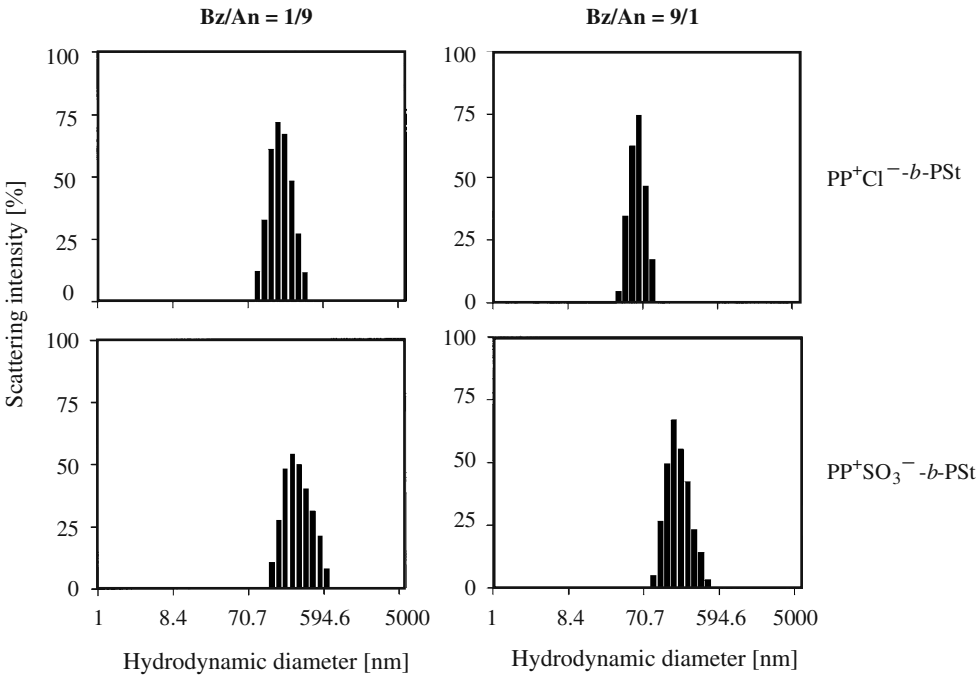

Fig. 3 Scattering intensity distribution for the hydrodynamic diameter of the PP^+Cl^--*b*-PSt and $PP^+SO_3^-$-*b*-PSt micelles in Bz/An=1/9 and 9/1. [PP^+Cl^--*b*-PSt] =0.143 g/L in 1/9 and 9/1, [$PP^+SO_3^-$-*b*-PSt]=0.200 g/L in 1/9 and 5.00×10^{-2} g/L in 9/1

in chloroform by DLS. As can be seen, the benzyl protons of PVBC-*b*-PSt completely disappeared after the reaction. The benzyl protons attached to the triphenylphosphonium cation were discerned at 5.5–6.3 ppm as a broad signal. The signals at 6.3–7.4 ppm were assigned to the protons of the aromatic groups attached to the main chain. The proton signals of the triphenyl groups were observed at 7.4–8.4 ppm. The conversion of the chlorobenzyl groups to the benzyl triphenylphosphonium chloride was estimated based on the relative integral intensity at 7.4–8.4 and 5.5–7.4 ppm, resulting in the quantitative conversion. This quantitative conversion was attained by a large excess (50 equiv.) of triphenylphosphine because 10 equiv. of triphenylphosphine was not enough to produce quantitative conversion of the copolymer. The copolymer was insoluble in water and organic solvents including methanol, ethanol, tetrahydrofuran, 1,4-dioxane, acetone, ethyl acetate, benzene, and hexane, with the exception of chloroform, dichloromethane, and acetonitrile.

The loading of methyl orange on PP^+Cl^--*b*-PSt was performed by ion exchange in acetonitrile at 30 °C for 20 h. Methyl orange showed a slight solubility in acetonitrile, however, it dissolved in the presence of a small amount of water. The ^{1}H NMR demonstrated that the chloride in PP^+Cl^--*b*-PSt was quantitatively exchanged with the 4-(4-dimethylamino)phenylazobenzenesulfonate in methyl orange to produce $PP^+SO_3^-$-*b*-PSt. Figure 1c shows the ^{1}H NMR spectrum of the $PP^+SO_3^-$-*b*-PSt copolymer in a mixed solvent of benzene-d_6/acetonitrile-d_3 (Bz/An-*d*)=5/5 (vol/vol). It was confirmed by DLS that the copolymer showed no self-assembly in the mixed solvent. The methyl protons based on the counter anion of 4-(4-dimethylamino) phenylazobenzenesulfonate ($DBSO_3^-$) were observed at 2.68 ppm. The aromatic protons located at the *ortho* positions to the amino group were discerned at 4.9–5.6 ppm as a broad signal, while the other aromatic protons in the counter anion were observed at 7.6–7.8 ppm. The degree of ion exchange was 100% based on the relative integral intensity at 4.9–5.6 ppm for the *ortho* protons of the counter anion and at 5.6–6.3 ppm for the benzyl protons attached to the triphenylphosphonium cation (Ph_3P^+). This quantitative exchange should be accounted for by the fact that the byproduct of sodium chloride was removed from the system due to the insolubility in the reaction solvent.

The ^{1}H NMR demonstrated that $PP^+SO_3^-$-*b*-PSt formed micelles in a mixed solvent with the composition of Bz/An-*d*=9/1. Figure 1d shows the ^{1}H NMR spectrum of the copolymer in Bz/An-*d*=9/1. The signals of the triphenyl groups of Ph_3P^+ were slightly discerned at 7.6–8.2 ppm, while the signals at 4.8–6.3 ppm for the benzyl protons and

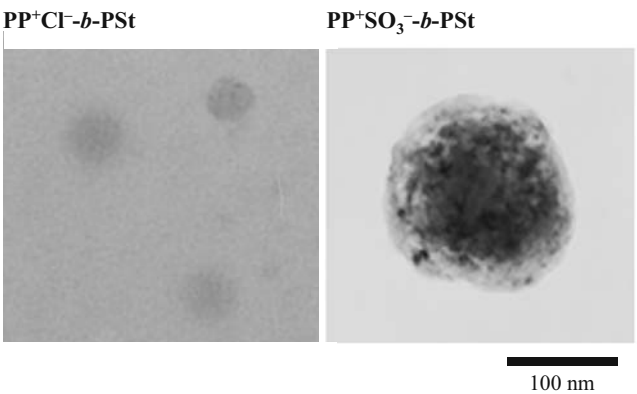

Fig. 4 TEM images of the PP^+Cl^--*b*-PSt and $PP^+SO_3^-$-*b*-PSt micelles

the *ortho* protons of $DBSO_3^-$ were shifted to 4.7–5.3 ppm along with a decrease in the signal intensity. In contrast to the $PP^+SO_3^-$ blocks that showed some changes in the signal intensity and chemical shift, the PSt blocks made a negligible difference, suggesting the formation of the micelles with the cores of the $PP^+SO_3^-$ blocks and the shells of the PSt blocks in the 9/1 mixed solvent.

When the copolymer was dissolved in a mixed solvent with the opposite composition, Bz/An-*d*=1/9, the signals of both the Ph_3P^+ and $DBSO_3^-$ were observed, accompanied by a decrease in the signal intensity of the PSt blocks (Fig. 1e). It was suggested that the copolymer formed the reversed micelles with the $PP^+SO_3^-$ shells and the PSt cores in the 1/9 mixed solvent.

A UV analysis supported the formation of the two different reversed micelles. Figure 2 shows the UV spectra of the $PP^+SO_3^-$-*b*-PSt micelles, methyl orange, and the PP^+Cl^--*b*-PSt micelles in the mixed solvents of Bz/An=1/9 and 9/1. The $PP^+SO_3^-$-*b*-PSt micelles had an absorption with λ_{max} at the same wavelength as the methyl orange in Bz/An=1/9. The micelles showed an increase in the absorbance when compared to that of the methyl orange itself, in spite of the fact that the $DBSO_3^-$ content was identical between the $PP^+SO_3^-$-*b*-PSt micelles and the methyl orange. More methyl orange dissolved by being loaded on the micellar shells. The copolymer in Bz/An=9/1 also showed an increase in the absorbance; however, it underwent a red shift in the wavelength for the methyl orange in Bz/An=9/1. The red shift may be accounted for by the fact that the methyl orange aggregated in Bz/An=9/1 as its very slight solubility was dissociated by being supported on the block copolymer.

The DLS analysis revealed that the ion exchange enlarged the micelles. The scattering intensity distributions for the hydrodynamic diameters of the PP^+Cl^--*b*-PSt and $PP^+SO_3^-$-*b*-PSt micelles placed in Bz/An=1/9 or 9/1 are shown in Fig. 3. The hydrodynamic diameters of the PP^+Cl^--*b*-PSt micelles were estimated to be 174.4 nm in the 1/9 Bz/An and 56.8 nm in the 9/1. The $PP^+SO_3^-$-*b*-PSt micelles had the hydrodynamic diameters of 280.2 nm in the 1/9 and 182.0 nm in the 9/1. The effect on the size increase was observed much more in the core-loading micelles than in the shell-loading ones. It is considered that the increase in the micellar size is attributed to the steric hindrance and solubility of $DBSO_3^-$.

The TEM observation revealed that the PP^+Cl^--*b*-PSt and $PP^+SO_3^-$-*b*-PSt micelles had the spherical shapes. The micelles prepared in the 1/9 Bz/An showed indistinct TEM images due to the fact that the micelles were immediately decomposed when exposed to a beam. On the other hand, the micelles obtained in the 9/1 Bz/An provided TEM images with an obvious contrast. Figure 4 shows the TEM images of the PP^+Cl^--*b*-PSt and $PP^+SO_3^-$-*b*-PSt micelles prepared in the 9/1 Bz/An solvent. The diameters of the micelles were 44.7 nm for PP^+Cl^--*b*-PSt and 176.1 nm for $PP^+SO_3^-$-*b*-PSt based on the TEM images. The size of the micelles estimated by TEM was smaller than that by the DLS. The micelles in the solution should have contracted when isolated in air. In the image of the $PP^+SO_3^-$-*b*-PSt micelles, the shells, like clouds, surround the dark cores containing many black spots. The black spots were attributed to the $DBSO_3^-$ anions because those were not found in the PP^+Cl^--*b*-PSt micelles. The thickness of the shells and the core size were approximately estimated to be 29.2 and 146.9 nm, respectively.

Conclusion

The synthesis of reversible micelles loading the methyl orange on the shells or cores was attained by ion exchange. The dye was quantitatively loaded on the micelles by the reaction. The loading of the dye on the micelles enhanced the solubility of the dye. The core-loading micelles produced a red shift in the UV wavelength of the methyl orange, whereas the shell-loading micelles produced no shift. The loading on the cores significantly expanded the micelles, as compared to that on the shells. The TEM observation demonstrated the formation of spherical micelles. This method of loading methyl orange on the micelles can be applied to insolubilization and nano-sizing of a variety of anionic commercial dyes.

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