

## Research Letter

# Reversible Control of Primary and Secondary Self-Assembly of Poly(4-allyloxystyrene)-Block-Polystyrene

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The reversible control of primary and secondary self-assemblies was attained using a poly(4-allyloxystyrene)-*block*-polystyrene diblock copolymer (PAST-*b*-PSt) through variations in temperature. The copolymer showed no self-assembly in cyclohexane over 35°C and existed as a unimer with a 37.1 nm hydrodynamic diameter. When the temperature was lowered to 30°C, the copolymer formed micelles with 269.9 nm by the primary self-assembly. As the result of further lowering the temperature to 20°C, the secondary self-assembly of the micelles occurred to produce ca. 2975.9 nm aggregates. The aggregates were dissociated into unimers by increasing the temperature up to 40°C. The light scattering studies demonstrated that the thermoresponsivity of the copolymer showed good hysteresis throughout the variation in the temperature in the range between 20 and 40°C, based on the Marquadt analysis of the hydrodynamic diameter distribution. It was found that the primary and secondary self-assemblies of the copolymer were perfectly controlled by the temperature.

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## 1. Introduction

The secondary self-assembly of molecular aggregates is important for effective control of the activity and function produced by the primary self-assembly of the molecules. The secondary aggregation spontaneously occurs through a number of attractive forces such as van der Waals interaction, solvation, depletion, bridging,  $\pi$ - $\pi$  stacking, hydrogen bond, and coordination bond [1]. The secondary self-assembly is well known as the formation of the quaternary structure of proteins with a high molecular weight over 100 000 daltons. Examples for the proteins include the hemoglobin [2], microtubules [3], pyruvate dehydrogenase complexes [4], influenza virus [5], tobacco mosaic virus [6–8], tomato bushy stunt virus [9, 10], and aspartate transcarbamylase [11]. Thus, the secondary aggregation has widely been discovered in the natural world, while it has been explored for artificial molecules such as dilauroylphosphatidylcholine with dipalmitoylphosphatidylethanolamine-conjugated biotin [12], polystyrene-*block*-poly(acrylic acid) [13], polystyrene-*block*-poly(4-vinylpyridine) onto silica

nanoparticles [14], poly(isobutene)-*grafted* boehmite rods [15], poly(ethylenimine)-*graft*-poly(ethylene glycol) [16], and a metallo-supramolecular diblock copolymer consisting of polystyrene block connected to a poly(ethylene oxide) by a bis(terpyridine)ruthenium complex [17]. In addition to these experimental results, there are many theoretical results concerning the secondary aggregation [18, 19].

We found that the primary and secondary self-assembly of a poly(4-allyloxystyrene)-*block*-polystyrene diblock copolymer (PAST-*b*-PSt) was reversibly controlled by a variation in temperature. The thermoresponsivity of this block copolymer showed good hysteresis in the range between 20 and 40°C. This study describes the reversible control of the primary and secondary self-assemblies of the PAST-*b*-PSt copolymer in cyclohexane.

## 2. Experiment

**2.1. Instrumentation.** The  $^1\text{H}$  NMR measurements were conducted using a Varian 300 FT NMR spectrometer. Light

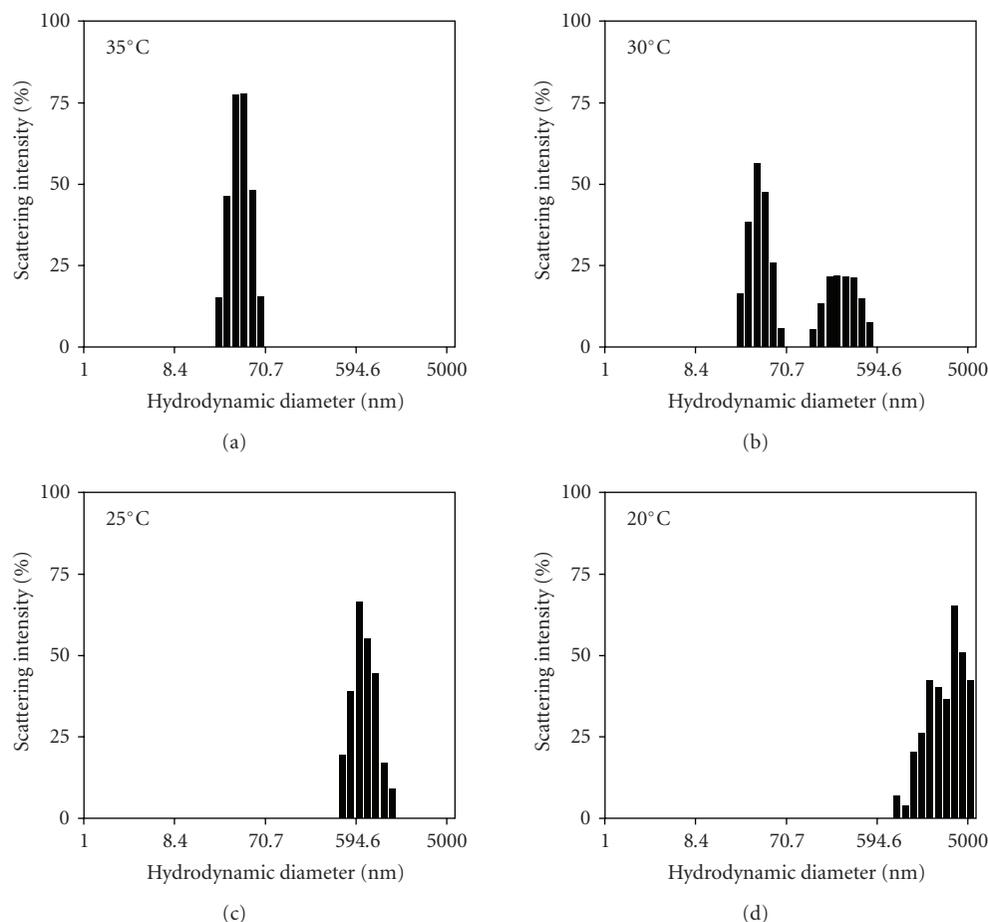


FIGURE 1: The variability in the hydrodynamic diameter distributions of the copolymer versus the temperature.  $[\text{copolymer}]_0 = 1.64 \text{ g/L}$ .

scattering measurements were performed with a Photal Otsuka Electronics ELS-8000 electrophoretic light scattering spectrophotometer equipped with a system controller, an ELS controller, and an He-Ne laser operating at  $\lambda = 632.8 \text{ nm}$ . UV analysis was performed with a Shimadzu UV-160A UV-Vis recording spectrophotometer.

**2.2. Materials.** A poly(4-*tert*-butoxystyrene)-*block*-polystyrene diblock copolymer (PBSt-*b*-PSt) was prepared as reported previously [20]. The molecular weight of the copolymer was  $M_n(\text{PBSt-}b\text{-PSt}) = 15\,400\text{-}b\text{-}96\,600$  by  $^1\text{H}$  NMR, while the molecular weight and its distribution were  $M_n = 58\,000$ , and  $M_w/M_n = 1.36$  by gel permeation chromatography based on polystyrene standards. Tetrahydrofuran (THF) and cyclohexane were distilled over sodium. *N,N*-Dimethylformamide (DMF) was distilled over calcium hydride under reduced pressure. Allyl chloride was also distilled over calcium hydride.

**2.3. Synthesis of PAST-*b*-PSt.** The PBSt-*b*-PSt (2.00 g) was dissolved in THF (70 mL). Concentrated hydrochloric acid (7 mL) was added to the copolymer solution. The mixture was heated at  $85^\circ\text{C}$  for 4.5 hours. The resulting solution was concentrated to ca. 30 mL by an evaporator, and was

poured into water (1 L) to precipitate a polymer. The precipitates were collected by filtration, then freeze-dried with 1,4-dioxane to obtain poly(vinylphenol)-*block*-polystyrene diblock copolymer (PVPh-*b*-PSt, 1.593 g). The PVPh-*b*-PSt (0.70 g) was dissolved in DMF (15 mL). Sodium hydride (0.414 g, 17.3 mmol) was added to the copolymer solution at  $0^\circ\text{C}$  under nitrogen atmosphere. The suspension was stirred at  $0^\circ\text{C}$  for 5 minutes and was further stirred at room temperature for 1 hour. Allyl chloride (1.41 g, 18.4 mmol) in DMF (5 mL) was added to the suspension at  $0^\circ\text{C}$ . The mixture was stirred at  $0^\circ\text{C}$  for 5 minutes and was further stirred at room temperature for 20 hours. The resulting solution was poured into methanol (1 L) to precipitate a polymer. The precipitates were collected by filtration, then dried in vacuo for several hours. PAST-*b*-PSt (0.68 g) was obtained. The molecular weight of the PAST-*b*-PSt was determined to be  $M_n(\text{PAST-}b\text{-PSt}) = 14\,000\text{-}b\text{-}96\,600$  by  $^1\text{H}$  NMR.

**2.4. Light Scattering Measurements.** Cyclohexane (5 mL) was added to PAST-*b*-PSt (8.2 mg), and the mixture was completely dissolved at  $40^\circ\text{C}$ . The solution was injected through a microporous filter into a cell using a syringe. The solution was subjected to light scattering at the angle  $\theta = 90^\circ$ .

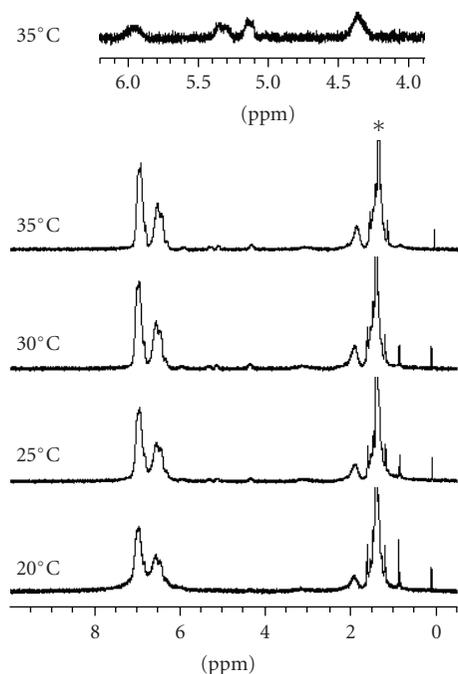


FIGURE 2: The variation in the  $^1\text{H}$  NMR spectra of the copolymer versus the temperature. Solvent: cyclohexane- $d_{12}$ .  $[\text{copolymer}]_0 = 1.6 \text{ g/L}$ , \* Cyclohexane.

The hydrodynamic diameter of the copolymer was estimated by the cumulant analysis, while the scattering intensity distribution of hydrodynamic diameter was obtained by the Marquadt analysis [21].

### 3. Results and Discussion

The PAST-*b*-PSt diblock copolymer showed no self-assembly in cyclohexane at  $40^\circ\text{C}$  and existed as a unimer of an isolated copolymer. The light scattering demonstrated that the copolymer formed aggregates by decreasing the temperature. Figure 1 shows a variation in the scattering intensity distribution for the hydrodynamic diameter of the copolymer when the temperature decreased. The scattering intensity distributions were obtained by the Marquadt analysis [21]. The copolymer still existed as a unimer at  $35^\circ\text{C}$ , so that only one distribution was observed around 35 nm. When the temperature was lowered to  $30^\circ\text{C}$ , another distribution was observed around 270 nm in addition to the unimer distribution. The distribution around 270 nm was attributed to the micelles formed by the primary self-assembly of the copolymer. The unimer distribution completely disappeared at  $25^\circ\text{C}$  and only distribution was observed around 700 nm. This distribution was further shifted to the hydrodynamic diameter around 3000 nm at  $20^\circ\text{C}$  and did not retain the Gauss distribution any longer. The formation of the aggregates with the hydrodynamic diameter over 1000 nm should be caused by the secondary aggregation of the micelles. In addition, the exact hydrodynamic diameters were estimated by the Marquadt analysis to be 37.1 nm ( $35^\circ\text{C}$ ),

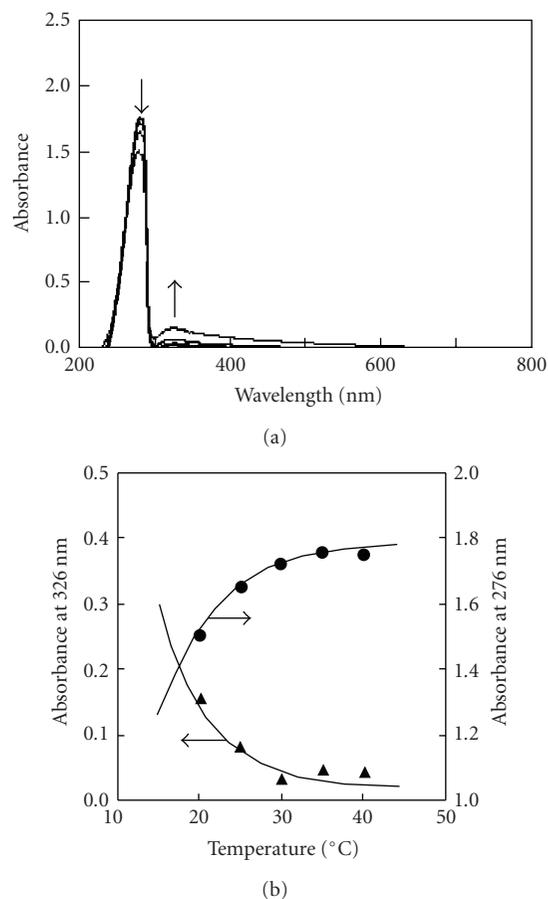


FIGURE 3: The variation in the UV spectra of the copolymer and the plots of the absorbance at (●) 276 and (▲) 326 nm versus the temperature.  $[\text{copolymer}]_0 = 1.64 \text{ g/L}$ .

35.1 nm for the unimer and 269.9 nm for the micelles ( $30^\circ\text{C}$ ), 698.0 nm ( $25^\circ\text{C}$ ), and 2975.9 nm ( $20^\circ\text{C}$ ).

The self-assembly of the copolymer was also explored by  $^1\text{H}$  NMR. Figure 2 shows the  $^1\text{H}$  NMR spectra obtained at each temperature using cyclohexane- $d_{12}$ . In the spectrum at  $35^\circ\text{C}$ , all the protons composing the copolymer were observed, since the copolymer existed as a unimer. The signals at 1.0–2.5, 4.3–4.5, 5.2–5.6, 5.8–6.1, and 6.2–7.5 ppm were assigned to the protons of the main chain, the allyl protons, the  $\alpha$ -proton of the vinyl, its  $\beta$ -protons, and the aromatic protons, respectively. The copolymer showed negligible changes at  $30^\circ\text{C}$ , although part of the aromatic proton signal at 6.3 ppm was slightly broadened. This signal was more broad at  $25^\circ\text{C}$  and was observed as a shoulder. At  $20^\circ\text{C}$ , all of the signals were broadened and, in particular, the protons of the allyl groups were barely discerned. The signal broadening was based on the protons shielded by the copolymer self-assembling. The marked effect of the shielding at  $20^\circ\text{C}$  indicates that not only the cores of the micelles but also the shells were shielded by the secondary aggregation.

The degree of the shielding was determined by UV analysis. Figure 3 shows the UV spectra of the copolymer at

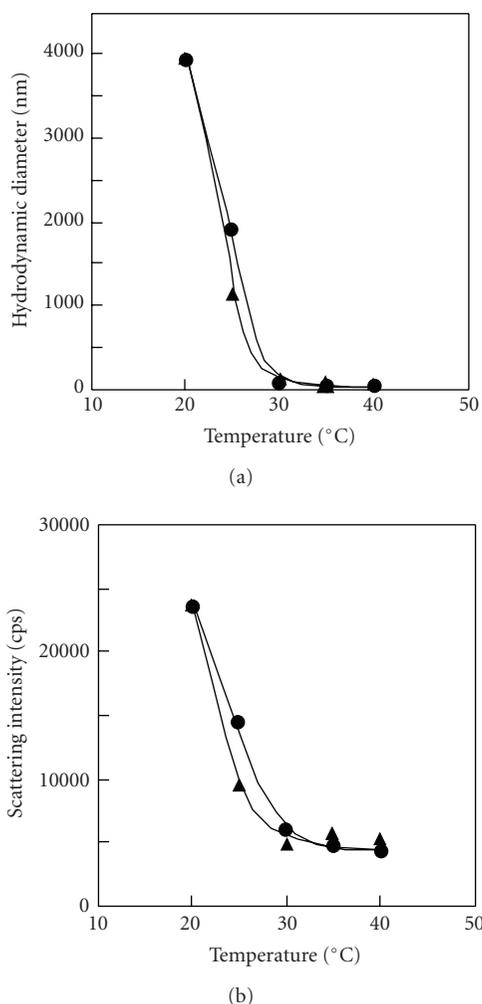


FIGURE 4: The variation in the hydrodynamic diameter and scattering intensity of the copolymer with (●) the decrease and (▲) increase in the temperature.  $[\text{copolymer}]_0 = 1.64 \text{ g/L}$ .

the respective temperatures and the plots of the absorbance at 276 and 326 nm versus the temperature. The absorbance at 326 nm increased as the temperature decreased. Contrarily, the absorbance at 276 nm decreased with the decreased temperature. Both the decrease and increase in the absorbances were accelerated below 30 °C. The decrease in the absorbance at 276 nm is based on the shielding of the aromatic groups and the olefins, while the increase at 326 nm is attributed to an increase in the interaction among the aromatic groups and the olefins. This is accounted for by the fact that the delocalization of electrons causes red shift.

The light scattering studies revealed that the primary and secondary aggregation of the copolymer was reversibly controlled by the temperature. The thermoresponsivity of the copolymer is shown in Figure 4. The hydrodynamic diameters were estimated by the cumulant analysis. The hydrodynamic diameter and scattering intensity increased by lowering the temperature, however, those reverted to the original values as a result of raising the temperature. The aggregates were dissociated into unimers. The course of the

dissociation was almost the same as that of the aggregation, indicating that the primary and secondary self-assemblies of the copolymer were perfectly controlled by the temperature.

#### 4. Conclusion

The reversible control of the primary and secondary self-assemblies was attained using the PAST-*b*-PSt diblock copolymer. The copolymer formed micelles with ca. 270 nm hydrodynamic diameter by the primary self-assembly. The micelles were further self-assembled into large aggregates with 3000 nm following the formation of 700 nm aggregates. The aggregates formed by the secondary assembly were dissociated into unimers by increasing the temperature. It was found that the primary and secondary self-assemblies of the copolymer were perfectly controlled by the temperature based on the fact that the thermoresponsivity of the copolymer showed good hysteresis. This is the first study demonstrating that the primary and secondary self-assemblies of the diblock copolymer were reversibly controlled by the variation in the temperature in the narrow range between 20 and 40 °C.

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