Micelle Formation of Nonamphiphilic Diblock Copolymers through Noncovalent Bond Cross-Linking

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ABSTRACT: Micelle formation was studied for a nonamphiphilic diblock copolymer consisting of only solventphilic polymer blocks. Poly(vinyl phenol)-*block*-polystyrene (PVPh-*b*-PSt) has no ability to form micelles in 1,4-dioxane, a good solvent for both the PVPh and PSt blocks. The copolymer showed micellization in the presence of 1,4-butanediamine (BDA). It was found that this micellization occurred by hydrogen bond cross-linking between the PVPh blocks in BDA on the basis of ^{1}H NMR analysis. The size of unimers (average 6.5 nm) and micelles (average 29.5 nm) prepared from the PVPh-*b*-PSt with 10K-*b*-70K as the molecular weight was almost independent of the copolymer concentration, while the aggregation numbers of the micelles increased as a result of increasing copolymer concentration. Dynamic and static light scattering demonstrated that the cmc for the PVPh-*b*-PSt was determined primarily by BDA concentration at a constant copolymer concentration and by copolymer concentration at a constant BDA concentration.

Introduction

Molecular self-assembly is the spontaneous association of molecules into stable, structually well-defined aggregates joined through noncovalent bonds, such as hydrogen bonds, van der Waals interactions, and the electrostatic force of attraction. In most cases, molecular association is dependent on the structure of the molecules and their concentration in solution and can be manipulated by external variations. Poly(L-lysine) oligomer, which itself has no ability to form an α-helix structure due to its low molecular weight, shows a pH-induced helix−coil transition in aqueous medium by conjugation with poly(ethylene glycol).[1] It has been reported that a coil−collapse transition of poly(*N*-vinylisobutyramide) and poly(*N*-propylacrylamide) is induced by both temperature and pressure.[2] The addition of inorganic salts also effectively brings about the coil−globule transition of these polymers.[3] Molecular association using this kind of external variation is based on entropic gain due to solvent release from the polymer strands and is known as coacervation. Molecular self-assembly forms highly ordered structures, and these structures are closely related to their functions. Some examples include protein (re)folding,[4] formation of the pyruvate dehydrogenase complex,[5] and self-assembly of the tobacco mosaic virus.[6] On the basis of such natural supramolecules, a variety of artificial nanoscale structures have been prepared: supramolecular cylinders; spherical supermolecular dendrimers;[7] cyanuric acid−melamine lattices;[8] molecular necklaces;[9] molecular shuttles;[10] both double[11] and triple helices.[12] Micelles are supramolecules formed by amphiphilicities self-assembling.[13] Formation of micelles is based on van der Waals interactions among the solventphobic moieties, sometimes induced by pH,[14] temperature,[15] or pressure.[16] Subsequently, the micelles have solventphobic cores and solventphilic coronas.

Amphiphilic diblock copolymer micelles are important in many applications including separation technologies[17] and drug delivery.[18] Use of diblock copolymers is the most popular and convenient method for preparing micelles. There are, however, limits to the molecular design of these copolymers. The amphiphilic diblock copolymers are comprised of soluble polymer blocks and insoluble polymer blocks that need to completely dissolve in solvent to form micelles. The solubility of the diblock copolymers is usually dependent on their block length. For the diblock copolymers to dissolve in a solvent, the length of the soluble polymer blocks cannot be shorter than that of the insoluble blocks. Hence, it is necessary to take the block length coupled with the ratios of their blocks into consideration in designing amphiphilic diblock copolymers for the preparation of micelles. We found that poly(vinyl phenol)-*b*-polystyrene (PVPh-*b*-PSt) diblock copolymer formed micelles in a solvent suitable for both PVPh and PSt blocks. This micellization was attained in 1,4-dioxane in the presence of α,ω-diamine of ethylenediamine, 1,4-butanediamine, or hexamethylenediamine.[19] We determined the dominant factors affecting the critical micelle concentration (cmc) for the micellization of the PVPh-*b*-PSt diblock copolymer in the presence of 1,4-butanediamine through light scattering studies. This paper describes how the cmc was determined by the observation of hydrodynamic radii and aggregation numbers during PVPh-*b*-PSt micellization. The preparation of crew-cut micelles from PVPh-*b*-PSt is also described.

Experimental Section

Measurement. Gel permeation chromatography (GPC) was performed with a Toso HLC-802A instrument equipped with a RI detector and with a Toso CP-8000 chromato processor. Two polystyrene gel columns, Toso TSK gel G4000H_8 and G2000H_8, were used with THF as the eluent at 42 °C. ^{1}H NMR spectra were obtained with a Bruker ARX-500 NMR spectrometer. Dynamic light scattering experiments were performed at 20 °C at an angle of 90°, with a Photal Otsuka Electronics DLS-7000 super dynamic light scattering spectrometer equipped with an LS-71 control unit, an LS-72 pump controller, and an argon ion laser operating at $\lambda = 488$ nm.

Materials. 4-Methoxy-2,2,6,6-tetramethylpiperidine-1-oxyl (4-methoxy-TEMPO) was prepared as reported previously.[20] Benzoyl peroxide (BPO) was precipitated from chloroform and

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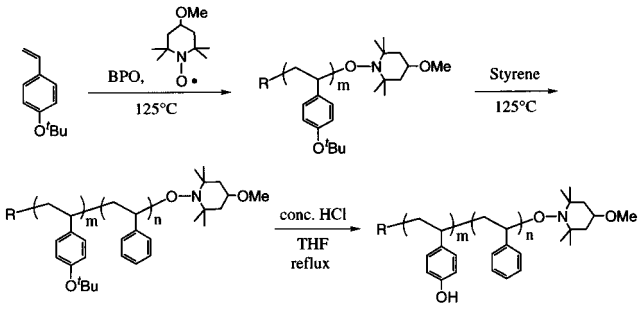
Scheme 1



crystallized in methanol at 0 °C. 4-*tert*-Butoxystyrene (*t*BOS) was supplied from Hokko Chemical Industry Co. Ltd. *t*BOS and commercial grade styrene were washed with aqueous alkaline solution and water and distilled over calcium hydride. 1,4-Butanediamine (BDA) was distilled over calcium hydride. THF and 1,4-dioxane were purified by refluxing on sodium for several hours and distilled over sodium.

Synthesis of a P*t*BOS-*block*-PSt Diblock Copolymer for Preparing Crew-Cut Micelles. St (6.00 g, 57.6 mmol), BPO (93 mg, 0.384 mmol), and 4-methoxy-TEMPO (93 mg, 0.500 mmol) were placed in an ampule. After the contents were degassed, the ampule was sealed in vacuo. The polymerization was carried out at 125 °C for 46 h and terminated by cooling with liquid nitrogen. The reaction mixture was dissolved into dichloromethane and poured into methanol to precipitate the polymer. The polymer was purified by repeated reprecipitation from dichloromethane into methanol. The precipitate was then dried in vacuo for several hours to obtain the prepolymer of PSt (5.71 g).

The PSt thus obtained (427 mg) and *t*BOS (5.00 g) were placed in an ampule. After the contents were degassed, the ampule was sealed in vacuo. Subsequent polymerization was carried out at 125 °C for 46 h. The product was isolated and purified in the same manner as utilized in the preparation of the PSt prepolymer. A total of 4.88 g of the copolymer was obtained.

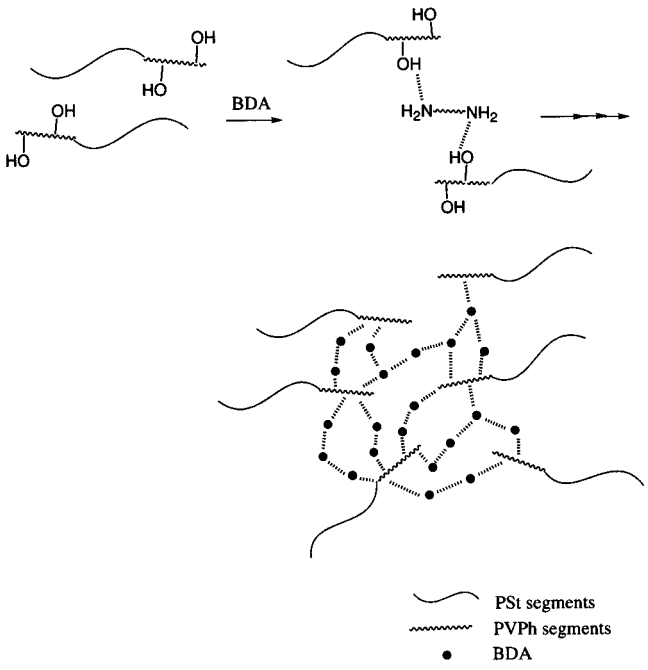
Synthesis of a PVPh-*block*-PSt Diblock Copolymer for Preparing Crew-Cut Micelles. P*t*BOS-*b*-PSt (500 mg) was dissolved in THF (15 mL), and concentrated HCl (1 mL) was added to the solution at room temperature. The mixture was kept at 85 °C for 4.5 h. The resulting mixture was then poured into water (300 mL) to precipitate the polymer. After the polymer was dried, the product was suspended in 200 mL of benzene and stirred for 4.5 h at room temperature to remove St homopolymer. The resulting precipitate was collected by filtration and then dried in vacuo for several hours. A total of 307 mg of PVPh-*b*-PSt diblock copolymer was obtained.

Light Scattering Measurements. PVPh-*b*-PSt (M_n = 10K-*b*-70K, 10 mg) was dissolved in 1,4-dioxane (3.1 mL), and using a syringe, the resulting solution was injected through a microporous filter into a cell. The solution was subjected to light scattering measurement at 20 °C. After the measurement, 6 μL of BDA solution (BDA, 132 mg, 1.50 mmol) in 1,4-dioxane (1 mL) was added to the copolymer solution in the cell, and the mixture was shaken vigorously. The solution was allowed to stand at 20 °C for 5 min and then subjected to light scattering again. This procedure was repeated until distribution due to the unimers was not observed as determined by nonnegatively constrained least-squares (NNLS) analysis.[21] Aggregation numbers were estimated by using scattering intensity of the micelles and the unimers.

Results and Discussion

A well-defined PVPh-*b*-PSt diblock copolymer was prepared by living radical polymerization mediated by 4-methoxy-TEMPO (Scheme 1).[19] The molecular weights of each block of the copolymer were estimated by ^{1}H NMR on the basis of the degree of polymerization (DP) for a P*t*BSt prepolymer. The DP of the prepolymer was

Scheme 2



determined by ^{1}H NMR on the basis of the integral intensity of proton signals due to the aromatic rings and proton signals attributed to the methoxy group, 4-methoxy-TEMPO, attached to the polymer chain end. The DP thus estimated was 80. It was assumed that the DP of the P*t*BSt prepolymer was equal to that of the PVPh blocks, and the molecular weight of the PVPh-*b*-PSt copolymer was estimated as 10K-*b*-70K on the basis of the integral intensity of proton signals attributed to the hydroxyl group of the VPh units and the aromatic rings. The polydispersity (M_w/M_n) of the copolymer was estimated as 1.40 through GPC. This somewhat broad polydispersity in comparison to that of the P*t*BSt prepolymer (M_w/M_n = 1.09) is accounted for by the fact that the PVPh blocks are adsorbed onto and interact with PSt gels in columns with THF as the eluent. The fact that the molecular weight and polydispersity of VPh homopolymer could not be estimated by GPC with THF as the eluent supports this inference.

The PVPh and PSt blocks are both solventphilic for 1,4-dioxane, so that the PVPh-*b*-PSt diblock copolymer showed no self-assembly into micelles in the solvent. Dynamic light scattering studies demonstrated that the PVPh-*b*-PSt diblock copolymer formed micelles with a size of 29.2 nm as a hydrodynamic radius in 1,4-dioxane when BDA was added to the copolymer solution. The addition of *n*-butylamine to the copolymer solution revealed no change in the hydrodynamic radius. This indicates that the monoamine did not promote micellization. The micelles should be formed by hydrogen bond cross-linking between the PVPh blocks by BDA (Scheme 2). Micellization does not occur by simple insolubilization of the PVPh blocks due to the amino groups. This is because VPh homopolymer yielded no precipitate in the presence of *n*-butylamine, while the polymer was immediately converted into a white precipitate by the addition of BDA.

The ^{1}H NMR analysis confirmed that the formation of micelles occurred through hydrogen bond cross-linking. Figure 1 shows the ^{1}H NMR spectra of BDA and the PVPh-*b*-PSt diblock copolymer in the process of micellization. The signals at 7.3−7.5 ppm originate

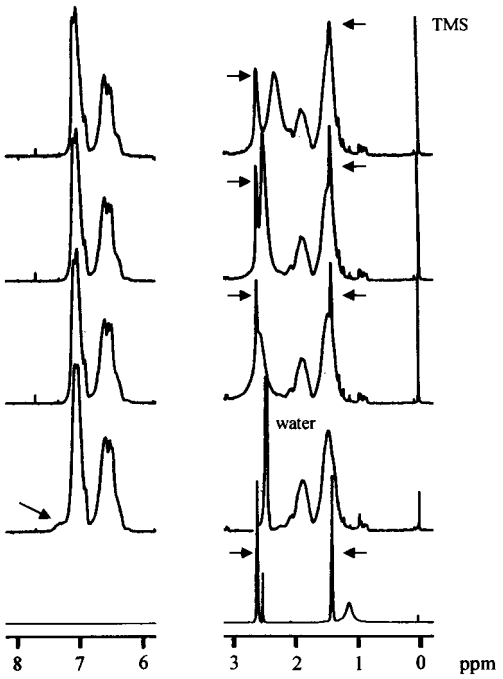


Figure 1. ^{1}H NMR spectra of (a) BDA and the PVPh-*b*-PSt diblock copolymer with various BDA/VPh ratios: (b) 0; (c) 0.5; (d) 1.0; (e) 2.0. Solvent: 1,4-dioxane-d_8.

from the hydroxyl group of the VPh units (Figure 1b). The signals were not observed when BDA was added to the copolymer solution (BDA/VPh = 0.5, Figure 1c). The broad signal at 0.8−1.3 ppm is due to the amino group of BDA (Figure 1a) and also disappeared after the addition of BDA to the copolymer solution (Figure 1c). No observation of the signal due to the amino group was made, even in the presence of an excess amount of BDA over VPh unit (Figure 1d,e). Further, the signals at 2.60 and 1.39 ppm due to two kinds of methylenes of BDA broadened as the molar ratio of BDA to VPh unit (BDA/VPh) was increased. These signals are attributed to a methylene bound to an amino group and another methylene neighboring this meythylene, respectively. The absence of the signal due to the amino protons and the broadening of the methylene protons indicate that all of the BDA molecules participated in the hydrogen bond cross-linking to form micelles. An excess of BDA should form complicated hydrogen bond networks in the cores of micelles.

The distributions of hydrodynamic radii are shown in Figure 2. The results were obtained for a copolymer concentration of 3.23×10^{-3} g/mL by applying the NNLS method of analysis to the data for the three different molar ratios of BDA to VPh units (BDA/VPh = 0, 2, and 4). The data correspond to three different states with the three molar ratios: no aggregation of the isolated copolymer chain at BDA/VPh = 0; a transition from unimers to micelles at BDA/VPh = 2; micelle formation at BDA/VPh = 4. We clearly distinguished one distribution representing unimers for BDA/VPh = 0 (Figure 2a), two distributions (unimers and micelles) at BDA/VPh = 2 (Figure 2b), and one distribution representing micelles at BDA/VPh = 4 (Figure 2c).

The driving force for micelle formation from the PVPh-*b*-PSt diblock copolymer clearly differs from that for micellization of amphiphilic copolymers. For PVPh-*b*-PSt, the hydrogen bond cross-linking assisted by BDA is the driving force to form micelles, while van der Waals

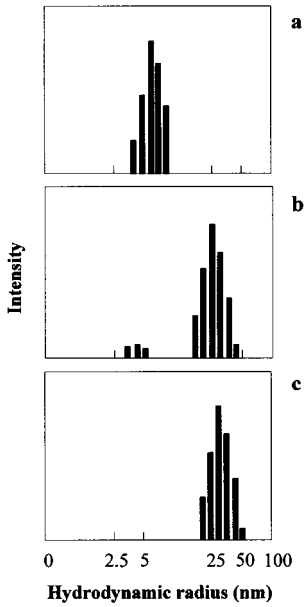


Figure 2. Intensity distribution obtained by NNLS analysis for the copolymer concentration $c = 3.23 \times 10^{-3}$ g/mL and the BDA/VPh ratios: (a) 0; (b) 2; (c) 4.

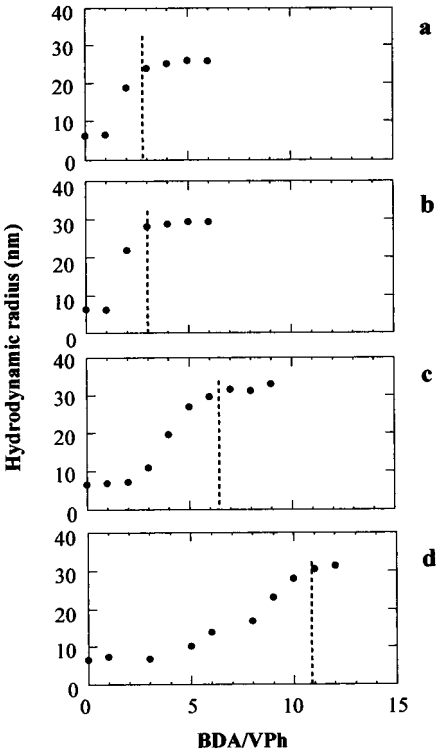


Figure 3. Plots of hydrodynamic radius over BDA/VPh ratio for the copolymer concentrations ($\times 10^{-3}$ g/mL): (a) 4.84; (b) 3.23; (c) 1.61; (d) 0.645.

interactions among solventphobic blocks plays a critical role in amphiphilic copolymer micellization. Subsequently the critical micelle concentration (cmc) for the amphiphilic copolymers is determined by the copolymer concentration. For the PVPh-*b*-PSt, the copolymer concentration is immutable, whereas the BDA concentration is variable during the micellization. We investigated the dominant factors affecting the cmc for the PVPh-*b*-PSt micellization. Figure 3 shows the hydrodynamic radius over the BDA/VPh ratio for the PVPh-*b*-PSt solution at T = 20 °C with the different copolymer concentrations: 0.645×10^{-3}, 1.61×10^{-3}, 3.23×10^{-3},

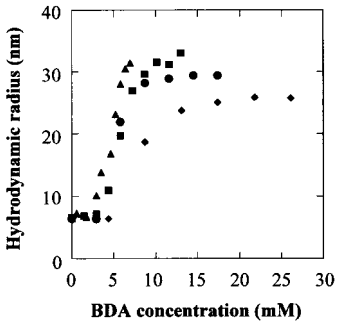


Figure 4. Plots of hydrodynamic radius over BDA concentration for the copolymer concentrations ($\times 10^{-3}$ g/mL): (◆) 4.84; (●) 3.23; (■) 1.61; (▲) 0.645.

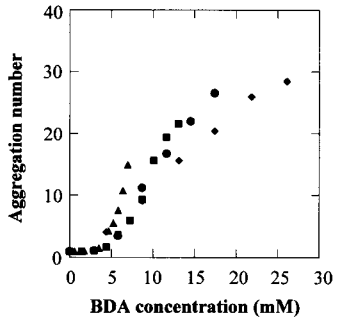


Figure 5. Variations of aggregation numbers versus BDA concentration for the copolymer concentrations ($\times 10^{-3}$ g/mL): (◆) 4.84; (●) 3.23; (■) 1.61; (▲) 0.645.

and 4.84×10^{-3} g/mL. The hydrodynamic radii were obtained using cumulant analysis. The size of the micelles (average 29.5 nm) and of the unimers (average 6.5 nm) is almost independent of the copolymer concentration. The width from the beginning to the end of the transition from unmers to micelles expanded with a decrease in copolymer concentration, indicating that the micellization occurs more slowly at lower copolymer concentrations. The unimers-to-micelles transition occurred at larger BDA/VPh ratios as the copolymer concentration was decreased. To form micelles at a lower copolymer concentration, more BDA had to be added to the solution. These results suggest that the cmc is determined by the BDA/VPh ratio when the copolymer concentration is constant.

The hydrodynamic radius versus the BDA concentration instead of the BDA/VPh ratio for the PVPh-*b*-PSt copolymer solution is represented in Figure 4 for the four different copolymer concentrations. The BDA concentration at which all the unimers were converted to the micelles increased with increased copolymer concentration. This suggests that the cmc is determined by the copolymer concentration at a constant BDA concentration. Figure 5 shows variability in aggregation numbers through micellization at the four different copolymer concentrations. At all copolymer concentrations, the aggregation numbers increased with an increase in BDA concentration. The micellization at a higher copolymer concentration occurred at a higher BDA concentration and showed larger aggregation numbers. The aggregation numbers of the micelles at copolymer concentrations of 0.645×10^{-3}, 1.61×10^{-3}, 3.23×10^{-3}, and 4.84×10^{-3} g/mL were estimated to be 15, 22, 27, and 29, respectively. Accordingly, we concluded that the cmc was determined by the BDA concentration at a constant copolymer concentration and was determined by copolymer concentration at a constant BDA concentration. This observation can be regarded as reasonable when it is taken into consideration that micellization is promoted by two components of BDA and the PVPh block.

Scheme 3

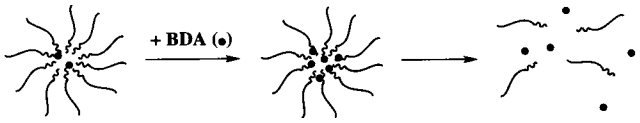


Table 1. PVPh-*b*-PSt Diblock Copolymer and BDA Concentrations at the Micelles-to-Unimers Transition[a]

BDA/VPh[b]	10^3[copolymer][c] (g/mL)	10^3[BDA][d] (mol/L)
2	3.33	6.01
4	1.67	6.01
8	0.833	6.01
16	0.417	6.01

[a] At 20 °C in 1,4-dioxane. The initial copolymer concentration $= 6.67 \times 10^{-3}$ g/mL. [b] The BDA/VPh ratio after BDA was added to the micelle solution. [c] A copolymer concentration at the micelles-to-unmers transition. [d] A BDA concentration at the transition.

To investigate the cmc in detail, reverse transition from micelles to unimers (Scheme 3) was explored by the following manipulation: BDA was added to a copolymer solution to form micelles (a copolymer concentration $= 6.67 \times 10^{-3}$ g/mL, BDA/VPh = 2). Another specific quantity of BDA was added to this micelle solution, and then the mixture was diluted with 1,4-dioxane. We determined both the copolymer concentration and the BDA concentration at the micelles-to-unimers transition, observing the resulting variations in hydrodynamic radius by dynamic light scattering. The results are shown in Table 1. It was found that the PVPh-*b*-PSt copolymer showed a transition from micelles to unimers with dilution of the micelle solution. The copolymer concentration at the micelles-to-unimers transition decreased as the amount of BDA added to the micelle solution increased. On the other hand, the BDA concentration at the transition was identical, independent of the amounts of BDA added. This interesting observation supports the conclusion that the cmc is dependent on the copolymer concentration at a constant BDA concentration.

Crew-cut micelles, which have long insoluble blocks in the cores and short soluble blocks in the coronas, are generally difficult to prepare from amphiphilic block copolymers using conventional methods. This is because amphiphilic block copolymers are mostly insoluble in solvents selective for short blocks. Eisenberg and coworkers succeeded in preparing crew-cut micelles from amphiphilic block copolymers using an unusual method based on the selectivity of solvents for the soluble and insoluble blocks.[22] PVPh-*b*-PSt diblock copolymers with molecular weights of 26K-*b*-13K, 65K-*b*-13K, and 100K-*b*-13K formed crew-cut micelles in the presence of BDA in 1,4-dioxane. Dynamic and static light scattering demonstrated that the micelles for the 26K-*b*-13K, 65K-*b*-13K, and 100K-*b*-13K samples had hydrodynamic radii of 30.1, 39.7, and 52.1 nm and aggregation numbers of 32, 63, and 42, respectively. The size of the micelles was dependent on the PVPh block length when the PSt block length was constant. Amphiphilic diblock copolymers form micelles by van der Waals interactions among solventphobic polymer blocks, so that the polymer chains of the solventphobic blocks are contracted in the cores. With PVPh-*b*-PSt, the micellization oc-

curred by hydrogen bond cross-linking between the solvent-soluble PVPh blocks via α,ω-diamines. The PVPh block chains in these micelles should be expanded in the cores even after micellization, with the result that the block length is reflected in the size of the micelles.

Conclusions

The PVPh-*b*-PSt diblock copolymer showed micellization in a solvent suitable for both the PVPh and PSt blocks, in the presence of 1,4-butanediamine. ^{1}H NMR analysis revealed that micellization occurred by hydrogen bond cross-linking between the PVPh blocks in BDA and that an excess of BDA to VPh units formed complicated hydrogen bond networks in the cores. Dynamic and static light scattering demonstrated that the cmc for the PVPh-*b*-PSt was determined predominantly by the BDA concentration at a constant copolymer concentration and also by copolymer concentration at a constant BDA concentration. As demonstrated in the preparation of crew-cut micelles, this method of micellization for nonamphiphilic diblock copolymers has advantages over the conventional method using amphiphilic diblock copolymers. The block length and ratios determining the solubility of the copolymers did not have to be taken into consideration in the molecular design of the block copolymers, since the nonamphiphilic block copolymers consisted entirely of solventphilic polymer blocks. This method of micellization from nonamphiphilic diblock copolymers may be useful for creating a great variety of nanoscale structures and should promote development in many fields using micelle technologies.

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