Notes

A One-Step Approach to the Highly Efficient Preparation of Core-Stabilized Polymeric Micelles with a Mixed Shell Formed by Two Incompatible Polymers

Taoran Hui, Daoyong Chen,* and Ming Jiang

Department of Macromolecular Science and The Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China

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Polymeric micelles are core−shell structured nanospheres with an aggregated core being surrounded and stabilized by a hairlike shell.[1,2] During past decades, polymeric micelles have attracted much attention due to their great potential applications for the use as nanocarriers for catalytic particles, molecules with electronic and photonic functions, biological and medical species, and so forth.[3−9] It is well-known that the applications of the polymeric micelles mainly depend on the properties of the core and the shell.[6] The changes in the structure and the composition of the core or shell will bring new properties to and further broaden the applications of the polymeric micelles.[10,11] It is expected that the polymeric micelles with a mixed shell (PMMS, i.e., the hairlike shell is composed of two linear polymers), particularly when the two shell-forming polymers have distinctly different properties and the cores are stabilized, are capable of meeting various requirements in applications. For examples, the PMMS with a shell of hydrophilic/hydrophobic polymers could be soluble in water as well as in low-polarity organic solvents; the PMMS with a shell of biocompatible/chemically active (capable of being modified for targeting) polymers may be a desirable precursor for the preparation of targeting drug delivery systems.[6] In the existing methods, the PMMS were prepared by micellization of an A-*b*-B-*b*-C triblock copolymer[12,13] or a pair of diblock copolymers A-*b*-B and C-*b*-B in the solvent being selective for both A and C blocks,[14,15] leading to B core and A/C shell.[11,16] In these methods, polymers A and C should be sufficiently compatible so that A and C chains can be fully mixed in the shell of the resultant micelles.[11] However, the overwhelming majority of polymers differing in chemical nature are incompatible, whereas, regarding applications, a sufficient difference in the properties between the shell-forming blocks is necessary. Besides, the micellization has to be carried out at low concentrations, and the preparation efficiency is low.[17]

In this paper, we describe a one-step approach to the preparation of core-stabilized PMMS with PEO/PS as the shell by directly cross-linking P2VP in PS-*b*-P2VP (polystyrene-*block*-poly(2-vinylpyridine)) and PEO-*b*-P2VP (poly(ethylene oxide)-*block*-poly(2-vinylpyridine)) mixture in DMF, which is the common solvent of the block copolymers, using 1,4-dibromobutane as the cross-linker. Although the PEO chains and the PS chains are strongly incompatible,[18] the cross-linking of P2VP blocks connects both the PEO and the PS chains to a common core and enables the sufficient mixing of the unlike blocks in the shell. In addition, the preparation efficiency is much higher than that of the existing methods.

Table 1. DLS Characterization Data of the Aggregates Produced by Cross-Linking P2VP of PS-*b*-P2VP and PEO-*b*-P2VP Mixture in DMF

sample	D_h (nm)[b]	$\mu_2/\langle\Gamma\rangle^2$ [c]
S1 in DMF[a]	80.50	0.169
S1 in H_2O	70.60	0.117
S1 in 1,4-dioxane/DMF 99/1	63.00	0.170
S2 in DMF[d]	102.0	0.260

[a] S1 = the aggregates prepared in DMF at total concentration of the two copolymers of 10.0 mg/mL (5.0 mg/mL for each of the copolymers). [b] The average hydrodynamic diameters of the aggregates. [c] The polydispersity indexes of the size distributions.[19] [d] S2 = the aggregates prepared at the total concentration of 50.0 mg/mL (25.0 mg/mL for each of the copolymers).

The block copolymers used for this study are PS-*b*-P2VP (PS: M_n = 23 600 g/mol, M_w = 25 900 g/mol; P2VP: M_n = 10 400 g/mol, M_w = 11 500 g/mol) and PEO-*b*-P2VP (PEO: M_n = 15 400 g/mol, M_w = 16 000 g/mol; P2VP: M_n = 14 200 g/mol, M_w = 14 800 g/mol). DMF is the common solvent of both the PS-*b*-P2VP and the PEO-*b*-P2VP. When the two block copolymers used in this study were mixed in DMF at different compositions and at the total concentrations lower than or equal to 50.0 mg/mL, no aggregation was detected by dynamic light scattering (DLS) at room temperature. It is also demonstrated that the block copolymers are molecularly dispersed in DMF at concentrations of the copolymers of 10.0 or 50.0 mg/mL and at 100 °C since no opalescence can be observed. However, with the addition of 1,4-dibromobutane in the solutions (at 100 °C), opalescence appeared gradually, indicating the occurrence of aggregation in the solutions. In this study, the weight ratio of PS-*b*-P2VP/PEG-*b*-P2VP in the solutions is fixed at 1/1 (the molar ratio is 1/1.14). The resultant aggregate solutions in DMF were diluted to the concentration of 0.1 mg/mL with DMF or 1,4-dioxane or dialyzed against water and then diluted to 0.1 mg/mL with water. These solutions at 0.1 mg/mL were characterized by DLS, and the results are listed in Table 1.

As indicated in Table 1, DLS measurements demonstrate the formation of aggregates via the cross-linking reaction. We have measured the aggregates (both S1 and S2 in Table 1) in DMF at the concentrations of 10.0, 1.0, and 0.1 mg/mL by DLS and found that the dilution has little effect on the structure of the resultant aggregates. Obviously, this could be attributed to their cross-linked structure. In addition, the angular dependence of laser light scattering was investigated for S1 and S2 (in DMF at 0.1 mg/mL). The linear relation of Γ

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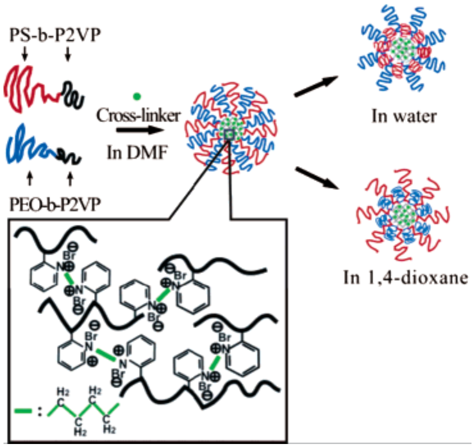
$\sim q^2$ (Supporting Information SI1) indicates that the micelles have an isotropic diffusive behavior and the micelles have a spherical structure.[20]

It has been proved that cross-linking P2VP homopolymer in DMF under the same conditions results in precipitation. In contrast, the cross-linking reaction of P2VP blocks of the copolymer mixture in DMF leads to the formation of regular aggregates, as shown by the data in Table 1. We have demonstrated that the quaternization of pyridine units cannot lead to the aggregation. The reason is that the pyridinium, which is produced by the reaction between pyridine units and the alkylogen molecules, can be well solubilized in DMF.[21] Additionally, in a control experiment, when the mixture of PEO-*b*-P2VP and PS-*b*-P2VP was reacted with 1-bromobutane in DMF under the identical conditions, no aggregation was detected by DLS. Therefore, we conclude that it is the cross-linking reaction that forces the aggregation of P2VP block chains and drives the formation of the aggregates. We have demonstrated in our previous study[21] that during the cross-linking reaction the cross-linked block chains are forced to aggregate to form the core, while the un-cross-linkable block chains will adjust their conformation to stretch out to surround the core, forming the shell.[21] Therefore, in this case, the core should be formed by cross-linked P2VP block chains and the shell by PEO and PS chains. This conclusion is demonstrated by the behaviors of the resultant aggregates in the selective solvents and the morphology observed by TEM.

The solution of the aggregates in water was obtained by dialyzing the aggregate solution in DMF (S1, at the concentration of 10.0 mg/mL) against water. Although the water is the solvent for PEO but nonsolvent for PS, it is found that the as-prepared aggregates can be well-dispersed in water, and no precipitates were detected after storing the sample for ca. 4 months. DLS measurements exhibit that both the average hydrodynamic diameters $\langle D_h \rangle$ of the aggregates and the PDI in water are less than those in DMF (Table 1), indicating the individual dispersion of the resultant PMMS and no further aggregation between the aggregates occurring due to the change of the solvent from DMF to water (Supporting Information, SI2). The fact that no precipitates were observed in the aqueous solution makes us believe that each of the aggregates should have enough PEO chains in the shell, as the aggregates with PS alone as the shell will precipitate in water.

The aggregate solution in 1,4-dioxane was prepared by diluting the solution in DMF (S1, at the concentration of 10.0 mg/mL) by 1,4-dioxane to the concentration of 0.1 mg/mL. Since the volume ratio of 1,4-dioxane/DMF in the resultant solvent mixture is 99/1, the behaviors of the aggregates should be similar to those in pure 1,4-dioxane (hereafter, we will use 1,4-dioxane instead of 1,4-dioxane/DMF (99/1) for simplicity). It is demonstrated that the aggregates can also be well-dispersed in 1,4-dioxane, which is the solvent for PS chains but nonsolvent for PEO chains. No precipitates were observed after storing the solution for months as well. In this solution, the $\langle D_h \rangle$ of the aggregates is less than the value in DMF, and the PDI is comparable to that of the aggregates in DMF (Table 1), indicating the individual dispersion of the PMMS in 1,4-dioxane. In principle, any aggregates with PEO alone as the shell will precipitate in the solvent, so it is believed that each of the aggregates should be with enough PS chains in its shell. Therefore, with the behaviors of the aggregates in 1,4-dioxane and in water being considered, each individual aggregate should have both PS chains and PEO chains in the shell. The cross-linking reaction leads to the formation of PMMS with a PS/PEO mixed shell. According to the mechanism regarding the formation of core–shell nanoparticles, the core should be formed by the cross-linked P2VP block chains.[21] This is consistent with our TEM observations.

Scheme 1. Schematic Description of the Formation of PMMS by This Approach and the Change in the Conformation of the Shell-Forming Polymer Chains in the Selective Solvents



It is imaginable that, in the selective solvents, the behaviors of the PMMS should depend on the initial mixing state (i.e., in a shell of an as-prepared PMMS, whether or not the PEO and PS chains are randomly attached to the common core) of the two shell-forming blocks since the core is cross-linked and the shell-forming chains are tethered on the core. The fact that in each of the selective solvents the as-prepared PMMS are individually dispersed makes us think that in the selective solvents the majority of insoluble polymer chains collapse by contracting radially so that they can be surrounded and protected by the soluble ones. This reflects that the PEO chains and PS chains are fully mixed (i.e., the shell-forming blocks' chains are randomly attached to the core) in the shell (Scheme 1). The reason is that if the PEO chains and the PS chains were separately attached to different regions of the core, the PMMS would tend to behave like an "amphiphilic Janus micelle" in the selective solvents; i.e., they will further associate with a very low critical association concentration.[22] The conclusion is further supported by our TEM observations as well.

TEM observations indicate that the cross-linking reaction leads to the formation of the spherical aggregates, whereas the copolymer mixture in DMF without being treated with the cross-linker does not show any regular aggregates (Supporting Information SI3). In the TEM images, the aggregates deposited from the solution in DMF show an average diameter about 40 nm, which is much less than the value in DMF by DLS. However, after being stained by RuO_4, the diameter is increased to ca. 80 nm (Supporting Information, SI4), close to the $\langle D_h \rangle$ listed in Table 1. The difference between the diameters of the aggregates before and after the staining indicates the core–shell structure of the aggregates. The PS/PEO mixed shell without staining has insufficient contrast to be viewed by TEM, whereas the core formed by 1,4-dibromobutane cross-

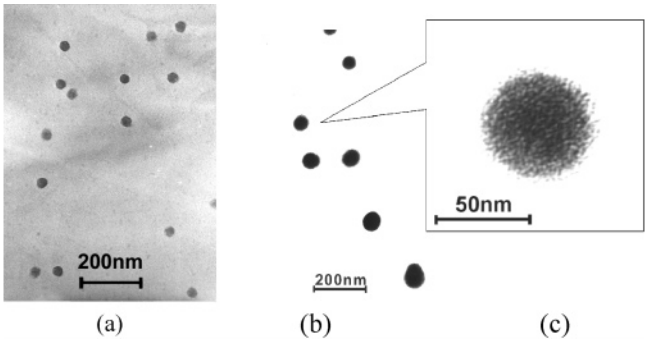


Figure 1. TEM images of the PMMS deposited from the solution in DMF: (a) the aggregates without staining; (b) stained by RuO_4; (c) the stained aggregates with a larger magnification.

linked P2VP chains contains bromide atoms and has enough contrast to be viewed in TEM images. Therefore, the size observed in the TEM images of the aggregates without staining (Figure 1a) represents the size of the core. However, after being stained by RuO_4, the shell becomes visible in the TEM images.[23] The 80 nm should be the size of the aggregates.

Taking a shell of the as-prepared PMMS into account, we expected that, when the PMMS were dried, the PEO chains and PS chains in the mixed shell would have a distinct phase separation whether or not the PEO chains and PS chains are fully mixed in the shell, since the interaction parameter (χN; 25 °C) between the PEO chains and the PS chains is estimated to be as large as 37.[24] It is reported that both PS chains and PEO chains can be stained by RuO_4 and PEO chains are more sensitive to the staining.[23−25] To observe the phase separation between the PEO chains and the PS chains in the shell of a PMMS, a short time staining procedure (30 min) was applied, which may cause nonuniformity during staining.[24] However, when the aggregates are deposited on the copper grid from DMF, there seems no clear phase separation observed in the TEM images of the stained aggregates (Figure 1b) when the magnification is not large enough. Nevertheless, the images of these stained aggregates with larger magnifications show a morphology of an interpenetrated structure with the average size of the domains being ca. 2 nm (Supporting Information SI5), as shown in Figure 1c. The domains in black should be the phase of stained PEO chains, and the domains with less contrast should be the PS phase. By carefully checking the images, one can see in each of the particles a percolated network constructed by thin PEO strands. The average size of the domains is much less than that observed in the morphology resulting from the phase separation of a block copolymer. This morphology tells us that in the shell of the as-prepared PMMS the PS chains and PEO chains are fully mixed; i.e., the PEO and PS chains are randomly attached to a common core. Since the core is cross-linked and the shell-forming chains are tethered to the core, the fully mixed PEO and PS chains have no freedom for phase separation into larger domains, rather than, a "fine phase separation" between the PEO chains and the PS chains are observed. Such "fine phase separation" can be observed for a polymer blend when there is specific interaction between the two polymeric components or for a interpenetrated networks (IPN),[26] whereas it has never been reported for a pair of immiscible polymers or a block copolymer with two incompatible blocks.

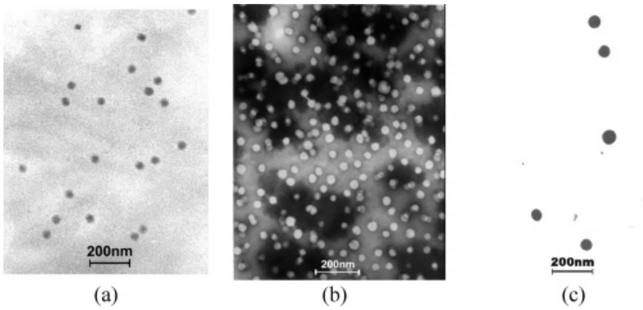


Figure 2. TEM images of (a) the aggregates deposited from water without staining, (b) the aggregates deposited from water negatively stained by phosphate tungstic acid, and (c) the aggregates deposited from 1,4-dioxane stained by RuO_4.

It should be mentioned that this approach to PMMS is in a mechanism different from that of the existing methods. In this approach, the driving force for the aggregation is the chemical cross-linking reaction so the phase separation during the formation of the PMMS between the two shell-forming polymers (PEO and PS in this case) is restricted. As the result, the chains of the two shell-forming polymers are fully mixed to form the shell of the PMMS although the two polymers are quite incompatible. This fully mixing cannot be realized by the existing methods when the two shell-forming polymers are so incompatible as PEO and PS.

The TEM images of as-prepared PMMS deposited from water or 1,4-dioxane without staining show spherical particles with an average size of ca. 40 nm as well (Figure 2a). As mentioned before, this is the size of the core. The aggregates either stained by RuO_4 or negatively stained by phosphate tungstic acid shown an average size in the TEM images (Figure 2, c and b, respectively) of ca. 60 nm, which are close to the respective values measured by DLS. These are quite expectable. No phase separation can be observed in the shell of the RuO_4 stained aggregates deposited from either water or 1,4-dioxane in the TEM images with high resolution even the time for staining is controlled. These supports our conclusion that in water or 1,4-dioxane the majority of insoluble polymer chains collapse by contracting radially and are surrounded and protected by the soluble ones; i.e., the outer shell should be formed by pure soluble chains in the selective solvents so no phase separation can be observed.

The cross-linking reaction can be carried out at the total concentrations of the two copolymers up to 50.0 mg/mL, leading to the formation of regular aggregates (S2 in DMF, Table 1), and the characteristics of the aggregates prepared at the concentration of 50.0 mg/mL are similar to those of the aggregates prepared at 10.0 mg/mL. This is related to the slow aggregation of P2VP chains induced by the cross-linking reaction, as compared with the aggregation of the insoluble chains of a block copolymer in its selective solvent. When the aggregation is slow, even at a high concentration, there is enough time for the shell-forming block chains to disentangle, stretch out, and surround the aggregated block and to play a role as a shield localizing the cross-linking induced aggregation.[21]

It is noted that the average size of the aggregates formed at 50.0 mg/mL is larger than that produced at 10.0 mg/mL. The explanation is given in the Supporting Information (SI6).

In conclusion, this paper describes a one-step approach to core-stabilized polymeric micelles with PS/

PEO as the mixed shell and cross-linked P2VP chains as the core by directly cross-linking P2VP blocks in the solutions of PEO-*b*-P2VP and PS-*b*-P2VP mixture in their common solvent DMF. This approach has two advantages over the existing methods: (1) the core-stabilized PMMS can be prepared by one step and at high solid contents (up to 50.0 mg/mL), so the preparation efficiency is high, and (2) in the shell of the resultant aggregates, PS chains and PEO chains are fully mixed although these two shell-forming polymers are quite incompatible. As a result of the full mixing, the resultant PMMS can be individually dispersed in either water or 1,4-dioxane (water (1,4-dioxane) is the solvent for PEO (PS) chains but the nonsolvent for PS (PEO) chains). Because of the full mixing as well as the fact that the core is stabilized, a "fine phase separation" between the PEO chains and the PS chains is observed; i.e., the TEM images of the RuO_4 stained aggregates deposited from their common solvent DMF show a morphology of a percolated network constructed by PEO strands with the width being ca. 2 nm. Such a "fine phase separation" has never been reported for such a pair of strongly incompatible polymers like PS and PEO or a block copolymer with two incompatible blocks.

Experimental Section

The block copolymers used for this study were purchased from Polymer Source Inc. For the preparation of the aggregates, the PS-*b*-P2VP and the PEO-*b*-P2VP with the weight ratio of PS-*b*-P2VP/PEG-*b*-P2VP being 1/1 were dissolved in DMF for 48 h at designated concentrations (10.0 and 50.0 mg/mL, respectively). Then, 1,4-dibromobutane, with its molar ratio to the pyridine units in each of the reaction systems being 8:1, was added into the solutions. The reaction systems were stirred at 100 °C for 24 h under an argon atmosphere.

A commercial laser light scattering (LLS) spectrometer (Malvern Autosizer 4700) equipped with a multi-τ digital time correlation (Malvern PCS7132) and a solid-state laser (ILT 5500QSL, output power 100 mW at $\lambda = 514.5$ nm) as light source was used. The measurements were preformed at 25.0 °C, where the relative error of $\langle D_h \rangle$ is less than 2%. The solutions were respectively clarified using a 0.45 μm Millipore filter before the measurements and then poured into the cell. In DLS, the line-width (Γ) distribution function $G(\Gamma)$ can be calculated from the Laplace inversion of the intensity–intensity time correlation function $G^{(2)}(q,t)$.[27] The inversion was carried out by the CONTIN program supplied with the Malvern PCS7132 digital time correlator. $G(\Gamma)$ can be converted into a translational diffusion coefficient distribution $G(D)$ or a hydrodynamic diameter distribution $f(D_h)$ via the Stokes–Einstein equation, $D_h = (k_BT/3\pi\eta)D^{-1}$, where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.

DLS measurements were performed at a fixed scattering angle (θ) of 90°, and the angular dependence of the scattering was investigated.[20]

TEM observations were conducted on a Philips CM 120 electron microscope at an acceleration voltage of 80 kV. The sample for the TEM observations was prepared by placing 5 μL micelle solutions on copper grids coated with thin films of Formvar and carbon successively. The staining was carried out by exposing the sample in vapor of ruthenium tetroxide (prepared by treating RuO_2 with excess sodium periodate) 1% (w/w) water solution. The aggregates deposited from water were negatively stained by phosphate tungstic acid (5% (w/w)).

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Supporting Information Available: Angular dependence of scattering; discussion on the morphology in TEM image of core–shell particles; discussion on the effect of contraction of one of the shell-forming polymers on the $\langle D_h \rangle$ of the as-prepared PMMS. This material is available free of charge via the Internet at http://pubs.acs.org.

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