Water-Dispersible, Uniform Nanospheres by Heating-Enabled Micellization of Amphiphilic Block Copolymers in Polar Solvents

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ABSTRACT: Uniform nanospheres with tunable size down to 30 nm were prepared simply by heating amphiphilic block copolymers in polar solvents. Unlike reverse micelles prepared in nonpolar, oily solvents, these nanospheres have a hydrophilic surface, giving them good dispersibility in water. Furthermore, they are present as individual, separated, rigid particles upon casting from the solution other than continuous thin films of merged micelles cast from micellar solution in nonpolar solvents. These nanospheres were generated by a heating-enabled micellization process in which the affinity between the solvent and the polymer chains as well as the segmental mobility of both hydrophilic and hydrophobic blocks was enhanced, triggering the micellization of the glassy copolymers in polar solvents. This heating-enabled micellization produces purely well-defined nanospheres without interference of other morphologies. The micelle sizes and corona thickness are tunable mainly by changing the lengths of the hydrophobic and hydrophilic blocks, respectively. The heating-enabled micellization route for the preparation of polymeric nanospheres is extremely simple, and is particularly advantageous in producing rigid, micellar nanospheres from block copolymers with long glassy, hydrophobic blocks which are otherwise difficult to prepare with high efficiency and purity. Furthermore, encapsulation of hydrophobic molecules (e.g., dyes) into micelle cores could be integrated into the heating-enabled micellization, leading to a simple and effective process for dye-labeled nanoparticles and drug carriers.

INTRODUCTION

Polymeric nanospheres have been gaining ever-increasing interests in diverse fields including drug delivery, sensing, optoelectronics, size determination, calibration, etc. Properties and functions of these spheres are significantly influenced by their shape, size, size distribution, and surface chemistry. A large variety of preparative strategies for polymeric nanospheres have emerged in recent years. These strategies are either physical methods starting from synthetic or natural polymers or chemical ones starting from monomers which are converted to polymers through different particle-forming polymerization mechanisms. Among these methods, the amphiphilic block copolymers (BCPs) micellization route is distinct from others because of its features including ultrasmall particle size down to sub-100 nm, narrow particle size distribution, and functionalizable sphere surfaces. Amphiphilic block copolymers are composed of two or more homopolymer chains which are covalently linked with each other and have strong contrast in hydrophilicity. They tend to microphase separate either in solid state or in selective solvents because of the thermodynamical incompatibility between the constituent blocks, generating a diverse range of well-defined nanostructures. For instance, amphiphilic BCPs dispersed in polar solvents may produce micellar spheres, cylinders, vesicles, etc., depending on the molecular structures of the copolymer as well as the micellization conditions.

Similar to small molecule surfactants, amphiphilic BCPs can form either regular micelles or reverse micelles in selective solvents depending on whether the micelle is prepared in polar or nonpolar solvents with the corona correspondingly composed of hydrophilic or hydrophobic blocks. BCP reverse micelles have long been prepared by direct dissolution in nonpolar solvents. These micelles possess a core of collapsed hydrophilic blocks and a corona of hydrophobic blocks extending to the solvent to stabilize the micelle. Upon casting on solid surface, they form a continuous thin film other than individual, separated micellar particles because of the fusion of neighboring micelles. In addition, BCP reverse micelles are unable to be dispersed in water for applications in aqueous environments since they are initially formed in oily, nonpolar solvents. In contrast, BCP regular micelles having a hydrophilic corona are prepared in polar solvents. It is possible to disperse them in water. BCP regular micelles can be collected from the solvent as dry nanoparticles, and their spherical shape in solution can be largely retained if the core-forming block is in the glassy state at the room temperature, although the solubilized coronae may undergo certain deformation in the process of deposition to the substrate and solvent evaporation. However, this deformation does not noticeably influence the integral spherical shape of the particle since the coronae are typically very thin (several nanometers). Micellization by direct dissolution of BCPs in a polar solvent is only feasible for BCPs...
with hydrophilic blocks as the major component or the hydrophobic blocks are flexible or have a low glass transition temperature \( (T_g) \), producing deformable, "soft" nanospheres. However, rigid nanospheres with stable size and shape are required and particle agglomeration should be avoided in many application circumstances. Furthermore, in most applications, these nanospheres need to be used in aqueous circumstances. Therefore, BCP reverse micelles prepared in nonpolar solvents or "soft" micelles from rubbery or low molecular weight BCPs cannot be applied in these cases.

One popular way to prepare rigid and water-dispersible BCP micellar spheres involves a two-step process. In this process, BCPs are first molecularly dissolved in a common solvent and then a polar solvent is added into the BCP solution to induce the micellization.\(^{17,18}\) This method is efficient in producing micelles with varying morphologies.\(^{9–11}\) However, if we exclusively need spheres without the interference of other structures (e.g., cylinders or vesicles), this method is not very reliable since different phases corresponding to specific morphologies occupy normally a relatively narrow region in the phase diagram and coexistence of different morphologies from neighboring phase regions occurs frequently. In this work, we demonstrate heating-enabled micellization of BCPs, which is a direct dissolution route for BCP micelles and leads to monodispersed polymer nanospheres with tunable sizes down to \( \sim 30 \) nm and hydrophilic surfaces. This method is particularly advantageous in producing rigid nanospheres from BCPs with a long, glassy hydrophobic block and a short hydrophilic block. Furthermore, hydrophobic dyes can be encapsulated into the micelle cores simultaneously in the micellization process, leading to dye-labeled nanospheres which are interesting for applications such as medical imaging and size determination and calibration.

**EXPERIMENTAL SECTION**

All the block copolymers (details are given in Table 1) were purchased from Polymer Source Inc., Canada, and used without further purification. Perylene was purchased from Aladdin Reagents with a purity of 98%. AR-grade acetic acid, ethanol, and 4-bromoethane were commercially obtained and used as received. To prepare the BCP micelles, we mixed the BCP with acetic acid or ethanol at a weight ratio of 1:4 and a polymer concentration of 0.5 wt %, and kept the mixture at 110 °C for 15 h. We mixed 4-bromoethane at a concentration of 3 wt % with the perylene-loaded micelles in ethanol and the quaternization was carried out at 60 °C for 10 h. The quaternized micelles were collected from ethanol by centrifugation (1, 4000 rpm for 10 min) and washed with ethanol three times to remove unreacted 4-bromoethane. The quaternized micelles were then redispersed in deionized water for further characterizations.

We diluted the BCP micellar solutions with the same solvent (acetic acid or ethanol) 5–50-fold for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) examinations. The diluted BCP micellar solutions were deposited on silicon wafer shards or carbon-coated copper grids for SEM and TEM examination, respectively. TEM observations were carried out on a Hitachi S4800 field-emission SEM operated at 10 keV after sputter coating a thin layer of gold. TEM images were taken on a JEOL 1010 microscope operated at 100 keV. For the micelles from PS-b-P2VP-3 and PS-b-P2VP-4, we exposed the micelles deposited on copper grids to iodine vapor at 60 °C for 30 min to selectively stain the P2VP domains prior to TEM probing. The dynamic light scattering (DLS) curves were recorded on an NPA152-31A Zetatrac particle size analyzer, and the fluorescent spectrum of the perylene-loaded micelles was obtained from a VARIAN Cary Eclipse fluorescent spectrometer excited at 400 nm. The corresponding fluorescent picture of the micelle solution in a quartz container was taken using a digital camera under the irradiation of 365 nm UV light.

### RESULTS AND DISCUSSION

For an amphiphilic block copolymer, for instance, PS-b-P2VP, with a hydrophobic block longer than the hydrophilic block, it can be directly dissolved in a nonpolar solvent by forming reverse micelles with the swollen PS as the corona and collapsed P2VP as the core (Scheme 1a). Upon spin coating, micelles self-assemble on a smooth surface as a micellar monolayer, and PS corona are dried and fused with neighboring ones while the P2VP cores remain intact, forming a continuous thin film with near-hexagonal patterned surface protrusions (Scheme 1b). In contrast, in the current work, we demonstrated the copolymer could also form regular micelles in polar solvents with.

<table>
<thead>
<tr>
<th>BCPs</th>
<th>( N_{PS} )</th>
<th>( M_{PS}^{w} )</th>
<th>( N_{P2VP} )</th>
<th>( M_{P2VP}^{w} )</th>
<th>micelle diameter/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-b-P2VP-1</td>
<td>168</td>
<td>17,500</td>
<td>90</td>
<td>9,500</td>
<td>30</td>
</tr>
<tr>
<td>PS-b-P2VP-2</td>
<td>313</td>
<td>32,500</td>
<td>114</td>
<td>12,000</td>
<td>50</td>
</tr>
<tr>
<td>PS-b-P2VP-3</td>
<td>481</td>
<td>50,000</td>
<td>157</td>
<td>16,500</td>
<td>60</td>
</tr>
<tr>
<td>PS-b-P2VP-4</td>
<td>981</td>
<td>102,000</td>
<td>924</td>
<td>97,000</td>
<td>68</td>
</tr>
<tr>
<td>PS-b-P2VP-5</td>
<td>2788</td>
<td>290,000</td>
<td>686</td>
<td>72,000</td>
<td>120</td>
</tr>
</tbody>
</table>

\( ^{\text{a}} \)The micelles were prepared in AA at 110 °C.
P2VP as the extended corona and PS the collapsed core (Scheme 1c). By casting on smooth substrates, one can obtain individual, separated micellar particles (Scheme 1d).

We first investigated the heating-enabled micellization of PS-\(b\)-P2VP with a moderate molecular weight (\(M_{w}^{PS} = 50,000, M_{w}^{P2VP} = 16,500\)). We heated the copolymer in acetic acid (AA) at 110 °C. After about 1 h, the polymer solids disappeared in the solvent, forming a slightly milky solution. We kept the mixture at 110 °C for 15 h and then cooled it to the room temperature naturally. We observed no change of the milky appearance of the solution during the heating and cooling process. We tried to probe the solids dispersed in the as-prepared solution without dilution using SEM; however, it tended to form a continuous film of particles sticking together. To observe the individual particles, we diluted the as-prepared BCP solution with acetic acid 5–50-fold and deposited the diluted solution on silicon wafer shards or carbon-coated copper grids for SEM and TEM examinations. As shown by the SEM images in Figure 1a,b, we obtained spherical particles from the BCP solution, and the particles were highly uniform in size with a mean diameter of 60 nm. Because of the near monodispersed size, these particles arranged on the surface as a monolayer of isolated spheres with a certain degree of short-range ordering and some hexagonal patterns could be found (dashed lines in Figure 1b).

TEM characterizations confirmed the spherical, uniform morphology of the particles and revealed the particles had a solid interior (Figure 1c). The BCP nanospheres are composed of two distinct blocks, PS and P2VP, and it is important to know the localization and distribution of each block. To this end, we exposed the BCP nanospheres deposited on a copper grid to iodine vapor and then examined them under TEM. As shown in Figure 1d, the spheres actually possessed a core/corona composite structure in which the thin corona appearing darker was the P2VP component since iodine selectively enriched in the pyridyl rings, and the gray core was undisturbed PS. The thickness of the P2VP corona was determined to be 5.3 nm. The PS core/P2VP corona structure suggested the BCP nanospheres were formed through the micellization mechanism. To further characterize the uniformity of the PS-\(b\)-P2VP nanospheres, we carried out dynamic light scattering (DLS) measurement on the nanospheres dispersed in AA. DLS results showed a narrow single peak centered around 100 nm, indicating that the spheres in AA were uniform in diameter with an average value of 100 nm (inset in Figure 1d). The diameter value measured from DLS is larger than that from SEM because DLS signals the particles in solution with an expanded volume.19

For comparison, we also prepared PS-\(b\)-P2VP reverse micelles by directly dissolving the copolymer in nonpolar solvents (e.g., toluene and \(o\)-xylenes) with a concentration of 0.5%. Upon being cast onto a smooth surface, the micelles formed a continuous film of connected micelles by the fusion of coronae of adjacent micelles. SEM and atomic force microscopy showed there were also protrusions on the film surface but with a very smaller height, ∼3 nm.20 We further diluted the micellar solution 10-, 100-, or 1000-fold with its solvent and all the diluted solutions appearing as continuous films upon casting. Furthermore, dilution at high folds may result in dissociation of the reverse micelle since there is a critical micelle concentration for the copolymer in the nonpolar solvent at the room temperature. The deposited morphology on a planar surface of both reverse micelles and regular micelles having glassy, hard cores is dominated by the nature of the corona including its thickness, the degree of swelling, the spreading ability of the swollen chains on the substrate, etc. For an asymmetric BCP with a nonpolar block longer than the polar block as we used in this work, the corona of the reverse micelle would be thicker than the corona of the regular micelle supposing both micelles have a similar aggregation number. In addition, the solvated,
nonpolar corona with lower surface energy of a reverse micelle has better wettability compared to that of polar chains with higher surface energy in a regular micelle. Therefore, the space among the deposited reverse micelles would be fully covered by the spreading chains of the nonpolar corona, leading to a continuous film of fused micelles upon casting on a planar surface. However, in the case of regular micelles, the corona is thin and the composing polar chains have a weaker wettability because of their high surface energy; therefore, regular micelles are deposited as separated, individual spheres.

We note that, instead of acetic acid, other solvents selective to P2VP and miscible with acetic acid, for instance, ethanol and water, could also be used to dilute the as-prepared PS-b-P2VP nanospheres. Furthermore, we observed no micelle dissociation or change in sphere morphology and size upon diluting with different diluents and with different dilution folds. This structure robustness is due to glassy micellar cores remaining kinetically frozen at the room temperature in polar solvents with changing types and concentrations, although the micellar corona may expand to different degrees in changing solvents. The flexibility in dilution offers great convenience in the applications of the nanospheres in different situations. Moreover, at higher BCP concentrations, for instance, 5%, uniform micellar nanospheres were still achievable at a much higher production yield and there was no noticeable difference in the morphology and size of micelles produced at the concentration of 0.5% and 5%. Therefore, the concentration effect should be very weak in the heating-enabled micellization. In addition, the PS-b-P2VP nanospheres can be easily separated from the solvent by precipitation with alkaline water in which the corona-forming P2VP chains lose their solubility and consequently their stabilizing ability as in acidic environment. The precipitated nanospheres can be further redispersed in ethanol, acetic acid, acidic water, and other P2VP-favorable solvents.

Heating is crucial for the micellization process. The temperature increase enables the micellization process by two main contributions: increase in the affinity between the solvent and the blocks and enhancement in the segmental mobility of both blocks. The solvent quality, that is, the affinity between a polymer and a solvent is characterized by the polymer–solvent interaction parameter, $\chi_{P-S}$ which can be estimated by the van Laar-Hildebrand equation,$^{21}$

$$\chi_{P-S} = \frac{V_{S}}{(RT)} \times (\delta_{P} - \delta_{S})^{2}$$

where $V_{S}$ is the molar volume of the solvent and $\delta_{P}$ and $\delta_{S}$ are solubility parameters of the polymer and the solvent, respectively. Since $\delta_{P}$, $\delta_{S}$ and $V_{S}$ are only slightly temperature-dependent and is negligible in most cases, this equation predicts a decrease in the value of $\chi_{P-S}$ with a temperature increase, suggesting higher temperature improves the solvent quality toward both blocks. Moreover, temperature increase enhances the segmental mobility of both blocks, providing further flexibility of polymer chains for bending to form either the cores or the coronae in the process of micellization.

There is a critical micellization temperature (CMT) at which the hydrophobic blocks begin to transit from the glassy state to the rubbery state and consequently microphase separation leading to micellization taking place in the solvent. Below CMT, for instance, at room temperature (25 °C), the copolymer could not be dissolved in AA, while at the temperature of 50 °C we were able to obtain well-developed spherical micelles. Therefore, the CMT for this specific copolymer should be somewhere between 25 and 50 °C. Above the critical temperature, the temperature effect is not obvious and heating to higher temperature does not change the spherical morphology of the micelles. Moreover, the heating temperature just slightly affects the micelle sizes because the micellization process is mainly dominated by the insoluble PS blocks other than the temperature when the PS block is long (>30 repeat units).$^{22}$ For instance, we always obtained similar spherical micelles by heating PS-b-P2VP-3 in AA at different heating temperatures of 50, 95, and 110 °C, while the sizes of these micelles were 65, 61, and 60 nm, respectively (Figure 2 and Figure 1c).

![Figure 2. TEM images of PS-b-P2VP-3 ($M_{w}^{PS} = 50,000$ and $M_{w}^{P2VP} = 16,500$) micelles prepared in acetic acid at 50 °C (a) and 95 °C (b). The TEM image of the micelle prepared from the same copolymer at 110 °C has already been shown in Figure 1c.](image-url)

The size of the micellar spheres can be efficiently tuned by changing the block length of the copolymers. We prepared micellar nanospheres with diameters ranging from ~30 to ~120 nm from PS-b-P2VP copolymers with different molecular weights (PS-b-P2VP-1 through PS-b-P2VP-5, Table 1). There is a scaling correlation between the micelle core radii ($R_{c}$) and the number of units of the soluble blocks ($N_{a}$) and the insoluble blocks ($N_{b}$): $R_{c} = N_{a}^{\alpha}N_{b}^{\gamma}$ (0.67 ≤ $\alpha$ ≥ 0.76 and 0.1 ≤ $\gamma$ ≥ 0).$^{23,24}$ For asymmetric BCPs with shorter soluble blocks, the thickness of the corona is very thin in the dry state and the micelle size is mainly dictated by the core diameter. Therefore, the micelle sizes are strongly related to the lengths of the PS block and longer PS chains lead to bigger sizes, while the micelle sizes are slightly related to the lengths of the P2VP block and longer P2VP chains result in smaller micelle size. This is the reason why the micelle size of PS-b-P2VP-4 ($M_{w}^{PS} = 102,000$ and $M_{w}^{P2VP} = 97,000$) is just slightly larger (68 nm vs 60 nm) than that of PS-b-P2VP-3 ($M_{w}^{PS} = 50,000$ and
$M_w^{PVP} = 16\,500$, although the first copolymer has significantly longer length of both blocks than the second one.

As shown in Figure 3, micelles from all the copolymers developed a well-defined spherical morphology with narrow size distribution though the block lengths vary in a wide range. In addition, we made DLS measurements to the micelles from the shortest copolymer, PS-b-P2VP-1, and the longest copolymer, PS-b-P2VP-5, and confirmed that both micelles were uniform in particle size also in the solution and the “wet” micelles are about 60% larger than their dry counterparts. Although the length of the soluble P2VP chains contributes slightly to the micelle size, it determines the thickness of the micellar corona. Inset in Figure 3c gives the TEM image of stained micelles from PS-b-P2VP-4, which has the longest P2VP blocks in all the five investigated copolymers, and the corona thickness is measured to be 14.7 nm, which is nearly 2 times thicker than the corona of micelles from PS-b-P2VP-3 containing a 5 times shorter P2VP block.

The selective solvent plays an important role for the generation of well-defined spherical micelles. The solvent needs to have a strong contrast in solubility toward different blocks of the copolymer. For instance, spherical micelles can be easily obtained from PS-b-P2VP-3 at a temperature as low as 50 °C using AA as the solvent, while the copolymer remained largely undissolved in ethanol even at a temperature of 180 °C. (The copolymer and ethanol were mixed and heated to 180 °C in an autoclave). Compared to ethanol, AA has a much stronger affinity toward P2VP because of the strong acid–base interaction between the carboxylic groups of AA and pyridine rings, and will bind onto the pyridine rings of the P2VP blocks, enhancing their incompatibility with PS blocks. Consequently, microphase separation leading to the formation of spherical micelles occurs at modest thermodynamic conditions. However, for copolymers with low molecular weight, for instance, PS-b-P2VP-1, spherical micelles are also obtainable using ethanol as the solvent at 60 °C.

This heating-enabled micellization strategy is relatively generic and we are able to produce well-defined nanospheres from block copolymers of polystyrene with poly(4-vinyl pyridine), poly(methyl methacrylate), and poly(ethylene oxide) (PS-b-P4VP, PS-b-PMMA, and PS-b-PEO, respectively) by dispersing these copolymers in AA and keeping them at 110 °C for hours. As shown in Figure 4, the micelles obtained from BCPs with different chemical compositions were all present as spherical nanoparticles with uniform morphology. It was reported that there was a transition of micelles from spheres, to cylinders, and then to vesicles with progressively weakening of solvent quality to the hydrophobic blocks, suggesting spherical micelles is the most stable morphologies formed in selective solvents. Therefore, in the heating-enabled micellization, we can adopt a temperature much higher than CMT to ensure the exclusive formation of micellar nanospheres independent of the chemical compositions and molecular weights of the copolymer. Thus, this method is robust and reproducible in producing uniform nanospheres.

Furthermore, we demonstrated perylene, a hydrophobic fluorophore, could be encapsulated into the micellar cores simultaneously in the heating-enabled micellization. We mixed perylene together with PS-b-P2VP-1 ($M_w^{PS} = 17\,500$, $M_w^{P2VP} = 9\,500$) in ethanol with a weight ratio of 1:4 and kept the mixture at 110 °C for 15 h. We obtained a homogeneous solution with slightly yellowish color. In contrast, in absence of the copolymer, perylene at the same concentration remained as insoluble precipitates in ethanol because of its very limited solubility in ethanol. Therefore, the solubilization of perylene was caused by the encapsulation of perylene in the cores of PS-b-P2VP micelles driven by the hydrophobic interactions between the perylene molecules and the PS blocks. The added perylene

Figure 3. SEM images of PS-b-P2VP with different molecular weights (a–d) (the molecular weights are given in the figures). Insets in (a) and (d) are the DLS curves of the corresponding micelles. Inset in (c) is the TEM image of micelles from PS-b-P2VP-4 stained with iodine.
should have been mostly encapsulated into the micelles since the affinity of perylene to polystyrene was stronger than that to ethanol, resulting in a ∼20% of loading percentage (perylene vs copolymer). As shown in Figure 5a,b, the PS-b-P2VP micelles after encapsulation with perylene remained as uniform spherical particles with a size enlarged to 40 nm, while the micelle from the pure copolymer was 25 nm in diameter. Furthermore, the perylene-encapsulated micelles could be quaternized to be water-dispersible. The quaternized micelles could be precipitated from the ethanol solution by centrifugation and well redispersed in water because P2VP turned out to be insoluble in ethanol but soluble in water after quaternization. As shown by the fluorescent spectrum in Figure 5c, the water-dispersible micelles were fluorescent and the characteristic peaks of perylene were present, indicating that not only perylene remained in the micelles after the quaternization and water dispersion process but also its fluorescent property was preserved. The inset in Figure 5c displayed the fluorescent picture of the micelle solution in a quartz container under the irradiation of 365 nm UV light. The micelle solution emitted a blue-colored glow, and the emission throughout the container is uniform, suggesting the micelles are dispersed in water evenly. The water-dispersible fluorescent nanoparticles are very useful in drug delivery, live cell imaging, biosensing, etc. in aqueous or biological systems.

**CONCLUSIONS**

We prepared uniform nanospheres by a one-step process in which amphiphilic block copolymers were heated in polar solvents to induce the micellization. These nanospheres have a glassy, hydrophobic core surrounded by a hydrophilic corona. The micellar spheres can be collected from the solution and redispersed in other polar solvents, or deposited onto solid surfaces as rigid, spherical nanoparticles. This direct dissolution method is capable of exclusively producing spherical micelles. The sphere sizes could be tuned effectively from 30 to 120 nm...
by using copolymers with different molecular weights, and the dissolution temperature played a minor role in affecting the micelle sizes. Hydrophobic dyes, for example, perylene, could be encapsulated in the micelles to make the micelle fluorescent. Furthermore, since the micelles have polar corona composed of P2VP blocks, they can be chemically modified to be more hydrophilic and water-dispersible by quaternizing the P2VP corona.

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