# Effects of Solvent Evaporation Rate and Poly(acrylic acid) on Formation of Poly(ethylene oxide)-*block*-polystyrene Micelles from Emulsion

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 Ⓢ *Supporting Information*

ABSTRACT: In this work, a solution of poly(ethylene oxide)-*block*-polystyrene (PEO-*b*-PS) block copolymer in an organic solvent was dispersed in water to form an emulsion in the presence of poly(acrylic acid) (PAA), which upon solvent evaporation produced micelles, and the "emulsion and solvent evaporation" process was studied. It was found that PAA interacted with the PEO corona of the micelles to reduce the curvature, transforming the aggregates from cylinders into vesicles when 1,2-dichloroethane was the solvent. However, when a more volatile solvent, dichloromethane, was used instead, cylindrical micelles were obtained. Even from 1,2-dichloroethane, cylinders were the predominant species when the initial solution concentration was higher (i.e., shorter evaporation process)

or when PAA with a much higher molecular weight was used. On the basis of these observations, the interplay between the solvent evaporation rate and the mass transport and chain reorganization at the interface is discussed. In addition, some intermediate structures were observed, which provided insight into the assembly process.
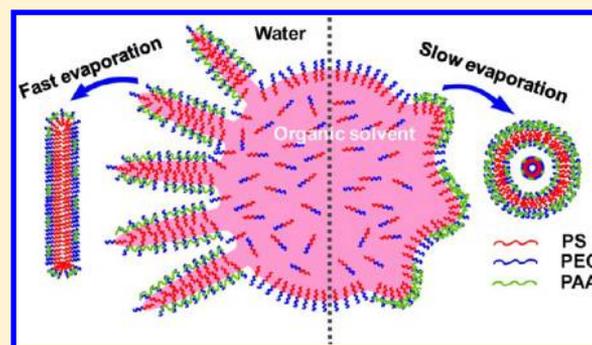
■ INTRODUCTION

Block copolymers can self-assemble in a selective solvent to form a range of structures, typically spherical micelles, cylindrical micelles, and bilayer vesicles, as dictated by the copolymer composition and the interactions between the components and the solvent. Owing to their enhanced mechanical stability as compared with small amphiphilic molecule counterparts, block copolymer assemblies have attracted considerable attention as drug delivery vehicles.[1−3] The dominant approach for processing block copolymer assemblies involves addition of a selective solvent into a miscible common solvent containing the block copolymer to gradually decrease the solvent quality for one block, resulting in formation of aggregates.[4−7] Unfortunately, this gradual addition method often leads to precipitation of ingredients and hence low encapsulation efficiency, which has become a bottleneck for the application of the copolymer assemblies. Prud'Homme and co-workers developed a method called "flash nanoprecipitation" to elegantly circumvent this problem, in which a solution of the copolymer and the drug in a water-miscible organic solvent is rapidly mixed with water, a poor solvent for the hydrophobic block and the drug, resulting in precipitation of the drug into nanoaggregates of the copolymer.[8−10] But only spherical micelle can be obtained by this method due to the short time scale of mixing, which prevents chain relaxation to form other structures. On the other hand, it was revealed recently that biodistribution, cellular uptake, and toxicity is closely associated with the shape of drug delivery vehicles, and structures other

than spheres may be advantageous to drug performance.[11,12] An alternative approach based on emulsion was recently developed to improve encapsulation efficiency[13] and to obtain nonspherical structures.[14] In this "emulsion and solvent evaporation" method, also called "co-solvent/evaporation" method, the copolymer solution in a volatile organic solvent immiscible with water is first dispersed in water as an emulsion, with the aid of a surfactant to stabilize the droplets, and upon gradual removal of the organic solvent by slow evaporation aggregates of the copolymer dispersed in water are produced.[13,14] Pioneering work done by Geng and Discher in 2005, the deepened and extended work by Zhu and Hayward more recently has brought new vigor to this method, including production of very long cylinders, efficient encapsulation of hydrophobic materials, especially nanoparticles, into the core of long cylinders, and the ability to produce composite cylinders with nanoscopic aqueous or external hydrophobic domains, *etc*.[14−16] However, many open questions remain to be answered to fully employ this method, especially the interplay between solvent removal rate and the kinetics of mass transport and interfacial chain reorganization.[17−19]

Through the introduction of strong secondary interactions, such as hydrogen bonding, one can enrich the methodology to manipulate ordered structures at nanometer scale.[20−25] For

instance, Shi and co-workers found that hydrogen bonding between poly(acrylic acid) (PAA) and poly(4-vinylpyridine) can induce aggregation of poly(ethylene oxide)-*block*-poly-(acrylic acid) (PEO-*b*-PAA) in a common solvent.[26,27] Several other groups reported that morphology of poly(ethylene oxide) (PEO)-based block copolymer aggregates in a selective solvent could be altered through introduction of PAA during or after the assembly process.[28,29] Besides, recent work by Wang and co-workers demonstrated that hydrogen bonding can slow down the morphology transformation of poly(ethylene oxide)-*block*-polystyrene (PEO-*b*-PS) micelles from spheres to vesicles and facilitate the observation of the cylinders in transition state.[30] Thus, hydrogen bonding can impact the thermodynamics as well as kinetics of the assembly of block copolymers.

In this report, we investigate the assembly of PEO-*b*-PS by the emulsion and solvent evaporation method in the presence of PAA, and explore the effects of solvent evaporation rate on the morphology obtained, in an attempt to understand the interplay between solvent evaporation and mass transport at the interface.

■ EXPERIMENTAL SECTION

Materials. PEO-*b*-PS, with number-average molecular weights of 5.0 kg/mol and 4.2 kg/mol for the PEO and PS blocks, respectively, and a polydipersity index of 1.5, was synthesized following a literature procedure.[31] PAAs (M_w = 2 kg/mol and 100 kg/mol) were purchased from Sigma-Aldrich. Dichloromethane and 1,2-dichloroethane (AR grade) were purchased from Beijing Chemical Works. All materials were used without further purification. Ultrapure water (18.2 MΩ cm) was obtained using a PGeneral GWA-UN Pure Water System and used for all experiments.

Micelle Preparation. The block copolymer was first dissolved in 1,2-dichloroethane (or dichloromethane) at a concentration of 10 mg/mL. Subsequently, under vigorous stirring 100 μL of the block copolymer solution were poured into 1 mL of an aqueous solution of PAA of different concentrations to yield an emulsion. The organic solvent was then evaporated with the aid of gentle stirring (about 100 rpm) and the emulsion droplets began to shrink. The stirring was maintained for more than 10 h until no noticeable amount of the emulsion was observed, as indicated by the disappearance of the strong scattering of visible light by the emulsion droplets.

Characterization. Transmission electron microscopy (TEM) characterization was performed on a JEOL JEM-1011 microscope operating at an acceleration voltage of 100 kV. A drop of the sample was placed onto a carbon-coated copper grid to allow the solvent to evaporate for 20 min, and the remaining solution was blotted away using a strip of filter paper. The specimen was then negatively stained using a phosphotungstic acid solution (1 wt %, for 2 min) before the TEM experiment. Atomic force microscopy (AFM) experiments were carried out on an Agilent Technologies 5500 scanning probe microscope. A drop of the sample solution was deposited onto a clean silicon wafer and was left to stand for 10 min before drying with a stream of N$_2$.

■ RESULTS AND DISCUSSION

When the solution of PEO-*b*-PS in 1,2-dichloroethane was added under stirring into water, which is immiscible with the solvent, the amphiphilic block copolymer and surfactant can stabilize the organic phase to form an emulsion. The volatile organic solvent then slowly evaporated, which was facilitated by gentle stirring, and the concentration of the block copolymer in the droplets and at the solvent/water interface increased gradually. The organic solvent/water interface became rough because of transient vanishing or even negative interfacial tension or preferred curvature of the block copolymer.[32,33] Finally, the organic solvent evaporated completely and the

copolymer was dispersed in water to form micelles. For micelles prepared by this "emulsion and solvent evaporation" method, it has been demonstrated that the copolymer assembly can be effectively tuned by addition of a surfactant, such as sodium dodecyl sulfate or poly(vinyl alcohol). The surfactant adsorbs to the organic/water interface and lowers the interfacial tension, and the interfacial curvature is altered because of the competitive adsorption of the surfactant with the copolymer at the interface. As a result, the assembly morphology can transform from microparticles to strings of vesicles to cylinders and then to nanoparticles upon increase of the surfactant concentration.[34] In the present study, the polymeric surfactant we employed was PAA, which can interact with PEO-*b*-PS and modify the morphology of the copolymer assembly.[28] Figure 1
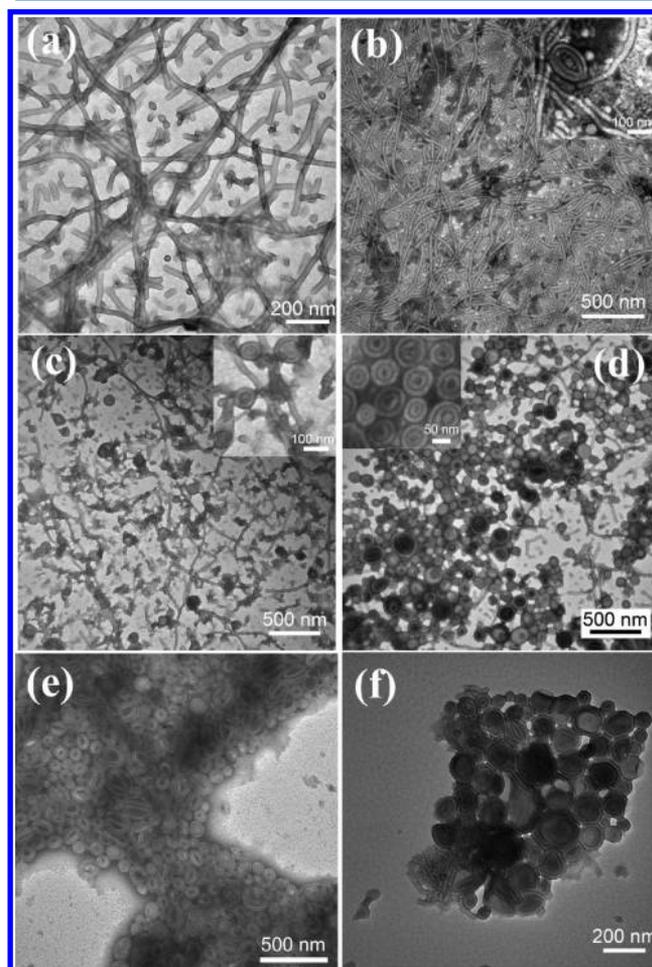

Figure 1. TEM images of PEO-*b*-PS aggregates formed from 1,2-dichloroethane solution in the presence of PAA of (a) 0, (b) 0.5, (c) 1.0, (d) 2.0, (e) 4.0, and (f) 10 mg/mL, respectively. The insets in parts b−d are enlarged views of the vesicles.

presents the TEM images of the PEO-*b*-PS copolymer aggregates produced at various concentrations of PAA. Starting with cylindrical micelles in the absence of PAA, vesicles appear concomitant with the introduction of PAA. The amount of vesicles increases with the augmentation of PAA concentration from 0.5 to 4 mg/mL, and the morphology becomes irregular at 10 mg/mL. The formation of vesicles at PAA concentration of 4 mg/mL was confirmed by AFM characterization (Figure S1, Supporting Information). This trend, however, contradicts the above-mentioned literature results where increasing

Table 1. Characteristic Parameters of the Organic Solvents Employed

solvent	$T_b/°C$	$S/\text{g L}^{-1}$	$\gamma/\text{mN m}^{-1}$	$\gamma_{inter}/\text{mN m}^{-1}$	$\delta/\text{MPa}^{1/2}$
dichloromethane	39.8	20.0	28.0	28.3	19.84
1,2-dichloroethane	83.5	8.7	27.0	28.4	20.05

T_b, boiling temperature; S, solubility in water at 20 °C; γ and γ_{inter}, surface tension and interfacial tension between organic solvent and water respectively;[37] δ, solubility parameter.[38]

surfactant concentration led to higher interfacial curvature and transformation from vesicles to cylinders. Therefore, the PAA did not appear to act as a typical surfactant in this case.

As reported in the literature, PAA can alter the assembly structure of PEO-b-PS by hydrogen bonding with the PEO units in the copolymer,[28,35] which reduces the swelling of the corona of the micelle (the PEO) and leads to lower curvatures, and the effects depend on the molar ratio of the acrylic acid vs the ether, $R_{[AA]/[EO]}$. With the copolymer composition and the molecular weight of each repeat unit, we calculated the molar ratio of acrylic acid vs ether for different concentrations of PAA, and the $R_{[AA]/[EO]}$ was 0.3, 0.6, 1.1, 2.2, and 5.5 for the PAA concentration of 0.5, 1.0, 2.0, 4.0, and 10.0 mg/mL, respectively. From Figure 1 it can be seen that cylindrical micelles dominate when $R_{[AA]/[EO]}$ is below 1, whereas the aggregates are almost exclusively vesicles at $R_{[AA]/[EO]}$ above 1, suggesting a critical role of the PAA/PEO complex formation. It should be pointed out that the pH of the PAA solutions used was ∼3.0, at which the PAA units are protonated and are effective hydrogen bond donors. In contrast, in a control experiment, when a PAA solution of 2.0 mg/mL ($R_{[AA]/[EO]}$ ∼1.1) with pH maintained at 8.2 was used instead, cylinders were again the predominant species (Figure S2, Supporting Information). Apparently at this pH, much higher than the pK_a of the carboxylic acid, the acrylic acid units were deprotonated and thus cannot hydrogen bond with the ether units in PEO, whereas the repulsion between the ionized groups dominated the interactions between PAA and PEO.[36] On the basis of these observations we conclude that PAA formed a complex with the PEO block of the copolymer via hydrogen bonding to induce the structural transformation of the copolymer aggregates.

In the assembly of copolymers, it has been suggested that the evaporation rate of the organic solvent plays an important role in the aggregate formation, but experimental evidence is lacking.[17−19] To explore this, we picked a pair of organic solvents of similar properties except volatility. From Table 1, it can be seen that dichloromethane and 1,2-dichloroethane have very close surface tension and interfacial tension (with water) values, and similar solvency as indicated by the solubility parameter, but differ significantly in volatility. In an emulsion the organic solvent can evaporate into the atmosphere either directly from the organic phase or by dissolution into the surrounding aqueous phase and evaporating thereafter. Dichloromethane exhibits a much lower boiling point and a significantly higher solubility in water as compared with 1,2-dichloroethane, therefore was expected to evaporate much faster than latter in the emulsion. In fact, under our experimental conditions, the emulsion droplets were observed to disappear in around 80 and 600 min, respectively, almost an order of magnitude difference when dichloromethane and 1,2-dichloroethane was the solvent. We then compared the morphology of the PEO-b-PS aggregates formed in the presence of 2.0 mg/mL PAA when dichloromethane and 1,2-dichloroethane was respectively employed as the solvent. As can be seen from Figure 2, the aggregates produced from 1,2-
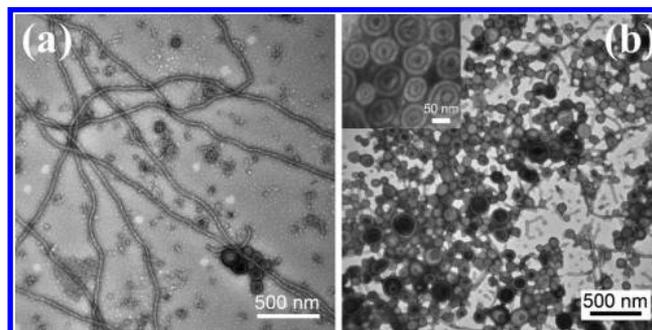

Figure 2. TEM images of PEO-b-PS aggregates formed in the presence of PAA ($R_{[AA]/[EO]}$ = 1.1) using (a) dichloromethane and (b) 1,2-dichloroethane as the organic solvent, respectively.

dichloroethane solution were mostly vesicles, as we have seen and discussed above; however, in sharp contrast, the aggregates were predominantly cylinders with dichloromethane as the solvent. This result clearly demonstrates the critical role solvent evaporation rate plays in the formation of the copolymer aggregates. During the assembly, the micelles are solidified when the organic phase becomes too viscous for the polymer chains to move as the concentration increases to a critical value due to solvent evaporation, therefore the time for mass transport and chain reorganization at the interface is limited by the solvent evaporation process.

Along this line, when starting with a higher PEO-b-PS concentration in the organic phase, less solvent is removed before the copolymer chains in the organic phase are too sluggish and the time available for the PEO blocks to find and form complex with PAA is thus shortened, and formation of a vesicle would be suppressed. This was exactly what we observed when the concentrations of PEO-b-PS and PAA were raised simultaneously by a factor of 5 while maintaining the same $R_{[AA]/[EO]}$ (Figure 3a). In all the experiments so far, low molecular weight PAA (M_w ∼ 2.0 kg/mol) was employed.

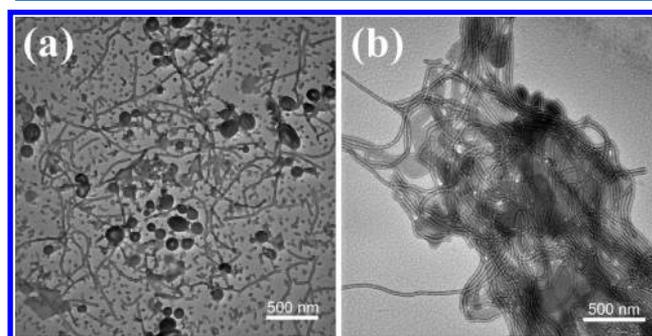**Figure 3.** TEM images of PEO-b-PS aggregates formed from 1,2-dichloroethane solution in the presence of PAA ($R_{[AA]/[EO]}$ = 1.1), (a) when one starts from a higher copolymer concentration of 50.0 mg/mL or (b) when PAA of a much higher molecular weight of 100 kg/mol was used.

When PAA of much higher molecular weight, 100 kg/mol, was used instead, almost exclusively cylinders were observed, with a very small amount of vesicles present (Figure 3b). As the vesicle formation requires the presence of PAA at the interface to associate with the PEO blocks, diffusion of PAA chains to the interface is much slower when the molecular weight is significantly greater, resulting in less PAA chains available at the interface before the organic phase reaches the critical concentration. Therefore, few vesicles form. Besides, a less compact structure of the complexes formed with high molecular weight PAA may also hinder the vesicle formation.[39,40] These results demonstrate the interplay between mass transport and chain reorganization at the interface and the solvent evaporation rate in the assembly.

In the course of the experiments, some interesting structures were captured, which probably were intermediate states, and they may provide some insight into the evolution process of micelle structure. Figure 4 presents two such examples. It can
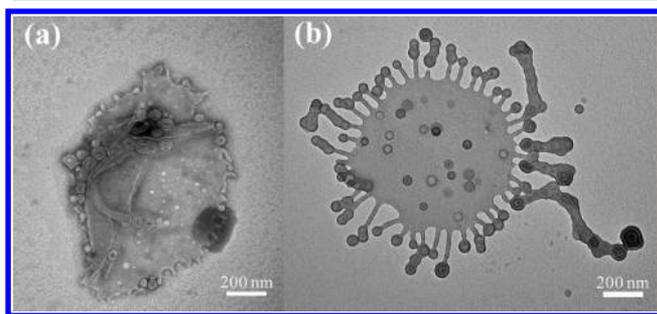

Figure 4. Representative TEM images of the intermediates in the assembly process of PEO-*b*-PS/PAA from 1,2-dichloroethane.

be seen that buds emerge simultaneously along the rim of the oblate vesicle. We notice that long buds are more likely to end up with vesicles of one or two layers than the shorter ones. This probably is because the time scale for long buds to evolve is much longer, allowing more PAA chains to associate with the PEO blocks, which in turn favors the formation of vesicles. Moreover, a string of vesicles are also observed when PAA/PEO association is more adequate (in the long bud, Figure 4b). Tsafrir and co-workers observed budding and tubulation in highly oblate vesicles of a small molecule surfactant when an amphiphilic polymer was introduced to modify the curvature, and attributed this to the spontaneous curvature.[41] The resemblance between our experiment results and that reported by Tsafrir suggests that it is the preferred curvature, not the vanishing or transiently negative interfacial tension,[34] that drives the copolymer assembly in the emulsion and solvent evaporation method. In these experiments we also noticed that these intermediate structures were more likely to be captured at higher PAA concentrations, which could be ascribed to the slowing down of the morphology evolution by the association of PAA,[30] making the observation of the intermediates easier. Despite these interesting observations, TEM characterization is still an ex situ method that is time-consuming and cannot provide statistical information on the whole, and other in situ methods based on spectrometry are needed to provide more real time information to further our understanding of the assembly process.

On the basis of the experimental results discussed above, we can now offer a schematic illustration to describe the effects of solvent evaporation rate on the assembly morphology (Scheme

1). Upon solvent evaporation and copolymer migration to the interface, the interfacial structure starts to evolve due to

Scheme 1. Schematic Illustration of the PEO-*b*-PS Micelle Formation in the Presence of PAA at Different Solvent Evaporation Rates
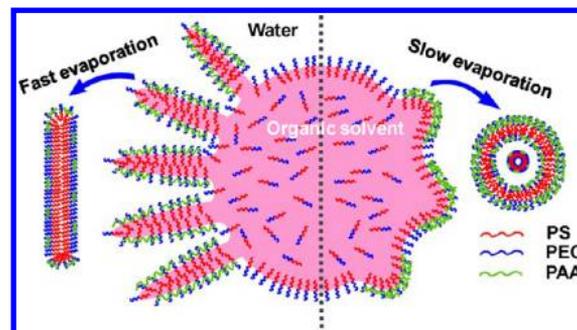
interfacial instability. When the organic solvent used is more volatile, such as dichloromethane, it evaporates more quickly and the copolymer concentration in the organic phase reaches the critical value where the chains are sluggish in a shorter time scale, so that the time for PAA to interact with the corona and for the copolymer chain to adjust their conformation is limited, leading to cylindrical micelles. In contrast, assembly from a less volatile solvent, such as 1,2-dichloroethane, evolves slowly, which allows more PAA to diffuse to the corona and interact with the PEO to form a more compact corona, resulting in lower curvatures and vesicle formation.

■ CONCLUSIONS

We have studied the micelle formation via the emulsion and solvent evaporation process of PEO-*b*-PS in the presence of PAA, from two organic solvents of different volatility, and found that the aggregates produced from the more volatile dichloromethane were cylindrical micelles, whereas mainly vesicles were obtained from a solution of 1,2-dichloroethane, which evaporates slower. Although in general micelle structure is governed by the copolymer composition, our experimental results demonstrate that solvent evaporation rate, more specifically the interplay between the solvent evaporation and the mass transport and chain reorganization at the interface, can critically impact the morphology of the micelles. Our findings offer new insight into the micelle formation process via the emulsion and solvent evaporation method. Further work based on in situ methods is needed to better understand the assembly process and to facilitate the effective utilization of this assembly method.

■ ASSOCIATED CONTENT

ⓢ Supporting Information
AFM image of the vesicles and TEM image of the aggregates obtained at high pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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