

Effect of Water Content on Encapsulation of SWNTs and Hydrophobic Dyes within PS-*b*-PAA Block Copolymer Micelles

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Due to their unique structural, mechanical and electronic properties, carbon nanotubes have been of great interest for last couple decades.¹ Numerous applications of carbon nanotubes have been investigated in various fields of science and technology.²⁻⁹ For example, carbon nanotubes have been investigated as a component of sensors, drug delivery vehicles and hyperthermic therapy agents.¹⁰⁻¹⁵ It is generally acknowledged that carbon nanotubes first have to be functionalized and solubilized in aqueous media for such applications. This was achieved not only by covalent attachment of long chain molecules,^{13,16} but also by formation of micelles around carbon nanotubes. Block copolymer surfactants have played a particularly important role in solubilization of hydrophobic molecules and nanomaterials in aqueous media due to their large loading capacity,¹⁷⁻²⁰ and high structural stability.²¹⁻²⁶ Various nanomaterials have been stabilized by block copolymer micelles. We recently reported single-walled carbon nanotubes (SWNTs) and gold nanoparticles encapsulated within block copolymer micelles in aqueous media.²⁷⁻³⁰ In this method, block copolymers spontaneously formed thick concentric shell structures around nanotube- or nanoparticle core when solvent was gradually exchanged from DMF to water. Unlike other polymer wrapping methods, the shell of the resulting nanostructures was very thick and completely isolated nanomaterials from outer environment.^{29,31} These features are very useful for the biomedical applications (especially for drug delivery) of SWNTs. Extending our previous works, herein, we are going to investigate the encapsulation behavior of SWNTs at different water content. Furthermore, partitioning behavior of hydrophobic molecules between encapsulated SWNTs and solvents with different water content was also investigated.

Results and Discussion

Highly asymmetric PS₁₅₉-*b*-PAA₅₈ block copolymers have been used for encapsulating SWNTs. It is known that PS₁₅₉-*b*-PAA₅₈ forms very uniform micelles around SWNTs.²⁷ SWNTs encapsulated within PS₁₅₉-*b*-PAA₅₈ block copolymers (SWNT@PS₁₅₉-*b*-PAA₅₈) have been prepared by the previously reported method.²⁷ Since both nanotubes and PS₁₅₉-*b*-PAA₅₈ block copolymers were not able to be directly solubilized in aqueous solvent, they were first co-suspended in DMF, and then H₂O was gradually added under vigorous sonication. As increasing water content (wc), originally insoluble SWNTs/co-polymer mixtures gradually formed homogeneous dispersion, which implies the formation of block copolymer micelles around SWNTs. Partitioning behavior of SWNTs has been first investigated as a function of water content. As shown in Figure 1a, solubility of SWNTs varied with water content giving the maximum value at wc = 20%; it sharply increased until water content reached to 20%, and then exponentially decreased thereafter. Solubility change with water content can be explained with swelling/deswelling and morphological change of micelle. Our light scattering data show that micelles were formed at very low water content, about wc = 4.5% (Figure 1b), and are consistent with previous results.³² At such low water content, it is expected that micelle cores are still swollen with significant amount of DMF, since DMF is a good solvent for PS blocks. The swollen micelles more effectively solubilize SWNTs because of their larger internal diameter, but obviously their structural integrity is weak when they are swollen.²⁷ Deswelling decreases the diameter of micelle, and consequently have SWNTs precipitate out of micelle core.

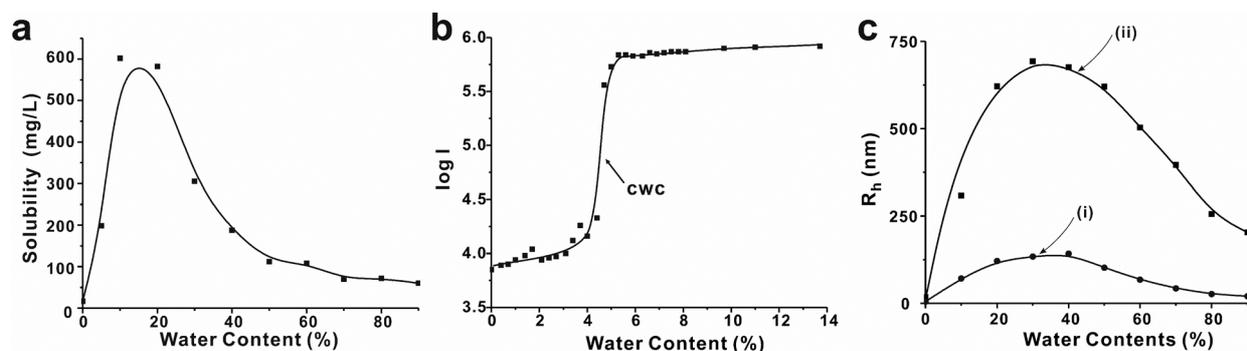


Figure 1. (a) Solubility change of SWNTs as a function of water content. (b) Intensity change of scattered light from PS₁₅₉-*b*-PAA₅₈ solution as a function of water content: Critical water content (cwc) which is defined as the minimum water content where micelle micelles form was measured at 4.5%. (c) Change of hydrodynamic radius of (i) PS₁₅₉-*b*-PAA₅₈ and (ii) SWNT@PS₁₅₉-*b*-PAA₅₈ as a function of water content.

Additionally it is also expected that micelle morphology affects the solubility of SWNTs with water content. It is known that the morphology of highly asymmetric PS-*b*-PAA block copolymer micelle varied with water content.^{33,34} Since vesicles and cylindrical micelles have much larger internal volume than spherical micelles, vesicles or cylindrical micelles can effectively increase the solubility of SWNTs at low water content. The measurements of hydrodynamic radius (R_h) with water content support these arguments. We attribute the large values of R_h at $wc = 10$ -60% to the formation of swollen cylindrical or vesicular micelles (Figure 1c). Similar to Figure 1a, R_h (both pure PS₁₅₉-*b*-PAA₅₈ micelles and PS₁₅₉-*b*-PAA₅₈/SWNT@PS₁₅₉-*b*-PAA₅₈) sharply increased at low water content, then dropped at higher water content giving a peak at $wc = 30\%$. R_h of pure PS₁₅₉-*b*-PAA₅₈ micelle was 20 nm at $wc = 90\%$, which is consistent with the radius of dry spherical micelle previously measured with AFM.²⁷ These data support that deswollen spherical micelles were formed at high water content ($wc = 90\%$). R_h of SWNT@PS₁₅₉-*b*-PAA₅₈ is much larger than that of pure PS₁₅₉-*b*-PAA₅₈, which is possibly because of the limitation of light scattering technique to 1D structure such as SWNT.

Partitioning behavior of hydrophobic molecules between SWNTs@PS-*b*-PAA and solvent has been monitored by using pyrene as a hydrophobic fluorescent probe. Pyrene was chosen because of its structural similarity to SWNT and unique fluorescent properties. The emission spectrum of pyrene shows some characteristic bands which are affected by the polarity of the surrounding environment. Especially, the relative intensity of the first (I_1) and third bands (I_3) in the pyrene emission spectrum can be used for monitoring the polarity of microenvironments.³⁵ When pyrene molecules are placed in more hydrophobic environment, the larger I_3/I_1 ratio is exhibited. Figure 2a shows the fluorescence spectra of pyrene solubilized with PS-*b*-PAA in DMF. When a certain amount of water ($wc = 88\%$) was added, the I_3/I_1 ratio of pyrene emission spectrum increased, which implies that pyrene molecules are located at more hydrophobic environment. The partitioning behavior of pyrene between

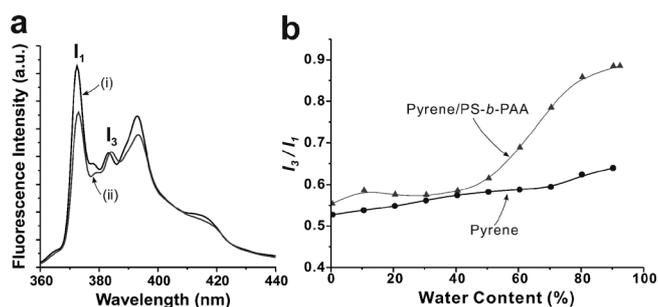
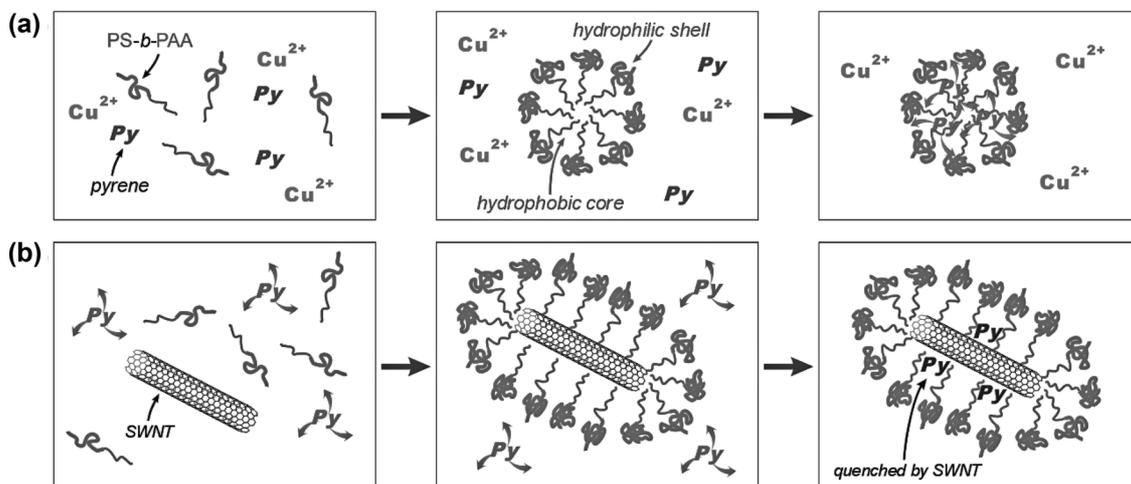


Figure 2. (a) The changes of the fluorescence spectrum for pyrene when different amount of water was added to the solution of PS-*b*-PAA in DMF. Emission spectrum was taken at (i) water content = 0% ($I_3/I_1 = 0.56$) and (ii) water content = 88% ($I_3/I_1 = 0.74$) respectively. (b) The change of the I_3/I_1 ratio of pyrene emission spectra as a function of water content ($[PS_{159}\text{-}b\text{-}PAA_{58}] = 10^{-4}$ g/mL, $[PS_{159}\text{-}b\text{-}PAA_{58}] = 0$ g/mL).

PS-*b*-PAA micelle core and H₂O/DMF solvent mixture can be monitored by observing the I_3/I_1 ratio as a function of water content.

The change of the I_3/I_1 ratio of pyrene emission was taken when PS-*b*-PAA micelles were formed by adding water (Figure 2b). The I_3/I_1 ratio was not significantly changed until 40% of water content, and gradually increased thereafter. These data represent that hydrophobic pyrene molecules start to be partitioned into micelle cores when the water content is higher than 40% although micelles are formed at much lower water content (4.5%). This was confirmed again by quenching experiments using copper ions (Figure 3). As shown in Scheme 1a, copper ions were added to the solution of containing pyrene/PS-*b*-PAA. Since copper ion is a good quencher for most fluorophores, the fluorescence of pyrene is quenched in the presence of copper ions.³⁶ Pyrene emits light only when pyrene molecules are partitioned within hydrophobic micellar core and isolated from copper ions present in the solution. Figure 3 shows that the fluorescent intensity of pyrene is effectively quenched at low water content (0-40%), and then sharply increase when water content is higher than 40%. These experiments



Scheme 1. Schematic illustration of pyrene quenching by (a) copper ions and (b) SWNTs.

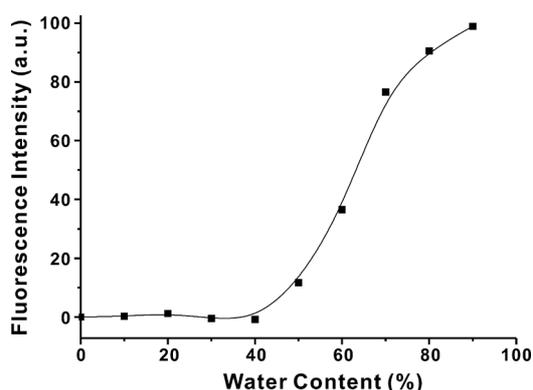


Figure 3. Change of the emission intensity of pyrene as function of water content in the presence of Cu^{2+} ions.

consist with the results shown in Figure 2b. It is interesting that partitioning of pyrene between micelle core and solvent is not in a simple linear relationship, but more likely shows discontinuous first order transition behavior.

Partitioning behavior of pyrene between SWNTs@PS-*b*-PAA and solvent has been investigated by fluorescence quenching technique which is similar to the previous one but SWNTs instead of copper ions were used as a quencher (Figure 4). Previous researches show that carbon nanotubes effectively quench the fluorescence of pyrene.³⁷ As shown in Scheme 1b, the fluorescence of pyrene can be quenched when pyrene molecules are localized into the micellar core of SWNTs@PS-*b*-PAA. The fluorescent readings of the suspension were taken at different water content. The suspension was left at room temperature without sonication for 2 hours prior to take the fluorescent readings. The effects of dilution and self absorbance by SWNTs were normalized. The fluorescence intensity of pyrene/SWNT/PS-*b*-PAA was slightly lower than that of pyrene/PS-*b*-PAA at $\text{wc} = 0\%$ (Figure 4a, c). However, they become equivalent as water content increased over the critical water content ($\text{cwc} = 5\%$) (Figure 4c). The lower fluorescence intensity at $\text{wc} = 0\%$ is attributed to the slight quenching effect by bare SWNTs which dynamically interact with pyrene molecules in solution. This quenching effect was disappeared when the water content was higher than the critical water content. Since SWNTs encapsulated within block copolymer micelles were

isolated from pyrene molecules in the solution, quenching process is effectively prohibited when water content is higher than the critical water content. The fluorescence intensity of pyrene/SWNT/PS-*b*-PAA sharply decreased when water content increased over 40%, while that of pyrene/PS-*b*-PAA increased with increase of water content. These results suggest that pyrene molecules were localized within micellar core and quenched by SWNTs as illustrated in Scheme 1b. The decrease of the fluorescence intensity in this regime was much larger than that at $\text{wc} = 0\%$. We attribute this to the fact that the fluorescence quenching is more effective when pyrene molecules are highly localized around SWNTs within formed micelles than when pyrene molecules are free to move in the solution. These observations are consistent with those previously described in Figure 2b and 3.

In summary, we have investigated partitioning behavior of SWNTs and hydrophobic pyrene molecules between PS-*b*-PAA micelles and DMF/water mixtures. Interestingly SWNTs and pyrene show non-linear partitioning behavior at different water content. SWNTs were effectively partitioned within micelle cores at low water content ($< 20\%$) because of swollen micelle core, but quickly precipitated out as micelle cores were deswollen with increasing water content. In contrast, pyrene showed first-order transition like behavior in partitioning. Pyrene molecules were exclusively partitioned in solvent mixture until $\text{wc} = 40\%$, and sharply got into micelle cores thereafter. The same behavior was observed for SWNT@PS-*b*-PAA.

Experimental Section

Materials and Instruments. PS₁₅₉-*b*-PAA₅₈ ($M_w = 22050$ g/mol, $M_w/M_n = 1.05$, $f_{\text{PS}} = 78.5\%$) which contains 159 styrene repeat units and 58 acrylic acid repeat units was purchased from Polymer Source Inc., Dorval, Canada. Single-walled carbon nanotubes (SWNTs) were purchased from Carbon Nanotechnologies, Houston, TX. All other chemicals were purchased from Sigma-Aldrich Chemical (USA). Fluorescence readings were taken on Quantmaster QM-2000-7 (PTI, Lawrenceville, NJ, USA) spectrofluorometer and conducted in 1.5 mL cuvettes at 25 °C.

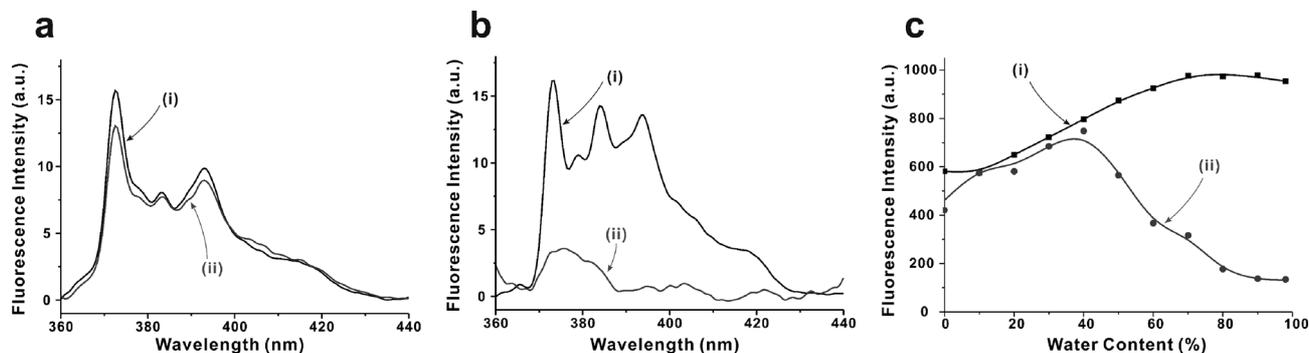


Figure 4. (a) Emission spectra of pyrene solubilized with (solid line) or without SWNTs (dashed line) at $\text{wc} = 30\%$. (b) Emission spectra of pyrene solubilized with (solid line) or without SWNTs (dashed line) at $\text{wc} = 90\%$. (c) Change of emission intensity of pyrene solubilized (i) without or (ii) with SWNTs as function of water content.

Formation of PS₁₅₉-*b*-PAA₅₈ Micelles in H₂O/DMF Mixture. PS-*b*-PAA block copolymer micelles were prepared by a method described elsewhere.³⁸ Briefly, stock solutions of PS₁₅₉-*b*-PAA₅₈ and pyrene were first dispersed in DMF. Since DMF is a good solvent for both PS and PAA blocks, no micelle was formed at this stage. The concentration was controlled to 10⁻³ g/mL for PS₁₅₉-*b*-PAA₅₈ and 10⁻⁶ M for pyrene. Micelles were induced by adding a certain amount of H₂O to the mixture. When H₂O, a selective solvent for PAA block was slowly added (~0.1 mL/min) over the critical water content (cwc), PS₁₅₉-*b*-PAA₅₈ were spontaneously assembled into micelle with hydrophobic PS core and hydrophilic corona. The resulting concentrations of polymer and pyrene are 10⁻⁴ g/mL and 10⁻⁷ M respectively and the water contents were varied from 0% to 90%. The emission spectra of the mixture were taken at different water contents.

Fluorescence Quenching by Cu²⁺. Copper ions were used as a quencher for pyrene fluorescence. Sample solutions with different water contents were first prepared as described above, and then aqueous copper chloride solution was added to the each sample solution. The amount of copper ion was chosen so as to give a copper ion concentration in the final solution of 10⁻⁵ M. The emission spectra of the mixture were taken at different water contents.

Encapsulation of SWNTs within Block Copolymer Micelles. SWNTs have been encapsulated within block copolymer micelles by the previously reported method.²⁷ Briefly, a small amount (< 0.1 mg) of SWNT soot was first suspended in 1 mL DMF in the presence of PS₁₅₉-*b*-PAA₅₈ (between 10⁻³ and 10⁻⁵ g/mL) and pyrene (10⁻⁶ M). This mixture was sonicated using a water-bath sonicator (Branson 1510 Ultrasonicator) at 50 °C for 30 min. Next, deionized water ($R = 18 \text{ M}\Omega/\text{cm}^2$) was gradually added at a uniform dropwise rate (~0.1 mL/min) until the solution consisted of the desired fraction of water while continuously sonicating the solution at 50 °C. The resulting concentrations of polymer and pyrene are 10⁻⁴ g/mL and 10⁻⁷ M respectively and the water contents were varied from 0% to 90%. Any nondispersed material was removed by filtering the suspension through cotton. The emission spectra of the mixture were taken at different water contents.

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