Zinc ion induced polymorphism in macromolecular self-assembly of diblock copolymers

Ming-Qiang Zhu, Alexander D.Q. Li *

Department of Chemistry, Washington State University, Pullman, WA 99164, USA

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Abstract

A novel interconnected cylindrical micellar network was prepared from a diblock copolymer, poly(maleic anhydride-*alt*-styrene)-*b*-polystyrene, in ethanol under a self-assembly directing agent: Zn^{2+} ions. The solution containing interconnected cylindrical network is bluish and transparent, which is stable for more than 6 months at room conditions without any observable macroscopic phase separation. In aqueous solution, however, hydrolysis of the anhydride yields hydrophilic carboxyl groups, which result in formation of uniform positive spherical micelles from the same diblock polymer. The nanostructures of both the spherical micelles and cylindrical assemblies are characterized with light scattering and transmission electron microscopy (TEM).
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1. Introduction

One of the great challenges in nanotechnology is to create interconnected nanostructures, preferably the constituent units are formed by functional macromolecular self-assembly. Block copolymers are at distinct advantages in this aspect because two disparate chemical and physical properties can be integrated into a single polymeric chain, yet they cannot undergo macroscopic phase separation due to covalent bonding between the two blocks. Alternatively, such block copolymers tend to self-organize into various nanostructures, such as spherical micelles, cylindrical tubes/rods, and lamella sheets [1–4]. For example, hydrophilic blocks usually aggregate together, forming occluded cores in hydrophobic media or organic solvents, whereas they will expand and interact with water molecules in aqueous solutions [5–7]. As a general rule, most block copolymers form positive micelles of various shapes and sizes in water with a hydrophobic core and a hydrophilic shell whereas they form inverse micelles in organic solvents. In some special cases, a block copolymer consisting of two hydrophobic blocks with different polarity can also form micelles in suitable organic solvents. Therefore, perfect balance of the interaction strengths between various polymeric blocks as well as between blocks and solvents, which mediate the formation of macromolecular assembly, will offer promising prospect for creation of organic supramolecular structures.

Indeed, miniaturized triblock polymers were found to self-organize into nanostructures with highly regular size and shape, such as supramolecular mushrooms [8,9]. Of particular interest is that such supramolecular mushrooms further self-stack into a layered structure with a polar arrangement and capable of second harmonic generation. Typically, amphiphilic block copolymers are rich in formation of microphase such as three-dimensional networks and worm-like rubber micelles, which can be subsequently crosslinked via a free radical polymerization to yield unusual viscoelastic properties [10–12].

Chemically distinct nanotubes were also prepared from triblock copolymers, bearing amino or carboxyl groups. An amidization reaction is used to join these nanotubes into multiple blocks of nanotubes [13]. Similarly, a nanotube can be connected to a nanosphere to generate dumbbell-like objects using similar coupling strategies of amidization [14]. As pointed out by Eisenberg et al. [15–17] that copolymers

* Corresponding author.
  E-mail address: dequan@wsu.edu (A.D.Q. Li).

mimic lipid amphiphilicity, but polymer molecular weights are orders of magnitude larger than those of lipids and hence structural features of vesicles and nanotubes are greatly influenced by the characteristics of the polymers. Future applications of copolymer nanostructured materials rely on exploitation of self-assembly characteristics and structure-property relationship. To this end, there has been limited exploitation of transition metal induced polymorphism of block copolymers in organic solvents as well as micellization of charged block copolymers in polar organic medium. Eisenberg et al. probed the effect of added metal ion on the aggregates in aqueous solution and found an unusual large compound vesicle [18–20]. In this paper, we will present our studies on zinc ion induced formation of nanostructures from diblock copolymer of poly(maleic anhydride-*alt*-styrene)-*b*-polystyrene in an organic solvent.

The hybrid supramolecular assemblies of polymer-transition metal ions are interesting because they can potentially serve as nanostructured precursors for preparation of metal sulfide or metal oxide materials as well as mesoporous materials. Theoretically, metal ion mediated self-assemblies of amphiphilic block copolymers have not been extensively investigated and offer a new playground for chemists to explore new nanostructured materials.

2. Experimental

2.1. Reagents

All reagents and solvents were purchased from Aldrich Chemical Co. and used as received unless stated otherwise. Maleic anhydride (MAn, 99%) and 2,2′-azobisisobutyronitrile (AIBN, 99%) were recrystallized from chloroform and methanol, respectively. Styrene (St, 99%) was distilled from CaH_2 under reduced pressure. *S*-benzyl dithiobenzoate (BDTB) was synthesized according to the literature procedure [21].

2.2. Polymerization

The block copolymer was synthesized via radical reversible addition-fragmentation chain transfer copolymerization (RAFT) according to the Ref. [22]. In a typical run, MAn, St, *S*-benzyl dithiobenzoate (BDTB), and AIBN ([MAn]:[St]:[BTBA]:[AIBN] = 100:900:2:1 in molar ratio, [MAn] = 0.2 mol/L) were charged in a polymerization tube. After the mixture was degassed three times, the tube was sealed under vacuum, and heated in an oil bath at 60 °C for 24 h and then at 80 °C for 48 h. The tube was broken and THF was added to halt the polymerization. The polymer was precipitated by pouring the THF solution into large amount of dry methanol (15 times excess). This precipitation procedure was repeated three times. The powdery polymer with light pink color was dried for 3 days under vacuum at 40 °C. The copolymer molecular weight and molecular weight

distribution were determined by gel permeation chromatography (GPC) using THF as a solvent and the molecular weight was calibrated against polystyrene (PS) standards. The block copolymer composition was calculated from the ratio of aromatic and alkyl resonances in ^{1}H NMR. According to the GPC results, the copolymer molecular weight and molecular weight distribution are 18,000 and 1.34, respectively. The copolymer composition is deduced from the combined analysis of GPC and ^{1}H NMR results and the diblock copolymer contains 50 units of MAn-*alt*-St and 80 units of St.

2.3. Characterization

Molecular weight and molecular weight distribution of polymer were determined by GPC, Waters 150CV, equipped with an RI detector using THF as an eluent at 25 °C. ^{1}H NMR spectra were recorded with a JEOL JNM-AL300 apparatus and the solvent is $CDCl_3$. The dynamic light scattering (DLS) measurements were carried out on a Beckman–Coulter instrument N4 plus using standard PS spheres as calibration standards. He–Ne laser (632.8 nm) was used as an excitation source and the scattering angle is fixed at 90°. The particle size distribution and average particle size were obtained from the autocorrelation function by CONTIN analysis using standard software package supplied by Beckman–Coulter. A JEOL 1010 transmission electron microscope (TEM) operated at 100 kV was employed to obtain TEM images. TEM samples were prepared by dipping a holey carbon film supported by Cu grids into a polymer solution followed by solvent evaporation at room temperature. For aqueous samples, negative staining by uranyl acetate was necessary for high contrast in TEM images.

2.4. Self-assembly of diblock copolymer

(a) The typical procedure for micelle preparation is described below: the block copolymer was dissolved in THF (5 mg/mL, 10 mL), followed by a slow dropwise addition of 25 mL acetonitrile. When about half of the total volume was added, the blue tint appeared. After the addition was complete, the solution became clearly bluish. The solution was allowed to stand overnight and THF was eliminated in vacuum. The solution was adjusted to a final concentration of 2 mg/mL. The copolymer micellar solution (dilute 100×, 2.0×10^{-2} mg/mL) was dropped onto a holey carbon film on a Cu grid and TEM images were obtained without any staining.

(b) The diblock copolymer was dissolved in THF (5 mg/mL, 10 mL), followed by a slow dropwise addition of NaOH aqueous solution (1 mM, 10 mL). When about half of the total volume is added, the blue tint appeared. After the addition is complete, THF was removed by dialysis. The concentration and pH value of the solution was then adjusted to 5 mg/mL and 9.0, respectively. The copolymer micellar solution (dilute 100×, 5.0×10^{-2} mg/mL) was dropped onto a holey carbon film on a Cu grid

followed by uranyl acetate staining before TEM imaging.

(c) The copolymer was firstly dissolved in THF (5.0 mg/mL, 10 mL). To it, a solution containing 100 mL zinc acetate in ethanol (1 mM) was added dropwise and the solution was stirred overnight at room temperature. Finally, THF was removed by distillation in vacuum. The concentration of final solution was adjusted to 0.5 mg/mL. The bluish solution is stable for more than 6 months at room temperature without any precipitation. The copolymer solution was dilute 100× for DLS measurement. The copolymer micellar solution (dilute 100×, 5.0×10^{-3} mg/mL) was dropped onto a holey carbon film on a Cu grid and TEM images were obtained without any staining.

3. Results and discussion

3.1. Formation of micelles in acetonitrile

Radical reversible addition-fragmentation chain transfer copolymerization (RAFT) was used to synthesize poly(maleic anhydride-*alt*-styrene)-*b*-polystyrene (PMS-*b*-PS) (Scheme 1). This block copolymer does not dissolve in acetonitrile, ethanol or water, but it does dissolve in THF. Water and ethanol are poor solvents for both PMS and PS blocks; however, acetonitrile is a good solvent for PMS blocks but a poor solvent for PS blocks. Therefore, micelles can be formed by dropwise addition of acetonitrile into a THF solution containing PMS-*b*-PS. Usually positive micelles from amphiphilic molecules, such as surfactants and block copolymers, are formed in aqueous solutions where the interactions between hydrophilic groups and

water molecules are optimized. In the system of PMS-*b*-PS in THF–acetonitrile, however, we also found positive micelles with a hydrophobic core and a hydrophobic shell. The hydrodynamic diameter of PMS-*b*-PS block copolymer micelles in acetonitrile measured by dynamic light scattering (DLS) was $\sim$32 ± 13 nm with polydispersity of 0.21. This indicates that PMS-*b*-PS block copolymer self-assembled into uniform spherical nanostructure with a narrow polydispersity. Transmission electron microscopy (TEM) image of PMS-*b*-PS block copolymer deposited from dilute acetonitrile solution was shown in Fig. 1a. The polymer micelles exhibit spherical shape with a mean diameter of about 25 nm. In comparison, the mean diameter of the micelles from DLS measurement is larger than the value determined by TEM, which indicates that micelles in solutions are swollen in acetonitrile.

3.2. Formation of spherical micelles in water

Hydrolysis of maleic anhydride affords two carboxyl groups, which render the PMS block water soluble. Because of this hydrophilicity, hydrolyzed PMS-*b*-PS block copolymer also form positive micelles in water. Compared to the micellization formation of charge neutral block copolymers, it is more difficult to study copolymers consisting of a hydrophobic block and a charged block because of their drastic difference in solubility. In many cases, direct dissolution of hydrophobic-charged block copolymers is difficult [16]. Our approach to prepare positive micelles from hydrophobic-charged block copolymers is to dissolve its neutral block copolymer precursor in a polar organic solvent, such as THF, DMF or dioxane followed by the addition of water to induce formation of charges during the micellization process. The organic solvent is then removed by dialysis. During
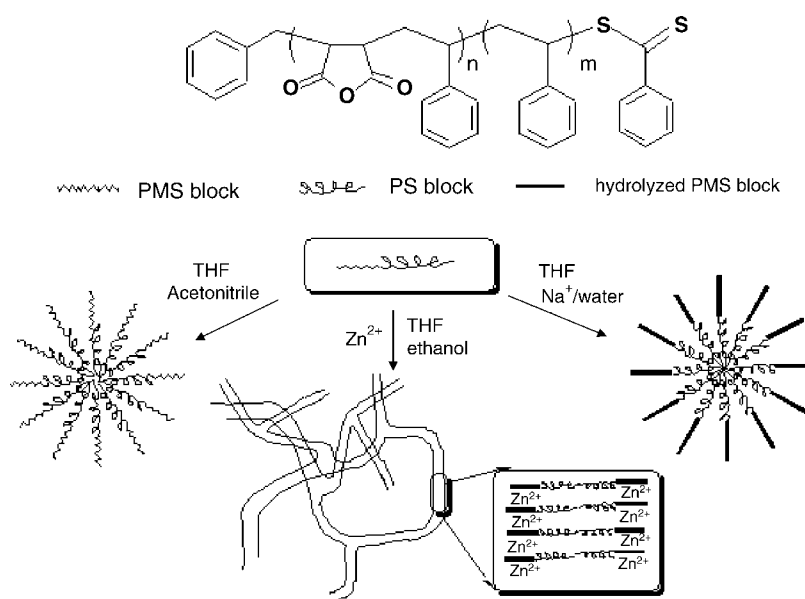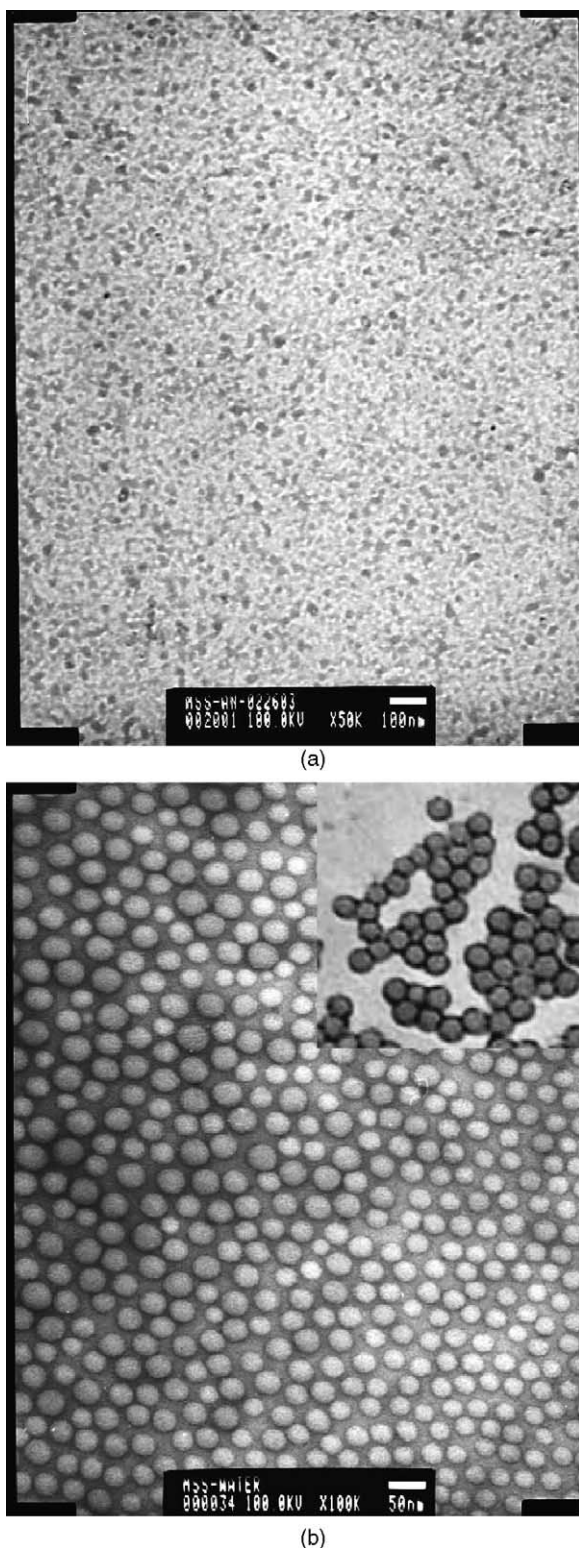

Scheme 1.

Fig. 1. Representative transmission electron micrographs of: (a) spherical micelles of PMS-*b*-PS formed in acetonitrile, and (b) their hydrolyzed product in water (pH 9). Inset: hydrophobic cores surrounded by hydrophilic shells (stained heavier) are clearly visible upon staining the micelles with uranyl acetate.

the dialysis process polystyrene cores become denser due to increased hydrophobic interaction and PS-water immiscibility as the organic molecules are removed and aqueous solution is established. The glass transition temperature (T_g) of PS is ∼100 °C, much higher than room temperature; therefore the newly formed PS core is very stable because it is in the glassy state of the polymer. Under these conditions, the micellar cores are considered frozen and can only be disrupted by introduction of good solvents for polystyrene or heating above the glass transition temperature. These nanostructures are robust enough and warrant experimental investigations by electron microscopy in the dry phase [16,20].

Based on the above analyses, we have prepared positive micelles from diblock copolymer PMS-*b*-PS by first dissolution of the polymer in THF, followed by hydrolysis-induced micellization with continuous addition of saline water. The micelles formed in water at pH 9 are shown in Fig. 1b; TEM images indicate that the polymer micelles exhibit well-defined spherical shape with a mean diameter of ∼30 nm. Furthermore, most of the micelles arrange in a regular hexagonal periodical array with a periodicity of ∼40 nm because of the electrostatic repulsive interaction between surface-charged micelles (Fig. 1b). In wide field of views, thousands of uniform spherical micelles were observed and in areas with high density of micelle arrays, the spheres are densely packed.

Negative staining by uranium acetate reveals the hydrophilic regions of the nanospheres, thus the core-shell structure of PMS-*b*-PS block copolymer micelles was observed in the properly stained area (inset of Fig. 1b). The charged block of copolymer in water is driven into an extended structure by the electronstatic repulsion between the neighbor anions within a single charged chain (intrachain repulsion). Similarly, charged blocks repeal each other, thus forming a maximally swollen structure (interchain repulsion) hold together only by the hydrophobic PS core. The second role of the interchain repulsion is that it stabilizes the polymeric colloidal solution. DLS results confirm that the hydrodynamic diameter of charged block copolymer micelles in aqueous solutions indeed increases to 99 ± 31 nm with the polydispersity index of 0.17; the hydrodynamic diameter is much larger than that of neutral micelles from the same block copolymer in acetonitrile prior hydrolysis.

3.3. Cylindrical network

Through trial and error, we found that cylindrical network micelles can be prepared in ethanolic medium using Zn^{2+} as a structural directing agent. First, the PMS-*b*-PS diblock copolymer was dissolved in THF. Second, ethanolic zinc acetate solution containing trace amount of water was slowly added to the PMS-*b*-PS copolymer in THF. Third, as THF was removed under reduced pressure, the hydrophobic PS cores begin to nuclear while PMS shells are hydrolyzed into hydrophilic blocks mediated by trace amount of water presence in ethanol. DLS measurements suggest that over

a three order of magnitude of concentration region, no spherical micelles as discussed previously were found, or we can conclude that the new nanostructure is not spherical. This result indicates that the nanostructure formed is quite stable and it does not undergo phase transition such as cylindrical micelles to spherical micelles as diblock polymer concentration is diluted, which is common for amphiphilic surfactants. Moreover, the new nanostructure exhibits great miscibility towards ethanol even though the aggregate size may well exceed traditional colloid region. TEM results show formation of a cylindrical and interconnected network structure in ethanolic medium even though the polymer concentration was as low as 1.0×10^{-5} mg/mL (Fig. 2). The cross-section average diameter of the cylindrical polymer micelles is ~20–30 nm and the interconnected nanostructures have largely well-defined shape and structure of cylindrical copolymer micelles. In consistent with DLS results, no spherical structures were found with TEM even at extremely low concentration of 1.0×10^{-5} mg/mL, which typically promotes the equilibrium to shift from cylindrical to spherical micelles. At a higher concentration (5.0×10^{-3} mg/mL), TEM images show that the block copolymers form high-density fibrous structures. It is generally accepted that equilibrium exists and mutual transformation between different micellar structures occurs regularly in aqueous media when the polymer concentration changes, such as spheres to cylinders, cylinders to spheres, even cylinders to network structure [7,11,16]. In the presence of Zn^{2+} ions, however, PMS-*b*-PS forms solely interconnected network; this result suggests that transition metal ions can play an key role, such as a carboxyl–Zn^{2+}–carboxyl bridge, in the self-assembly of charged diblock copolymer and therefore the metal ion effect on macromolecular assembly should be considered in future theoretical modeling.

In order to gain some insights about the formation mechanism of interconnected cylindrical micelles, ethanol was replaced by water. The micelle was prepared through hydrolysis of the copolymer in THF in the presence of an aqueous zinc acetate solution. The result shows that both interconnected cylindrical network and spherical micelles coexist at pH 7 aqueous solutions (Fig. 3a), but micelles formed under these conditions are not very uniform.

If Zn^{2+} ions are replaced with Na^+ ions, micelles formed in ethanol are mostly spherical with a slight a tendency of aggregations (Fig. 3b). These results indicate that both divalent Zn^{2+} and ethanol are important in the formation of interconnected cylindrical micelles. Another similar divalent cation Cd^{2+} also induces the formation of interconnected cylindrical structure as demonstrated by transmission electron microscopy, which further strengthens the interchain bridge role for the divalent cations.

Such morphology changes of the copolymers can be potentially used to monitor the presence of divalent or monovalent metal ions such as Zn^{2+} and Na^+. Therefore, metal ion sensors based on structural changes can be envisioned using functional block copolymers.
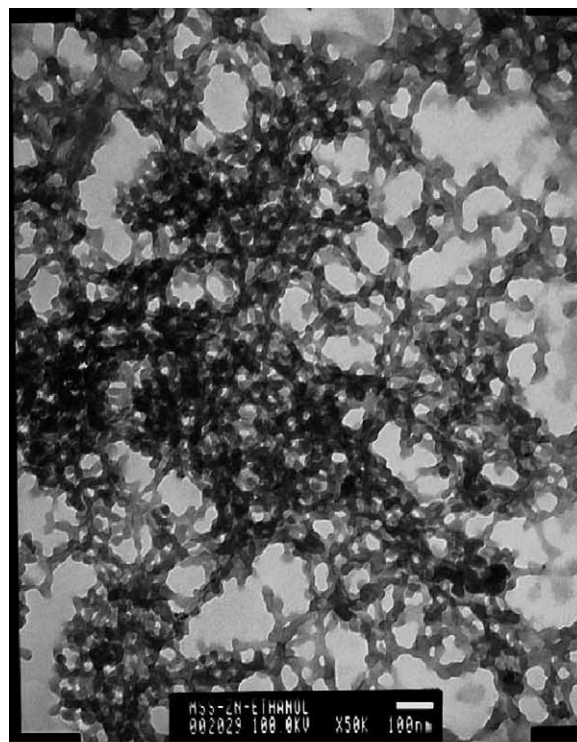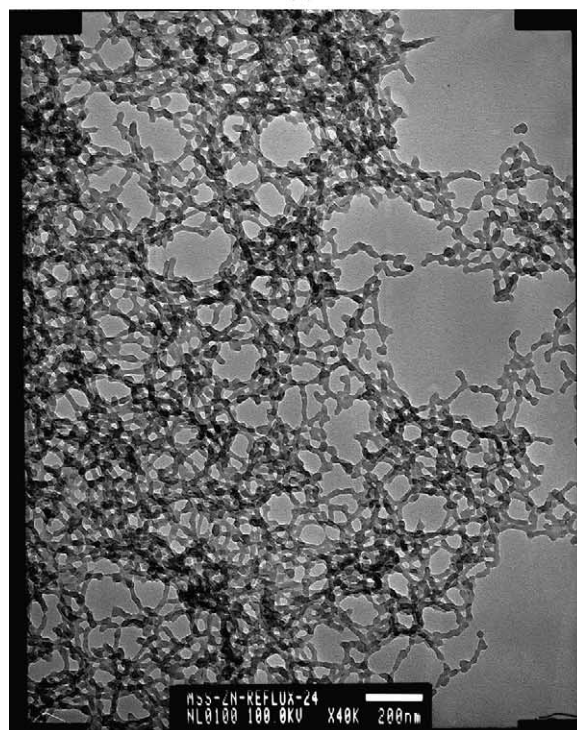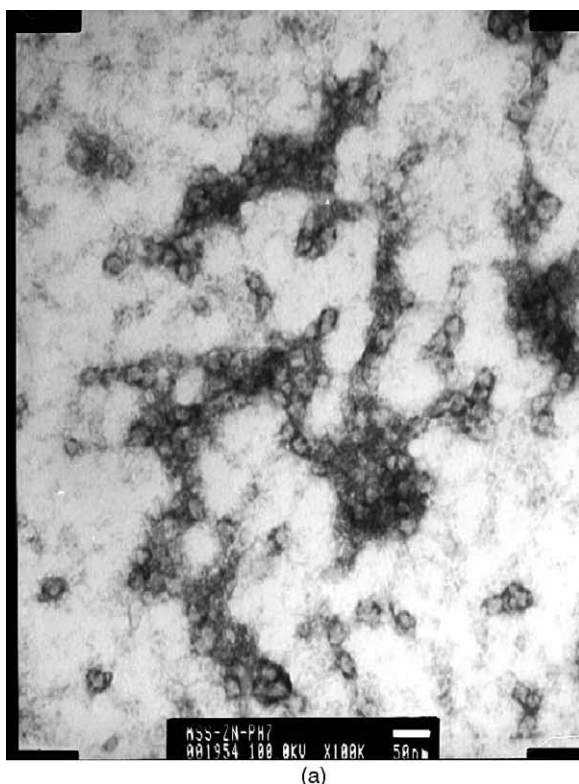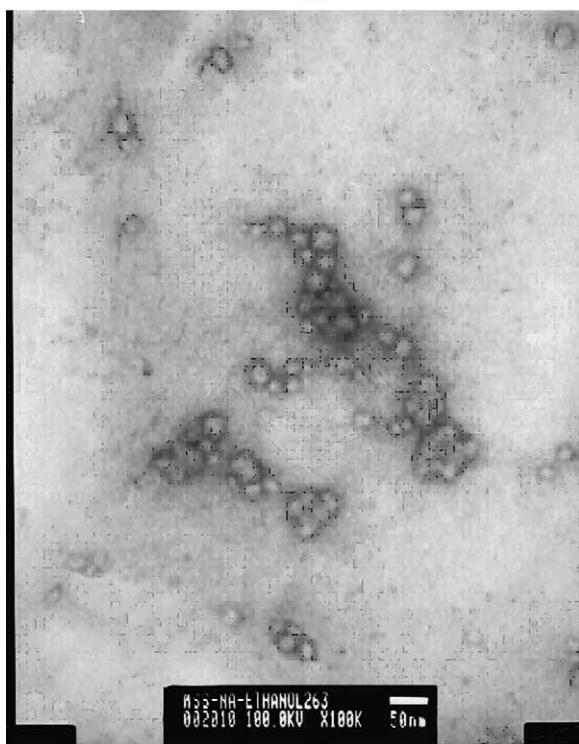

Fig. 2. Representative transmission electron micrograghs of Zn^{2+} ion-induced interconnected cylindrical micelles from PMS-*b*-PS block copolymer in ethanol. The Zn^{2+} copolymer system was allowed to: (a) stand at room temperature for 2 days, and (b) reflux for 24 h in ethanol.

Fig. 3. Representative transmission electron micrograghs of a mixture of spherical micelles and induced interconnected cylindrical micelles from PMS-*b*-PS block copolymer in water or ethanol under the conditions of: (a) Zn(OAc)$_2$ in water (pH 7–8), and (b) NaOAc in ethanol.

Another analytical application of nanostructured block copolymers is using them as a fluorescent tag for biological detection and measurements. Fluorescent dyes can be incorporated into the side chains of the copolymers. As a result, fluorescence microscopy can be employed to determine the binding of polymeric tags to biomolecules. Since many fluorescent chromophores can be grafted onto the copolymers and micelle structures contain many polymer chains, therefore the sensitivity of polymer micelle fluorescence detection is expected to be high.

4. Conclusion

In conclusion, a novel interconnected cylindrical micellar network was prepared from diblock copolymer PMS-*b*-PS in ethanol under the structural directing agent: Zn^{2+} ions. The bluish transparent solution containing interconnected cylindrical micellar network is rather stable and can be stored up to 6 months without any macroscopic phase separation. In addition, uniform positive spherical micelles were also prepared from the same copolymer in water through hydrolysis-induced self-assembly.

Acknowledgements

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