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Temperature-responsive multilayered micelles formed from the complexation of PNIPAM-*b*-P4VP block-copolymer and PS-*b*-PAA core–shell micelles

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ABSTRACT

Multilayered micelles with a polystyrene (PS) core, a swollen poly(acrylic acid) (PAA)/poly(4-vinyl pyridine) (P4VP) complex shell and a poly(4-vinyl pyridine)-*block*-poly(isopropyl acryl amide) (P4VP-*b*-PNIPAM) block-copolymer corona was synthesized by complexation between $PNIPAM_{53}$-*b*-$P4VP_{109}$ block-copolymers and the PS_{120}-*b*-PAA_{47} diblock-copolymer core–shell micelles in ethanol due to the hydrogen bonding between the AA units and 4VP units. The surface of the micelle has been modified and a temperature sensitive block PNIPAM was introduced into the corona of the micelles. After being dialyzed against acidic water, PNIPAM corona would collapse onto the PAA/P4VP shell and the excessive P4VP shell would extend into the acidic solution to form the corona reversed micelles when the micelle aqueous solution was heated to 45 °C. The whole process was performed using dynamic light scattering (DLS), static light scattering (SLS), atom force microscope (AFM) and nuclear magnetic resonance (NMR).

1. Introduction

Self-assembling of block-copolymers has been widely studied in the past decades [1–4]. Besides traditional spherical crew-cut or star micelles, much more novel morphologies of the polymeric micelles have been prepared or synthesized in order to meet the application of the micelles [5–7]. It is well-known that the structure and the morphology of the core and the shell of the micelles have great effects on the properties and the applications of the micelles [8]. The change in the structure and the composition of the core or the shell will bring new properties and further broaden the applications of the polymeric micelles [9,10]. Recently, polymer scientists have taken much effort on the modification of the micelles shell. Chen et al. have prepared the core-stabilized polymeric micelles with a mixed shell formed by two incompatible polymers using a one-step approach by cross-linking the poly(2-vinyl pyridine) (P2VP) blocks of poly(styrene)-*block*-poly(2-vinyl pyridine) (PS-*b*-P2VP) and poly(ethylene oxide)-*block*-poly(2-vinylpyridine) (PEO-*b*-P2VP) in their common solvent, DMF [11]. Micelles with a janus shell were synthesized by cross-linking the B block of A–B–C tri-block copolymer in bulk [12], self-assembling of star copolymer A_nB_n [13] and cross-linking the polybutadiene (PB) block of PS-*b*-PB-*b*-PMMA tri-block copolymer [14]. A type of complex micelles with tunable channels were formed through the self-assembly of a binary mixture of poly(*tert*-butylacrylate)-*block*-poly(isopropyl acrylamide) ($PtBA_{45}$-*b*-$PNIPAM_{91}$) and poly(*tert*-butylacrylate)-*block*-(4-vinyl pyridine) ($PtBA_{60}$-*b*-$P4VP_{80}$) diblock copolymers upon increasing the temperature or pH value of the solution [15]. Similarly, micelles with mixed poly(glyceryl methacrylate)/succinated poly(glyceryl methacrylate) (PGMA/PSGMA) shell formed when cross-linked poly(2-cinnamoyloxyethyl methacrylate) (PCEMA) block in PECMA-*b*-PSGMA and PECMA-*b*-PGMA block-copolymers in their solution mixture. The polymer blocks of PGMA and PSGMA are incompatible and it would drive the segregation of one type of chains from chains of the other to form nanospheres or microspheres with segregated surface chains [16]. Looking for a new method to modify the surface of the block copolymer micelles is still valuable for the application.

Core–shell–corona micelles were synthesized by the self-assembly of A–B–C tri-block copolymer in selective solvent and thus act as good vehicles for controlled drug release [17–21]. The most direct method to prepare core–shell–corona micelles is the self-assembling of tri-block copolymer in selective solvent [19,22]. Up to now, it has not been easy to synthesize tri-block copolymer, especially to synthesize the copolymer with different properties. Herein, another method of the co-precipitation of a core–shell micelle of AB block copolymer and a linear BC block copolymer was employed by Kabanov et al. to synthesize the multilayer micelles [23,24]. In this process, linear BC block would also precipitate to form BC core–shell micelles. The core–shell–corona micelles would be prepared much more easily by the adsorption or the aggregation of block-copolymer chains onto the core–shell micelles. Recently, we have prepared three-layered core–shell–corona micelles by the aggregation of PEO-*b*-P4VP block-

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copolymer onto the PS-*b*-PAA block-copolymer micelles in ethanol [25,26].

In this paper, we extended our study to prepare multilayered micelles with temperature sensitive and pH sensitive polymer blocks. PS_{120}-*b*-PAA_{47} core–shell micelles was first prepared in ethanol, and then the aggregation of $PNIPAM_{53}$-*b*-$P4VP_{109}$ block-copolymer onto the PS_{120}-*b*-PAA_{47} micelles occurred when $PNIPAM_{53}$-*b*-$P4VP_{109}$ block-copolymer ethanol solution was added into the PS_{120}-*b*-PAA_{47} micelles ethanol solution due to the hydrogen bonding between the AA units and 4VP units, resulted in the formation of core–shell–corona three-layered micelles. In this process, the surface of the micelle has been modified and a temperature sensitive block, PNIPAM, was introduced onto the surface of the micelles. After being dialyzed against acidic water, the modified temperature sensitive PNIPAM corona would shrink and collapse onto the PAA/P4VP complex core and the excessive P4VP shell would extend into the acidic solution when the micelle aqueous solution was heated to 45 °C. The resulted corona reversed micelles have a compact PS core, a swelling PAA/P4VP complex inner shell, a shrunk PNIPAM outer shell and an excessive P4VP corona. The PAA/P4VP complex layer would disengage when the pH value of the solution is lower than 2.0 or higher than 12.0, and the excessive P4VP layer would also shrink when the pH value of the solution is increased, and accordingly, the micelles have great potential applications in drug delivery, carrying catalyst, template and so on.

2. Experimental section

2.1. Materials

Amphiphilic block copolymer of polystyrene-*block*-poly(acrylic acid) (PS_{120}-*b*-PAA_{47}) was prepared from hydrolysis of polystyrene-*block*-poly(*tert*-butylacrylate) (PS_{120}-*b*-$PtBA_{47}$) in NaOH aqueous solution. PS_{120}-*b*-$PtBA_{47}$ was synthesized by atom-transfer radical polymerization (ATRP) using PS–Cl as macro-initiator [27]. The number average molecular weight $\overline{M}_n$ and the polydispersity index (PDI) of the PS–Cl macro-initiator measured by gel permeation chromatography (GPC) are 1.40×10^4 g/mol and 1.20, respectively. The $\overline{M}_n$ and PDI of PS-*b*-PtBA block-copolymer are 2.0×10^4 g/mol and 1.36. The hydrolysis of PS_{120}-*b*-$PtBA_{47}$ in NaOH aqueous solution can be seen elsewhere [28]. Poly(*N*-isopropylacrylamide)-*block*-poly(4-vinylpyridine) ($PNIPAM_{53}$-*b*-$P4VP_{109}$) was synthesized by ATRP using PNIPAM–Cl as macro-initiator and the detailed process can be found in Ref. [7]. The $\overline{M}_n$ and the PDI of the PNIPAM–Cl measured by GPC are 6×10^3 g/mol and 1.13, respectively. The $\overline{M}_n$ of the P4VP block in PNIPAM-*b*-P4VP block-copolymer measured by the ^{1}H NMR was 8.4×10^3 g/mol. The other reagents were of analysis grade and used without further treatment.

2.2. Preparation of the PS_{120}-b-PAA_{47} micelles

The process of preparing PS_{120}-*b*-PAA_{47} micelles was the same as that described in the Ref. [28] except that the concentration of the micelles was 0.05 mg/mL.

2.3. Preparation of the multilayered micelles

$PNIPAM_{53}$-*b*-$P4VP_{109}$ was first dissolved in ethanol to make the solution with a concentration of 0.15 mg/mL. Then $PNIPAM_{53}$-*b*-$P4VP_{109}$ ethanol solution was slowly added to an equal volume of the micelle solution of PS_{120}-*b*-PAA_{47} micelles. The mixed solution was characterized by SLS and DLS before being kept for 3 days at room temperature to ensure full complexation between P4VP blocks and PS-*b*-PAA micelles. It should be mentioned that the DMF concentration in all complexes is 5 vol%.

2.4. Preparation of the corona reversed micelles

Core–shell–corona three-layered micelles ethanol solution was first diluted with equal volume of acidic water (which has a pH value of 4.0) and then dialyzed against acidic water for more than 7 days to remove the ethanol and DMF. The aqueous solution was heated to 45 °C and kept for more than 2 h before characterization.

2.5. Characterizing of the micelles

In this study, dynamic light scattering (DLS) and static light scattering (SLS) experiments were performed on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 514 nm. All the samples were treated by centrifugal machine at a rotation speed of 3500 r/min for 25 min to remove the dust in the solution before light scattering measurements. The ^{1}H NMR spectra of the polymers in $CDCl_3$ and D_2O were recorded on a NITYplus 400 MHz for protons. Molecular weights and polydispersities were characterized by a Waters 600E GPC analysis system, where $CHCl_3$ and tetrahydrofuran (THF) were used as eluents and polystyrene as the calibration standard. All AFM was performed on a Digital Instruments Nanoscope multimode atomic force microscope (Veeco Metrology Group) in tapping mode.

3. Results and discussion

3.1. Characterizing of PS_{120}-b-PAA_{47} core–shell micelles

As is known to all of us, the PS-*b*-PAA block-copolymer can form core–shell micelles with a PS core and a PAA shell in ethanol because ethanol is a precipitator for PS block and a good solvent for PAA block [25–27]. Here, the spherical micelles formed when ethanol was slowly added into the PS_{120}-*b*-PAA_{47} DMF solution. The micelles ethanol solution is characterized by dynamic light scattering and the hydrodynamic diameters and the distribution of the spherical micelles are shown in Fig. 1a. The hydrodynamic diameters of the micelles are about 120 nm. The inset of Fig. 2 is the concentration dependence of the PS-*b*-PAA micelles in the mixture of ethanol and DMF. When the scattering angle changed from 45 to 135°, the hydrodynamic diameters changed from 126 to 117 nm and it revealed that the dimension of PS-*b*-PAA micelles have no angle dependence.

Static light scattering was used to measure the gyration radius of the micelles. From the fit line of Berry plot in Fig. 2, the gyration radius of the micelles can be calculated. The gyration radius of the PS_{120}-*b*-PAA_{47} micelles is about 65 nm. The value of R_g/R_h (the ratio of gyration radius to hydro dynamic radius) can be calculated based on the R_g and R_h values measured above. The value is 1.08, and it is well in accordance with that of the core–shell micelles with an incompact core. Atom force microscope (AFM) was also employed here to study the morphology of the PS_{120}-*b*-PAA_{47} micelles. As shown in Fig. 3A, spherical micelles with a diameter of about 90 nm formed and the diameters of the micelles are almost uniform. It is reasonable that the diameter measured by AFM is smaller than the light scattering. The collapse of the incompact core and the shell in the process of AFM sample preparation, leads to the shrink of both the core and the shell.

3.2. Complexation of $PNIPAM_{53}$-b-$P4VP_{109}$ block-copolymers with PS_{120}-b-PAA_{47} micelles in ethanol

Polymer chains or block-copolymers can form complex micelles or aggregates through hydrogen bonding or electrostatic affinity in solution [5,29–31]. In ethanol, $PNIPAM_{53}$-*b*-$P4VP_{109}$ chains would aggregate onto the shell of the PS_{120}-*b*-PAA_{47} micelles to form core–

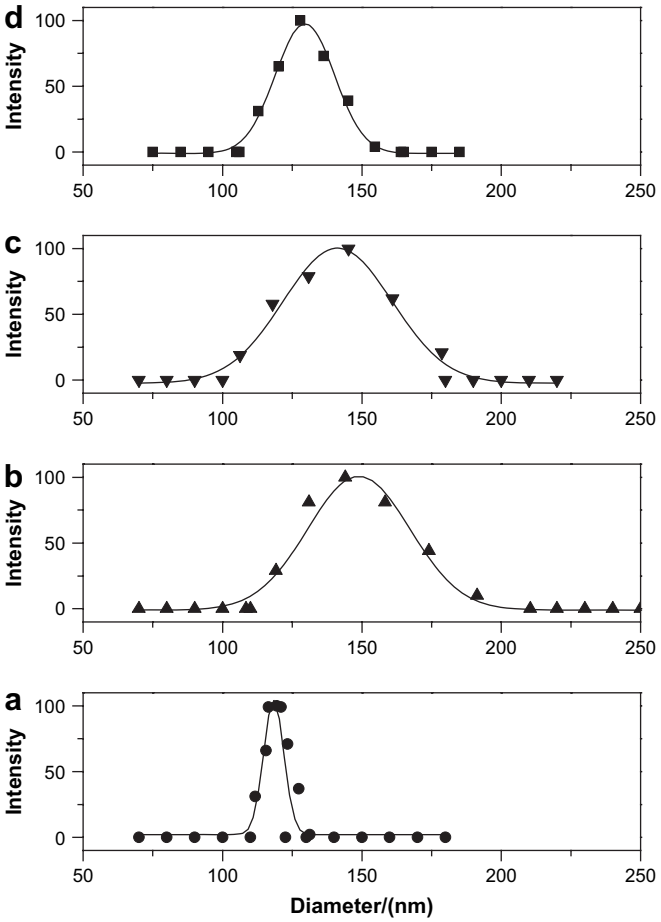


Fig. 1. Hydrodynamic diameters and distributions of micelles solutions. (a) PS_{120}-*b*-PAA_{47} micelles in ethanol; (b) PS-*b*-PAA/P4VP-*b*-PNIPAM complex micelles in ethanol; (c) PS-*b*-PAA/P4VP-*b*-PNIPAM complex micelles in acidic aqueous solution at 25 °C; (d) PS-*b*-PAA/P4VP-*b*-PNIPAM complex micelles in acidic aqueous solution at 45 °C.

shell–corona spherical micelles when added into the PS_{120}-*b*-PAA_{47} micelles solution due to the hydrogen bonding between PAA blocks and P4VP blocks. Fig. 1b shows the hydrodynamic diameters and the distribution of the complex core–shell–corona micelles. The diameter is about 140 nm, a little larger than the PS-*b*-PAA core–shell

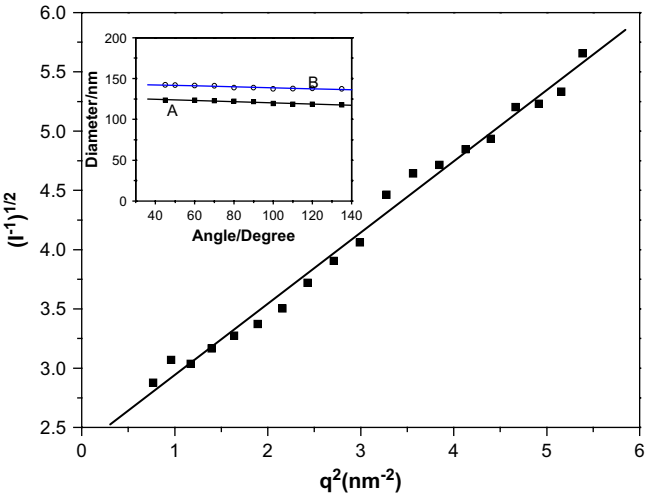


Fig. 2. Berry plot of PS_{120}-*b*-PAA_{47} micelles solutions in ethanol and the angle dependency of the diameters of the aggregates: (A) PS-*b*-PAA micelles in the mixture of ethanol and DMF; (B) the complex micelles in acidic water.

micelles and it has no angle dependence (as shown in the inset of Fig. 2). Commonly, the diameters of the complex micelles could be affected by two factors: one is that the newly added polymer would extend the chain length and it would lead to the increase of the diameters; and the other is that the complexation would lead to the shrinkage of the polymer chain and it would lead to the decrease of the diameters. The diameters of the micelles decreased or kept unchanged when the complex micelles formed in our former paper [25,27]. Here, the length of P4VP block is much larger than the PAA block, and the P4VP chain is excessive. So only a part of 4VP segments of the P4VP block could form the complexation and collapse onto the PS core in solution. The excessive disengaged 4VP segments and the well dissolved PNIPAM block would extend into the solvent, leading to the increasing of the diameter of the complex micelles.

The gyration radius of the core–shell–corona complex micelles was calculated from the Berry plot, and it is about 72 nm. The R_g/R_h value is also about 1.0, which is the same as that of the PS_{120}-*b*-PAA_{47} core–shell micelles. AFM image of the core–shell–corona complex micelles is shown in Fig. 3B. Spherical micelles with a diameter around 120 nm formed.

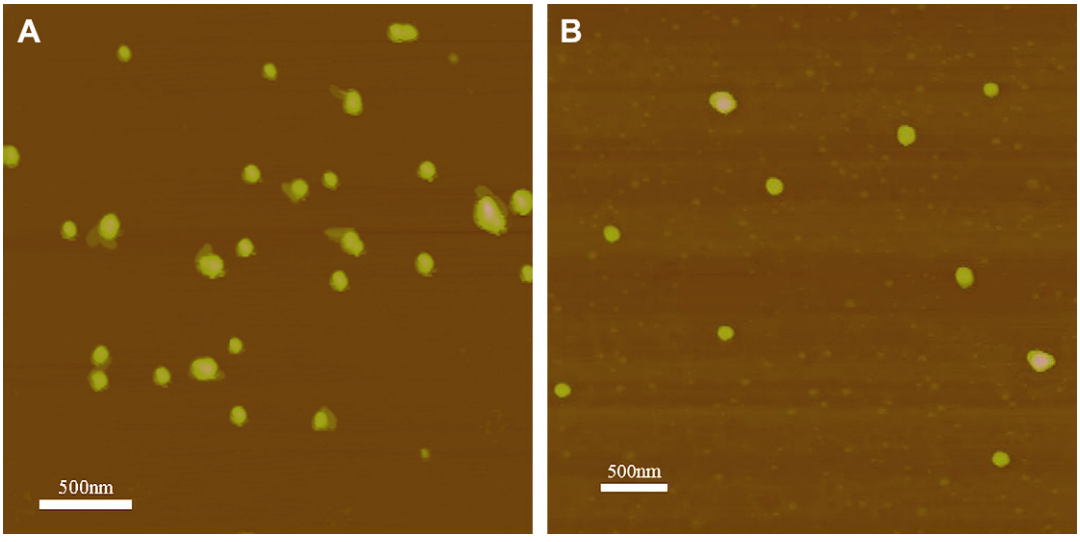


Fig. 3. AFM image of PS_{120}-*b*-PAA_{47} block-copolymer micelles in the mixture of ethanol and DMF (A), and PS-*b*-PAA/PNIPAM-*b*-P4VP complex micelles (B).

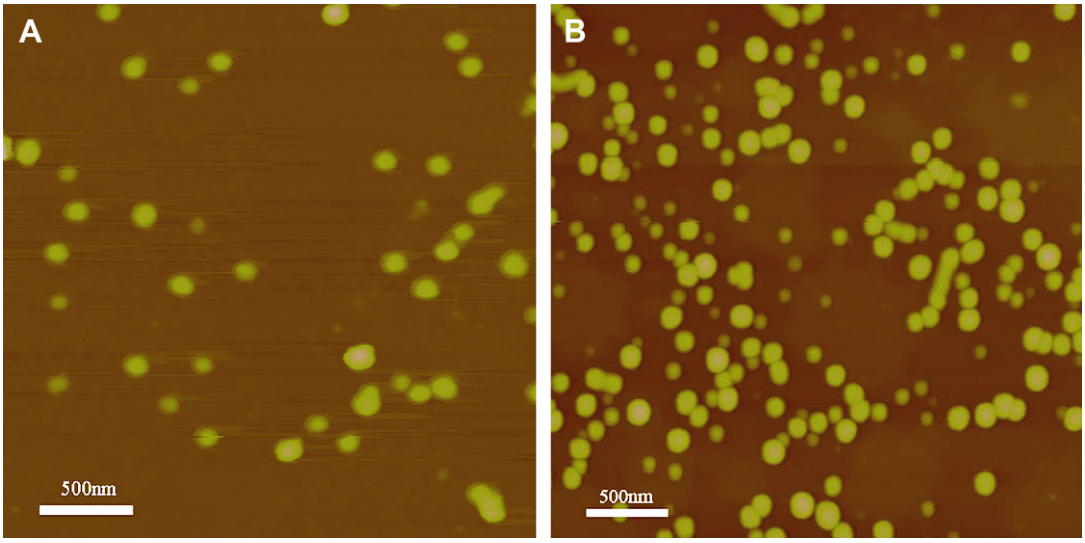


Fig. 4. AFM image of PS-*b*-PAA/PNIPAM-*b*-P4VP complex micelles in aqueous solution (A) and the shell reversed micelles in aqueous solution (B).

3.3. Characterizing of the modified PS_{120}-b-PAA_{47}/$P4VP_{109}$-b-$PNIPAM_{53}$ multilayered micelles in acidic aqueous solution

In the process of complexation, PNIPAM, a temperature sensitive polymer was introduced into the micelles, and it would be much more applicable if it was dialyzed against water. Firstly, a proper amount of acidic water (which has a pH value of 4.0) was added into the micelles ethanol solution, for that the dialysis bag cannot be used in the organic solvent. And then, the solution was dialyzed against the acidic water to remove the ethanol and DMF. The resulted complex micelles were studied by light scattering and atom force microscopy. The hydrodynamic diameters and the distribution are shown in Fig. 1c. It shows no obvious difference with that measured in the ethanol. And also, the diameters shown in the AFM (Fig. 4A) image are also well in accordance with that in the ethanol. It suggested that the core–shell–corona morphology did not change in the process of dialysis.

3.4. The formation of corona reversed micelles through thermal responsive corona of PNIPAM block

The outer corona of the PS_{120}-*b*-PAA_{47}/$P4VP_{109}$-*b*-$PNIPAM_{53}$ complex micelles is temperature responsive and it would shrink when the solution was heated above its lower critical solution temperature (LCST). The acidic solution was directly heated to 45 °C in this experiment. The shrunk PNIPAM chains would collapse onto the PAA/P4VP complex shell gradually and at the same time, the excessive P4VP block would still extend into the acidic aqueous solution. Finally, the corona reversed micelles formed in this process. Fig. 1d shows the hydrodynamic diameters and the distribution of the corona reversed micelles. The hydrodynamic diameter of the micelles decreased from 140 nm to about 130 nm. The decrease of the micelles is the result of the shrunk and the collapse of PNIPAM block and the pucker of the excessive P4VP block. AFM images of the corona reversed micelles are shown in Fig. 4B. There is no obvious difference between Fig. 4A and B and it is because the entire extended P4VP block and the PNIPAM block collapsed onto the core of the micelles in the process of AFM sample preparing.

3.5. The molecular mass measurement of the micelles

In order to further demonstrate the whole process of micellization and complexation, static light scattering was used to measure the molecular mass of the micelles. The molecular mass of the PS_{120}-*b*-PAA_{47} micelles in ethanol was about 6.31×10^7 g/mol, and it increased up to about 9.36×10^7 g/mol when $PNIPAM_{53}$-*b*-$P4VP_{109}$ ethanol solution was added into the micelles ethanol solution, and it did not change much when dialyzed against water and heated to 45 °C.

3.6. Nuclear magnetic resonance (NMR) analysis of micellization, complexation and temperature responses of the micelles

Fig. 5 is the ^{1}H NMR spectra of the micelles at different stages. The peak with a maximum around 4.0 ppm (peak A) can be assigned to the proton bound to the carbon atoms next to nitrogen atom and the peaks between 6.0 ppm and 8.0 ppm (peak B) can be assigned to the protons bound to the pyridine ring. In chloroform (as line a shown in Fig. 5), the area ratio of peak A to peak B (the summary of peak B1 and peak B2) is 1:8.21. The ratio changed to about 1:5.01 when the $PNIPAM_{53}$-*b*-$P4VP_{109}$ chains aggregate onto the PS_{120}-*b*-PAA_{47} block-copolymer micelles and that's because the PAA/P4VP complex would collapse onto the PS core. The length of P4VP block used here is much greater than the PAA block and the P4VP solution is excessive; so in the acidic solution, the excessive 4VP units would extend into the solution and peak B of line c in

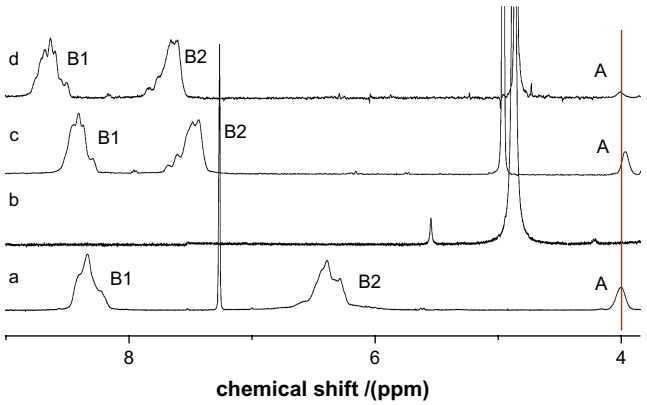
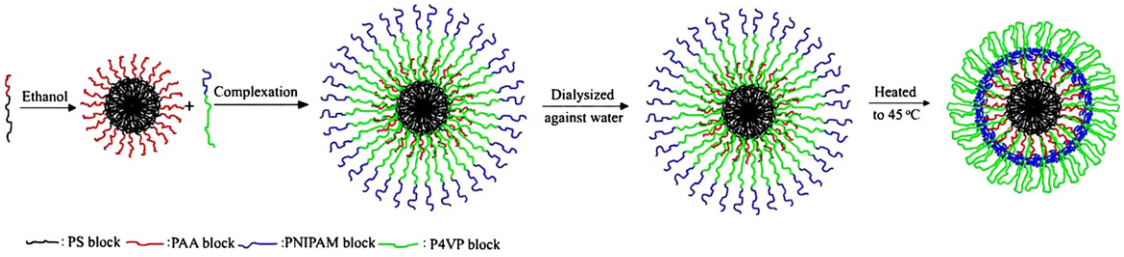


Fig. 5. The ^{1}H NMR spectra of the micelles: (a) $PNIPAM_{53}$-*b*-$P4VP_{109}$ block-polymer chains in chloroform; (b) PS_{120}-*b*-PAA_{47} micelles in D_2O; (c) PS_{120}-*b*-PAA_{47}/$P4VP_{109}$-*b*-$PNIPAM_{53}$ complex micelles in hydrochloric acid D1; (d) complex micelles in hydrochloric acid D1 at 45 °C.



Scheme 1. The schematic representation of the micellization, complexation and the temperature response.

Fig. 5 would just decrease but not disappear. In aqueous solution, PNIPAM block would shrink if the solution was heated to 45 °C. The area ratio changed to about 1:300 when the solution is heated to 45 °C (line d in Fig. 5).

4. Conclusion

In ethanol, PS_{120}-*b*-PAA_{47} block-copolymers would form core–shell micelles for it is a good solvent for PAA block and a precipitator for PS block. PS_{120}-*b*-PAA_{47}/$P4VP_{109}$-*b*-$PNIPAM_{53}$ core–shell–corona micelles formed through the complexation between $PNIPAM_{53}$-*b*-$P4VP_{109}$ block-copolymer and the PS_{120}-*b*-PAA_{47} micelle. The morphology of the micelles did not change when the solution was dialyzed against acidic water. The modified temperature sensitive corona of PNIPAM shrunk and the excessive P4VP block still extended into the acidic water when the aqueous solution was heated to 45 °C, and corona reversed micelles with a shrunk PNIPAM outer shell and excess extended P4VP block corona formed. The corona reversed micelles have an incompact PS core, the swollen PAA/P4VP complex layer, the compact shrunk PNIPAM outer shell and a P4VP corona. The shrink of PNIPAM outer shell is reversible and it would dissolve into the acidic water again when the solution is cooled below its LCST. The PNIPAM block or the P4VP block in the corona would shrink to form a compact outer shell when the temperature or the pH value of the acidic solution is changed, if needed in the application. The whole process of the complexation and the formation of the corona reversed micelles are shown in Scheme 1.

Acknowledgements

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