Stimuli-Responsive Aqueous Micelles from an ABC Metallo-Supramolecular Triblock Copolymer

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ABSTRACT: A metallo-supramolecular ABC triblock copolymer containing a bis(2,2′:6′,2″-terpyridine)-ruthenium(II) complex as a supramolecular connection between the constituting poly(styrene)-*block*-poly-(2-vinylpyridine) diblock and poly(ethylene oxide) block (PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70}) has been prepared. This copolymer was further utilized in order to prepare aqueous micelles consisting of a PS core, a pH-sensitive P2VP shell, and a PEO corona. The accordingly formed micelles have been characterized by a combination of dynamic light scattering, transmission electron microscopy, and atomic force microscopy. Spherical micelles and a minor fraction of bigger aggregates were observed by these three independent techniques. The pH sensitivity of the P2VP shell could be demonstrated and the reversibility of the metallo-supramolecular complex investigated utilizing a strong competitive ligand.

Introduction

The exact control over block copolymer morphologies is of great importance regarding the fine-tuning of performance, structure, and functionality as well as for the preparation of nanoscale devices. Utilization of self-aggregation and microphase separation of block copolymers resulted in a variety of well-defined supramolecular organizations both in the bulk and in a selective solvent of one of the blocks.[1] In this respect, ABC triblock copolymers have proven to be extremely useful precursors for very complex self-assembled structures in the bulk state.[2] Nevertheless, little is known up to now about their association behavior in selective solvents of one (or two) of the constituting block(s). In this respect, "three-layer" micelles were previously reported in aqueous media.[3] In addition, "crew-cut" micelles were prepared from highly asymmetric ABC copolymers.[4] Recently, an asymmetrical micelle was reported by Erhard et al. in organic solvents.[5] The so-called "Janus" micelle consists of a cross-linked poly(butadiene) core and a corona with a "southern" poly(styrene) and a "northern" poly(methyl methacrylate) hemisphere. Since the "Janus" micelle is the result of the stabilization of a supramolecular organization existing in the bulk state which is then transferred in solution, it must be therefore distinguished from the systems resulting from the direct solubilization of the ABC triblock copolymer in a selective solvent of one of the blocks.

Another important issue regarding the preparation of these self-assembled systems is their capability to respond to external stimuli such as temperature and/or pH.[6] In this respect, pH-sensitive "three-layer" aqueous micelles were prepared by coprecipitation of poly(styrene)-*block*-poly(2-vinylpyridine) and poly(2-vinylpyridine)-*block*-poly(ethylene oxide) diblocks. However, the coprecipitation procedure impeded the complete formation of "three-layer" micelles, which were then coexisting with micelles formed by the isolated diblocks.[7] Recently, the synthesis and characterization of pH-sensitive core−shell−corona (CSC) aqueous micelles made from poly(styrene)-*block*-poly(2-vinylpyridine)-*block*-poly(ethylene oxide) ABC triblock copolymers (PS-*b*-P2VP-*b*-PEO) have been described.[8] The pH sensitivity of the P2VP shell was used to tune the size of this system from a hydrodynamic diameter of 75 nm at pH > 5 to 135 nm at pH < 5. This effect was attributed to electrostatic repulsion between the charged P2VP blocks at low pH. Temperature- and pH-sensitive CSC micelles were also very recently reported by Armes et al.[9] Moreover, the shell of these CSC micelles could be selectively cross-linked.

In this contribution, we report on the preparation of responsive CSC micelles based on a novel type of ABC copolymer, namely the metallo-supramolecular PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer (see Figure 1 for structure and synthesis). The combination of metallo-supramolecular and polymer chemistry has been recently proven to be an unique tool for the synthesis of a new class of block copolymers and nanomaterials.[10] In these block copolymers, a metal−ligand complex is utilized as a supramolecular linker between the different blocks, giving rise to a metallo-supramolecular block copolymer. This strategy has been very recently implemented for the synthesis of PS-[Ru]-PEO diblock copolymers, which were used to prepare aqueous micelles.[11,12] The high stability of the bis(2,2′:6′,2″-terpyridine)ruthenium(II) complex impeded any ligand exchange, and therefore the integrity of the supramolecular block copolymer was kept in an aqueous environment even under extreme pH, temperature, and salt conditions.[12]

Compared to the covalent PS-*b*-P2VP-*b*-PEO counterpart, the metallo-supramolecular PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer introduces new interesting features

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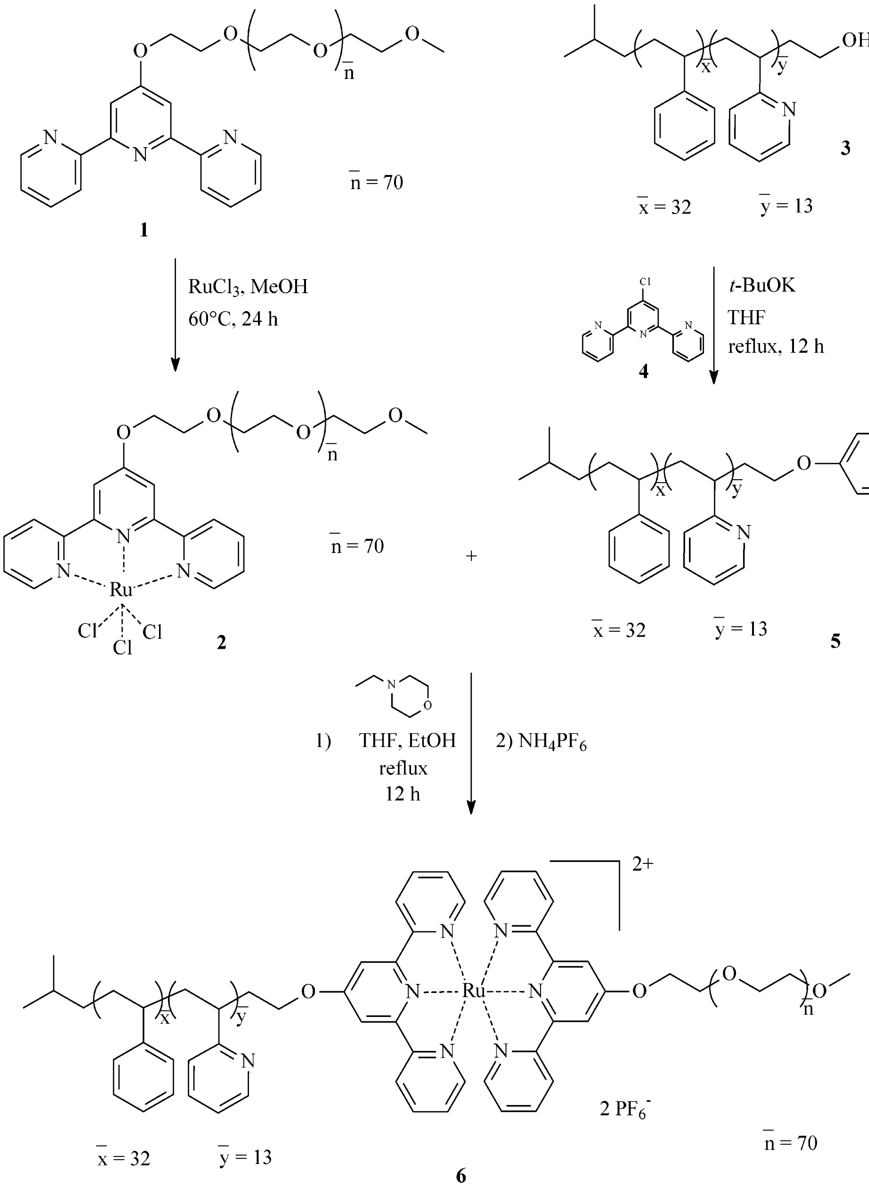


PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70}

Figure 1. Synthetic pathway to the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} metallo-supramolecular copolymer.

such as the reversibility of the metal−ligand complex under certain conditions,[13] the well-known interesting photophysical and electrochemical properties of the ruthenium−terpyridine complex,[14] and the possibility to easily construct a library of triblock copolymers of different compositions by varying the molecular weight of the terpyridine-functionalized PEO during the last step of the synthesis of the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer (see Figure 1). On the other hand, the bulky charged bis(2,2′:6′,2″-terpyridine)ruthenium(II) complex and their associated counterions are expected to strongly influence the phase separation between the different blocks and in turn the overall morphology of the micellar objects.[12]

Experimental Section

Chemicals were received from BASF, Aldrich, Fluka, Polymersource, and Shearwater and used without further purification. 4′-Chloroterpyridine (compound **4**) was prepared as described elsewhere.[15] Solvents were bought from Biosolve. THF was distilled over Na/K/benzophenone. The synthetic route to the metallo-supramolecular copolymer is depicted in Figure 1.

For preparative size exclusion chromatography Bio-Rad SX-1 beads swollen in CH_2Cl_2 or THF were used. ^1H NMR and ^{13}C NMR were recorded on a Varian Mercury spectrometer with frequencies of 400.1 and 100.6 MHz, respectively. Chemical shifts are given in ppm downfield from TMS. UV/vis spectra were recorded on a Perkin-Elmer Lambda 45P spectrophotometer. Size exclusion chromatography (SEC) was performed on a Shimadzu apparatus equipped with a mixed D-column from Polymer Laboratories and with UV and RI detectors. Chloroform was used as the eluent at a flow of 1.0 mL/min. Polystyrene standards were used for calibration.

Preparation of Terpyridine-Terminated PEO_{70} (1). See ref 16 for the detailed synthetic procedure. Yield: 95%. Selected analytical data: ^1H NMR ($CDCl_3$): δ = 8.68, 8.61, 8.04, 7.85, 7.34, 4.40, 3.93, 3.83−3.45, 3.38. ^{13}C NMR ($CDCl_3$): δ = 166.6, 156.7, 155.6, 148.7, 136.4, 123.5, 120.9, 107.0, 72.2−67.4, 58.6 53.3. UV/vis (H_2O): λ_{max}/nm (ϵ/L mol^{-1}

cm^{-1}) = 278 (13 200), 234 (17 000). SEC (RI): $M_n = 2360$, PDI = 1.21.

Preparation of the Ruthenium(III) Complex (2). See refs 16−18 for details. Yield: 99%. Selected analytical data: ^{1}H NMR ($CDCl_3$): $\delta = 3.92-3.46$, 3.38. UV/vis (H_2O): λ_{max}/nm (ϵ/L mol^{-1} cm^{-1}) = 272 (71 700), 375 (16 100).

Preparation of Hydroxy-Terminated PS_{32}-*b*-$P2VP_{13}$ (3). A hydroxy-terminated PS_{32}-*b*-$P2VP_{13}$ covalent block copolymer **3** was prepared by sequential living anionic polymerization of the two comonomers in THF at −78 °C as described elsewhere.[19] When the PS block was polymerized, an aliquot was withdrawn from the reaction media for size exclusion chromatography (SEC (RI): $M_n = 3200$, PDI = 1.15). 2-Vinylpyridine was then added. At the end of the polymerization reaction, the living PS_{32}-*b*-$P2VP_{13}^-$ macroanions were end-capped by an ethylene oxide unit. The resulting oxyanion was then converted into a hydroxyl group by addition of methanol. **3** was isolated by precipitation in methanol and dried in a vacuum oven. Yield: 99%. SEC (RI): $M_n = 4500$, PDI = 1.28.

Preparation of Terpyridine-Terminated PS_{32}-*b*-$P2VP_{13}$ (5). **3** (0.250 g, 0.0556 mmol) ($M_n = 4500$ g/mol) and *t*-BuOK (0.019 g, 0.167 mmol) were stirred at reflux in dry THF (5 mL). After 15 min, 4′-chloro-2,2′:6′,2″-terpyridine (**4**) (0.030 g, 0.111 mmol) in dry THF (5 mL) was added dropwise. The reaction mixture was stirred overnight, after which the solvent was removed in vacuo. The resulting solid was partitioned between water and chloroform. The chloroform layer was washed with water (3 × 25 mL). The organic layer, containing the product, was dried over Na_2SO_4 and removed in vacuo. The compound was purified by a double size exclusion chromatography (BioBeads SX-1, first column in THF, second column in CH_2Cl_2), yielding 0.187 g of **5** (72%). ^{1}H NMR ($CDCl_3$): $\delta = 8.65$, 8.57, 8.43−8.07, 7.79, 7.29−6.47, 3.96, 3.79, 3.64, 2.42−0.56. UV/vis (CH_2Cl_2): λ_{max}/nm (ϵ/L mol^{-1} cm^{-1}) = 263 (69 800). SEC (UV): $M_n = 3500$ g/mol, PDI = 1.28.

Preparation of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} (6). A solution of **2** (0.070 g, 0.020 mmol) and **5** (0.100 g, 0.021 mmol) in a mixture of THF:EtOH (7:3, 5 mL) was stirred for 30 min under reflux. A few drops of *N*-ethylmorpholine were added, and the solution turned from orange to red. Stirring under reflux was continued overnight, after which an excess of NH_4PF_6 (45 mg, 0.27 mmol) was added. Stirring was continued for 1 h, and subsequently the solvent was removed in vacuo. The reaction mixture was partitioned between 25 mL of water and 25 mL of CH_2Cl_2. The organic layer was washed with water (3 × 25 mL), dried over Na_2SO_4, and finally removed in vacuo, affording a reddish brown crude product. This was further purified by preparative size exclusion chromatography (BioBeads SX-1, first column in THF, second column in CH_2Cl_2), yielding 40 mg of **6** (22%). ^{1}H NMR (CD_3CN): $\delta = 8.67-7.84$, 7.54−6.51, 4.82, 4.13, 3.82−3.55, 3.38, 2.29−1.26, 0.88−0.57. UV/vis (CH_3CN): λ_{max}/nm (ϵ/L mol^{-1} cm^{-1}) = 486 (5800), 305 (22 860), 263 (62 100).

Preparation of the Micelles. The PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer was not readily soluble in water. Therefore, the preparation method previously introduced by Eisenberg et al. for "crew-cut" micelles was applied.[20] Addition of pure water resulted in the precipitation of the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer, so an aqueous 0.1 mol/L HCl solution was utilized in order to protonate the P2VP block and therefore to increase the hydrophilic character of the copolymer. First, an initial solution of each copolymer in *N*,*N*-dimethylformamide (DMF) was prepared (concentration of 1 g/L), and then an aqueous 0.1 mol/L HCl solution was dropwise added to induce aggregation of the insoluble polystyrene block. Subsequently, the DMF/water solution was dialyzed several times against a 0.1 mol/L HCl aqueous solution (Spectra-Por dialysis bags, cutoff 6000−8000 Da). The final concentration of the copolymer was set to 0.5 g/L. In a subsequent experiment, a very large excess (10^4 mol per mole of terpyridine ligand) of a strong competitive ligand (hydroxyethyl-ethylenediaminotriacetic acid sodium salt or HEEDTA) was added to the aqueous micellar solution which was further heated to 60 °C for 4 h. That resulted in a colorless solution because of the opening of the bis(2,2′:6′,2″-terpyridine)ruthenium(II) complex. The excess of HEEDTA was then eliminated by dialysis against pure water, and 20 mg of $FeSO_4$ was added, resulting in a violet-colored solution.

Dynamic Light Scattering (DLS). DLS measurements were performed with a Malvern 4700C apparatus equipped with a Malvern 7032 digital correlator and an Ion Laser Technology argon laser with a wavelength of 488 nm. Although the metallo-supramolecular copolymer was absorbing light at this wavelength, sufficient light was scattered to allow data with good statistics to be obtained. The samples were directly measured without any filtration. Each DLS data resulted from the averaging of 10 measurements. Measurements perturbed by dust particles were systematically removed before the averaging. The scattering angle used for the measurements was 90°. A temperature-controlled refractive index matching bath surrounded the scattering cell.

The experimental correlation function was analyzed by the Contin routine, a constrained regularization method program for the inverse Laplace transformation of dynamic light scattering data.[21] The Contin program gave access to the distribution of the relaxation times in the experimental time correlation functions. The *Z*-averaged distribution of the hydrodynamic diameter (D_h) was then calculated.

Transmission Electron Microscopy (TEM). TEM images were recorded on a JEOL 2000FX microscope working at 200 kV. TEM samples were prepared by drop-casting of a diluted micellar solution on a Formvar-coated copper grid. No contrasting agent was used for the observation.

Atomic Force Microscopy (AFM). AFM images were recorded with a Digital Instruments Multimode operated in air with the Tapping Mode. Micelles were adsorbed on a silicon wafer substrate which was previously cleaned by chloroform. The silicon substrate was first immersed in the micellar solution and washed with pure water and then dried under ambient atmosphere. The mean diameter, *D*, of the micelles was measured from the height contrast AFM image by using the "particle analysis" option from the Digital Instruments software. For each sample, *D* was averaged from the analysis performed on three different images. In each of these images, the number of micelles was around 100.

Results and Discussion

The PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} metallo-supramolecular copolymer has been prepared as described in detail in the Experimental Section and shown in Figure 1. The mono-chelic PEO_{70} end-capped with the 2,2′:6′,2″-terpyridine ligand (**1** in Figure 1) is selectively complexed with Ru(III) ions to exclusively form a mono-complex (**2**). A hydroxy-terminated PS_{32}-*b*-$P2VP_{13}$ diblock **3** is prepared by living anionic polymerization. The hydroxy end group of **3** is then reacted with **4** in order to yield the terpyridine-terminated PS_{32}-*b*-$P2VP_{13}$ diblock **5** in 72%. Subsequently, **2** is reacted with **5** via self-assembly processes, resulting in the metallo-supramolecular PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer **6**. This last reaction is accompanied by a reduction of Ru(III) into Ru(II) ions. The obtained ABC triblock copolymer was purified by two preparative size exclusion columns. The exclusive formation of the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} and of the intermediates **1**, **2**, and **5** was proven by several analytical techniques (see Experimental Part for details). The formation of the mono(2,2′:6′,2″-terpyridine)ruthenium(III) complex is evidenced by the metal-to-ligand charge-transfer (MLCT) band at 375 nm in the spectrum of compound **2**, while the MLCT band at 486 nm in the spectrum of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} is characteristic of the bis(2,2′:6′,2″-terpyridine)ruthenium(II) complex.

The PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer was then utilized in order to prepare aqueous micelles. Since this ABC triblock was not readily soluble in water, the method previously introduced by Eisenberg et al. for the

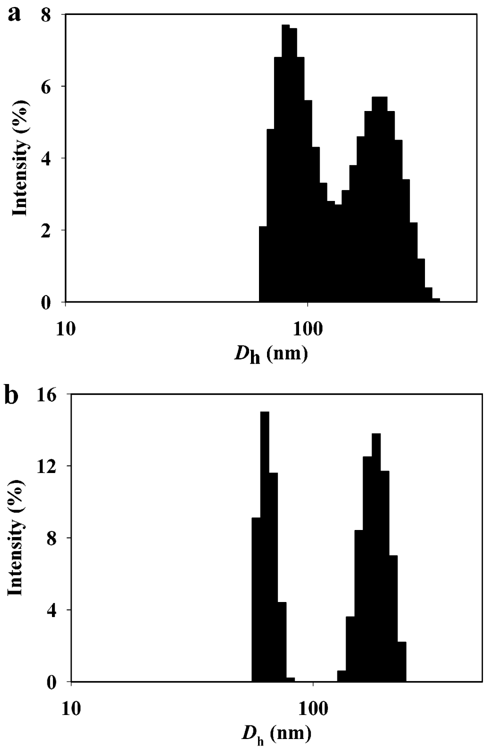


Figure 2. CONTIN size distribution histogram of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles at pH = 1 (a) and pH = 7 (b).

preparation of “crew-cut” micelles was used.[20] This results in our case in kinetically frozen micelles consisting of a PS core, a P2VP shell, and a PEO corona. In a previous report, we have described the formation and characterization of nearly monodisperse core−shell−corona (CSC) micelles made from the covalent PS_{200}-*b*-$P2VP_{130}$-*b*-PEO_{495} copolymer.[8] In sharp contrast, it was not possible to prepare micelles in neutral water from the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer. This is thought to be due to the short PEO block that could prevent an effective steric stabilization of the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles. One way to increase the hydrophilic character of the triblock is to protonate the P2VP block in order to introduce an electrosteric stabilization effect.[22] In our case, this has been achieved by adding a 0.1 mol/L HCl aqueous solution to the ABC triblock initially dissolved in DMF. A stable micellar solution was then obtained. That the integrity of the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} copolymer was kept in this acidic aqueous environment was verified by UV/vis spectroscopy, revealing the characteristic MLCT band of the bis-complex at 486 nm. Moreover, the micellar solution was found to be stable (no flocculation, creaming, or precipitation) with time (at least in a 3 month period).

The accordingly prepared micellar solution was analyzed by dynamic light scattering (DLS). The CONTIN size distribution histogram revealed two populations, as previously observed for other metallo-supramolecular micelles (Figure 2a).[11−13] According to these previous investigations, the smaller population with a D_h of 81 nm is attributed to primary micelles while the second one with a D_h of 191 nm is thought to result from the aggregation of individual micelles into larger aggregates. The size of the primary micelles does not fall in the usual range of block copolymer micelles,[1] regarding the low molecular weight of the constituting metallo-supramolecular triblocks. For this behavior several explanations can be provided: (I) protonation of the P2VP block, resulting in highly stretched segments; (II) formation of micelles which have a morphology (e.g., rodlike micelles or small vesicles) that differs from the classical spherical starlike shape; (III) formation of nonequilibrium micelles with a loose structure, which require time to reorganize into more compact spherical micelles. In this respect, the presence of the charged and bulky bis(terpyridine)ruthenium complexes at the interface between the PS-*b*-P2VP and PEO could strongly perturb the packing of the individual amphiphilic macromolecules into a compact micelle, as previously discussed.[12]

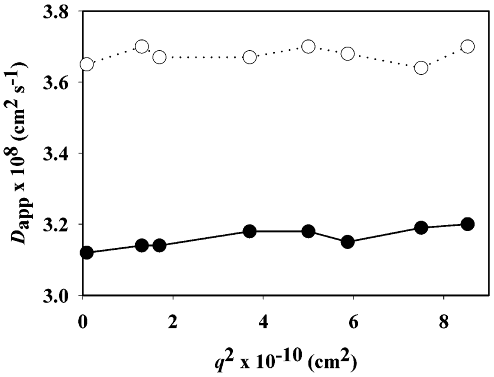
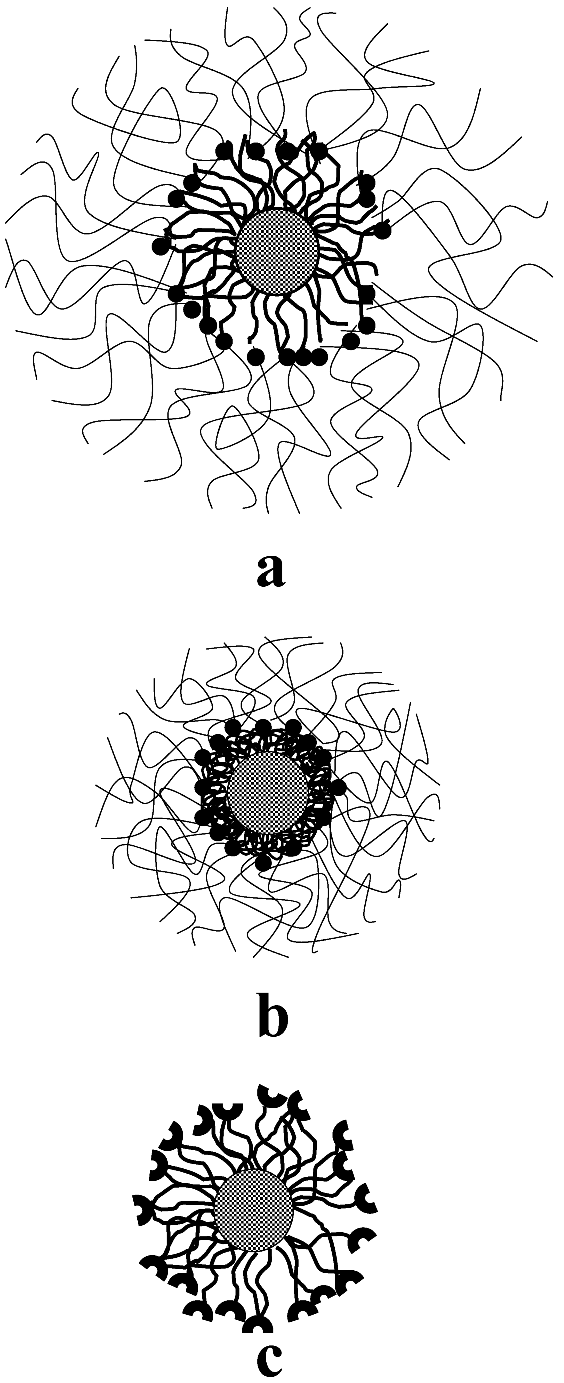


Figure 3. Angular dependence of the apparent diffusion coefficient (D_{app}) of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles and aggregates at pH = 1 (black dots) and pH = 7 (open dots).

To gain additional information about the micellar structure, complementary DLS measurements have been performed. In this respect, the angular dependence of the mean apparent diffusion coefficient (D_{app}) extrapolated to zero concentration has been investigated for the metallo-supramolecular micelles (Figure 3). The slope of the angular dependence of D_{app} can be related to the shape of the diffusing species.[22] In the present case, the constancy of D_{app} with q^2 can be accounted for the formation of spherical micelles and rules out other morphologies. Indeed, for spherical particles, the rotational motion is not detectable, and D_{app} is independent of q^2.[23] Spherical metallo-supramolecular micelles with stretched P2VP blocks are schematically depicted in Scheme 1a.

The morphology of the metallo-supramolecular micelles has been examined by TEM (Figure 4). Spherical micelles were observed together with larger structures which seem to result from the merging of invididual micelles. No contrasting agent was used for the TEM experiments. The electronic contrast is thought to originate from the ruthenium ions found in the block copolymer structure. This contrast was found to be very uniform within the metallo-supramolecular micelles. Since the micelles were observed in a dried, collapsed state for TEM experiments, the characteristic size of metallo-supramolecular micelles is much smaller in TEM (ca. 20 nm) than the D_h measured by DLS. Moreover, the larger aggregates of micelles were observed to evolve under electron microscopy, resulting in highly contrasted structures which were identified as salt crystallites by performing electron diffraction within the TEM. This leads to the hypothesis that some residual salt is entrapped in the PEO corona of these metallo-supramolecular micelles, as was already stated in a previous study on metallo-supramolecular micelles.[12] In this respect, the entrapped salt coordinated to ethylene oxide units could explain why uncharged

Scheme 1. Schematic Representation of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} Metallo-Supramolecular Micelles at pH < 5 (a), pH > 5 (b), and after Addition of a Large Excess of HEEDTA (c)[a]



[a] PS core: central sphere; P2VP chains in thick black; PEO chains in thin black; bis(terpyridine)ruthenium complex: black dots in (a) and (b); terpyridine ligand: half-circles in (c).

supramolecular micelles behave to some extent as polyelectrolyte micelles.[12,24]

The same micelles were subsequently adsorbed on a silicon wafer and investigated utilizing atomic force microscopy (Figure 5a). Individual micelles and aggregates of micelles were observed, in agreement with the TEM and DLS results. The mean lateral size of the individual micelles was estimated to be around 35 nm in the dried state and is larger than the characteristic size observed by TEM. This is thought to be due to tip convolution effects and to a possible interaction of the adsorbed charged micelles with the charged substrate. In this respect, the height of the adsorbed micelles was estimated to be 18 nm, a value which is in good agreement with the lateral dimensions observed by TEM. In contrast to the morphological characterizations previously obtained on the PS_{200}-*b*-$P2VP_{130}$-*b*-PEO_{495} covalent CSC micelles,[8] the protonated P2VP shell could not be distinguished from the PS core for the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} sample. This is thought to originate from the smaller molecular weight of the PS and P2VP blocks in the metallo-supramolecular sample. This could explain a less-developed phase separation between these two small blocks and thus why TEM and AFM experiments fail to visualize them separately.

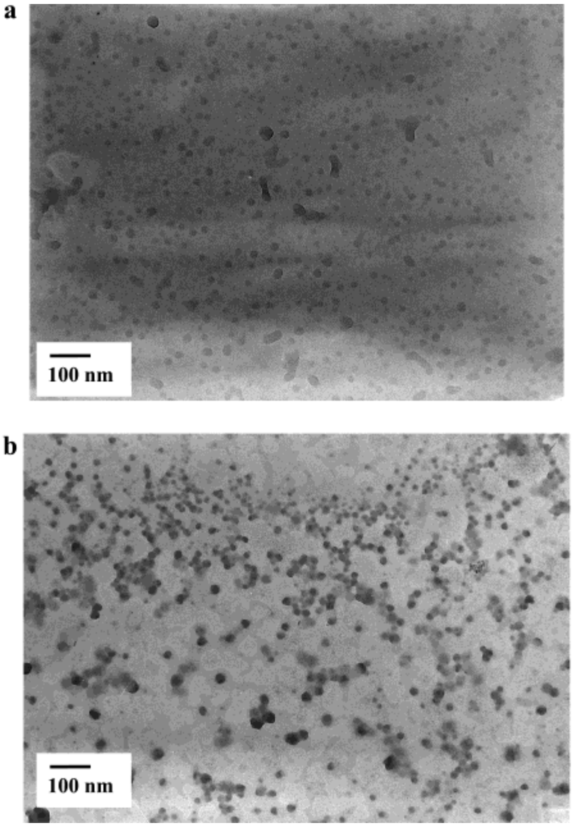


Figure 4. TEM pictures of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles at pH = 1 (a) and pH = 7 (b).

In further experiments, the pH of the acidic micellar PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} solution was adjusted to neutral value, resulting in the deprotonation of the P2VP blocks. A schematic representation of these “neutral” metallo-supramolecular PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles is shown in Scheme 1b. The deprotonated micelles were found to be stable under these conditions, although it was not possible to prepare them directly in neutral water. These “neutral” PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles were analyzed by DLS, and two populations were once again detected in the CONTIN analysis of the experimental correlation function (see Figure 2b). The mean size of the primary micelles (D_h = 63 nm) falls in the same range as the results previously obtained on metallo-supramolecular AB diblock copolymers.[11] The relatively large size of the metallo-supramolecular micelles in regard to their total molecular weight has been attributed to electrostatic repulsion between neighboring bis(2,2′:6′,2″-terpyridine)ruthenium(II) complexes and between salt-loaded PEO blocks that then behave as polyelectrolyte chains, as

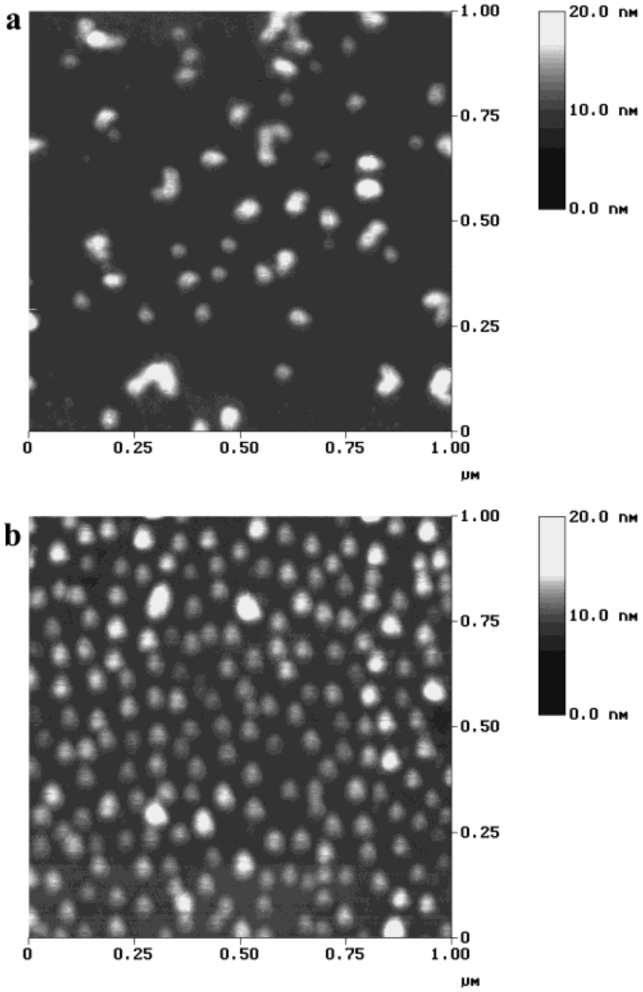


Figure 5. AFM height contrast images of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles adsorbed on a silicon wafer at pH = 1 (a) and pH = 7 (b).

previously discussed.[12] The D_h of the micelles at neutral pH was lower than the one observed at pH < 5, in agreement with the collapse of the deprotonated P2VP blocks, which are now hydrophobic. The magnitude of the pH-driven size variation effect is much lower for metallo-supramolecular PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles than for the previously studied covalent PS_{200}-*b*-$P2VP_{130}$-*b*-PEO_{495} system.[8] This is thought to be due to the length of the P2VP block, which is 10 times shorter in the case of the metallo-supramolecular micelles. To prove the reversibility of the size-variation effect, the pH was cycled by adding equimolar amounts of HCl and NaOH to the micellar solution. After each addition of acid or base, the D_h of the micelles was measured (see Table 1). The size-variation effect was not found to be reversible. Moreover, a macroscopic precipitation was observed during the second cycle. This could result from the decreased solubility of the PEO block in the presence of salt. Actually, NaCl is formed during the pH-variation cycles, and it is well-known that the addition of salt decreases the cloud point of PEO.[25,26] Elimination of the accordingly formed NaCl by dialysis was not successful to restore the solubility of the micelles. The D_h of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles has also been measured as a function of pH at a constant ionic strength (Figure 6). Two regimes are clearly observed: the “acidic” one with micelles with a D_h of approximately 80 nm and protonated P2VP shell and the “neutral” one with a hydrophobic P2VP shell and a D_h of approximately 65 nm. A sharp transition between both regimes was noted at pH = 5, in agreement with the pK_a of P2VP.[22]

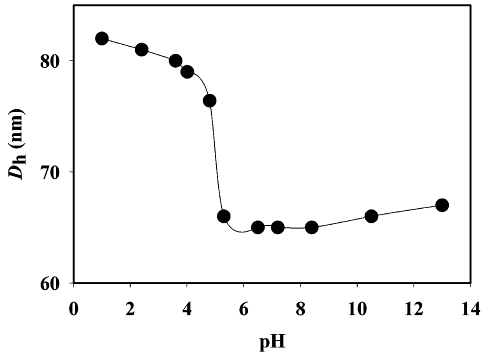


Figure 6. D_h of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles as a function of pH.

Table 1. Evolution of the D_h of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} Individual Micelles Observed When Cycling the pH

	initial (pH = 1)	+ 1 mmol of NaOH	+ 1 mmol of HCl	+ 1 mmol of NaOH
D_h (nm)	81	63	76	precipitation

The morphology of the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles with the collapsed P2VP shell at neutral pH was also examined by TEM and AFM (see Figures 4b and 5b). Individual micelles and aggregates were again observed. The number of aggregates could be overestimated due to the drying process required for TEM sample preparation. Because of polydisperse spherical micelles, it was not possible to see much difference in the size of the micelles formed at pH = 1 or pH = 7 (compare parts a and b of Figure 4). The same conclusion holds on for the AFM observation. However, the neutral micelles seem to be more regular in shape compared to the protonated ones. For protonated micelles, electrostatic interactions between the micelles and the substrate could modify their shape compared to the actual situation in solution.[27] The mean lateral size of the adsorbed neutral micelles was determined to be approximately 30 nm by image analysis. In conclusion, both AFM and TEM characterization techniques were suited to visualize the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles but failed to evidence the structural differences between neutral and protonated micelles. This is thought to mainly result from the low molecular weight of the P2VP block, as discussed earlier.

That electrostatic interactions play a major role in the aggregation behavior of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} chains was evidenced by varying the ionic strength in the aqueous micellar solution. For that purpose, different amounts of NaCl were added, and the D_h of the micelles was measured, as shown in Figure 7. A very significant decrease in micelle size was observed when NaCl was added, independent of the actual pH. This feature was previously observed for other metallo-supramolecular micelles and is thought to result from decreasing repulsion among coronal chains as salt is added.[12] The final size of the neutral and protonated PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles is in the range of common block copolymer micelles when 1 mol/L of NaCl is added. The addition of salt has also an influence on the ratio of micelles to aggregates (see Figure 8). Indeed, the addition of a small amount of salt results in an increase of the percentage of micelles. For the protonated micelles, aggregation was totally suppressed (Figure 8).

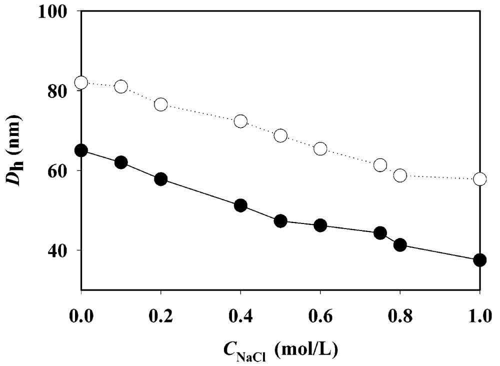


Figure 7. D_h of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles as a function of added NaCl concentration at pH = 1 (open dots) and pH = 7 (black dots).

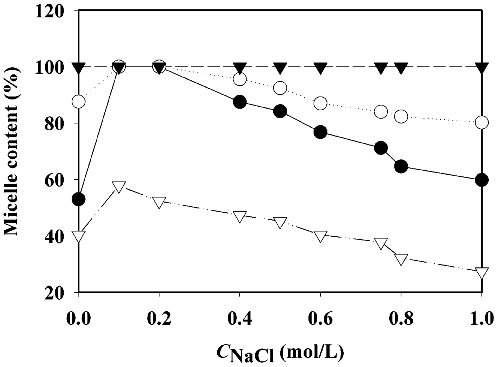


Figure 8. Content of micelles vs aggregates for PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles at pH = 1 (black triangles and black dots) and pH = 7 (open triangles and open dots). For the sake of comparison, "intensity" percentage (open triangles and black dots) and "number" percentage (black triangles and open dots) are shown.

This suggests an equilibrium between micelles and aggregates of micelles which is governed by electrostatic interactions. In this respect, it was previously shown that metallo-supramolecular micelles were behaving similarly to polyelectrolyte micelles.[12] This characteristic feature is again observed for PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles. Residual ions tightly bound to PEO could explain why neutral micelles are also behaving as a polyelectrolyte system. Further addition of salt results in an increase of the percentage of aggregates. This is thought to be due to the reduced solubility of PEO segments as a large amount of salt is added. This results turn in a decreased water solubility of PEO and thus to a less effective steric stabilization of the micelles.[28] At this point, it should be recalled that the percentage of micelles/aggregates strongly depends on how the DLS data have been computed. Indeed, "intensity" percentage obtained by integration of the corresponding population in the CONTIN histogram are clearly overestimating the actual percentage of aggregate. This is due to the fact that larger entities (aggregates) are much more strongly diffusing light than smaller ones (micelles). Therefore, "number" percentages have to be calculated as shown in Figure 8. The DLS software allows a rough conversion of "intensity" into "number" distribution histograms. Both data have been presented in Figure 8. "Number" distribution histograms indicate that very few aggregates are observed in the neutral PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles while they are negligible for protonated ones.

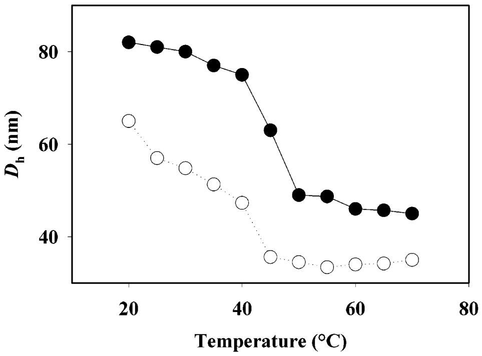


Figure 9. D_h of PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles as a function of temperature at pH = 1 (black dots) and pH = 7 (open dots).

In parallel to the effect of salt, the effect of temperature on PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles has been investigated as well, as shown in Figure 9. These results are qualitatively in agreement with previous experiments carried out on other supramolecular micelles.[12] The input of thermal energy allows a relaxation of the stretched PEO segments. This is accompanied by a progressive dehydration of PEO, which reduces the steric stabilization due to the PEO corona.

The reversibility of the salt and temperature effects was also checked. For that purpose, salt was eliminated by dialysis and the D_h of the metallo-supramolecular micelles was measured. The initial D_h was not restored, meaning that nonequilibrium effects are involved. In the same vain, the initial D_h was not restored when temperature was lowered from 70 to 20 °C, in agreement with nonequilibrium effects. The nonreversibility of the pH effect has been already discussed above. Moreover, the ratio of micelles to aggregates was also varying with time. All these observations clearly point out that PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles are existing in a kinetically frozen state and do not represent a thermodynamic equilibrium state.

In a recent paper, it has been demonstrated that the bis(2,2′:6′,2″-terpyridine)ruthenium(II) complex found in metallo-supramolecular micelles could be opened in an aqueous environment under relatively harsh conditions.[13] Heating an aqueous solution of metallo-supramolecular micelles in the presence of a large excess of hydroxyethyl-ethylenediaminotriacetic acid sodium salt (HEEDTA), which represents a strong competitive ligand for transition metal ions, resulted in the opening of the terpyridine bis-complex, as evidenced by the formation of a colorless solution and an important decrease of the D_h of the micelles. The same experiment was performed on the PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles, as macroscopically illustrated in Figure 10. The initial orange-colored micellar solution transformed into a colorless one after the addition of a large excess of HEEDTA and a heating procedure (see Figure 10). In parallel, the D_h of the micelles dropped to 27 nm. The structure of the resulting micelles is schematically depicted in Scheme 1c. The excess of HEEDTA and the free PEO_{70} blocks were eliminated by dialysis against pure water. In a final step, Fe(II) ions were added to the dialyzed micellar solution, resulting in a violet color. This color is characteristic for the corresponding Fe(II)−terpyridine complex[29] and indicates that some free terpyridine ligands are present at the surface of the nano-objects tentatively depicted in Scheme 1c.

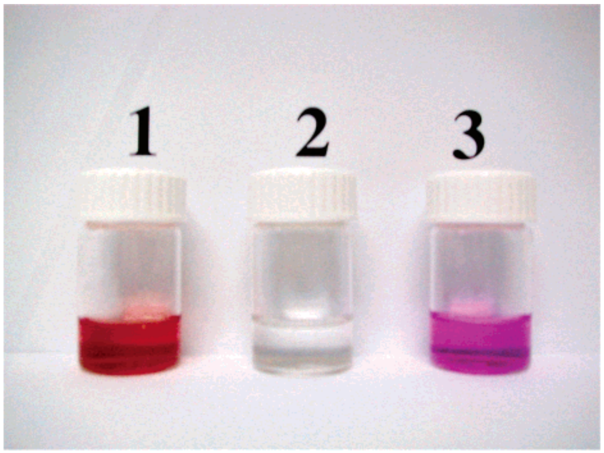


Figure 10. Macroscopic aspect of the initial PS_{32}-*b*-$P2VP_{13}$-[Ru]-PEO_{70} micelles in water (1), after the addition of a large excess of HEEDTA (2), and after elimination of the excess of HEEDTA by dialysis and subsequent addition of Fe^{2+} ions (3).

Conclusions

In this paper, we have successfully synthesized an amphiphilic metallo-supramolecular ABC triblock copolymer. In this compound, a supramolecular connection has been used between a PS-*b*-P2VP diblock and a PEO block. The resulting PS-*b*-P2VP-[Ru]-PEO copolymer was used to prepare aqueous micelles consisting of a PS core, a P2VP shell, and a PEO corona. Beside individual micelles, aggregates of micelles have also been detected. The pH sensitivity of the P2VP shell was verified by pH variations from 1 to 7. This feature makes these micelles attractive for encapsulation and/or release of active species. The bis(2,2′:6′,2″-terpyridine)-ruthenium(II) connection was found to be very stable, and the integrity of the triblock copolymer was kept in the aqueous environment even at extreme pH values. This complex could, however, be opened whenever a large excess of a strong competitive ligand was added. This allowed the release of the PEO coronal chains of the metallo-supramolecular micelles. In addition, the metal complex could be re-formed, even by using a different kind of metal ion. This last feature will be used in the near future as a tool to manipulate or to chemically modify nano-objects such as micelles.

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