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SHORT COMMUNICATION

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Dynamic light scattering and cryogenic transmission electron microscopy investigations on metallo-supramolecular aqueous micelles: evidence of secondary aggregation

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Abstract Metallo-supramolecular diblock copolymers consisting of a polystyrene (PS) block connected to a poly(ethylene oxide) (PEO) block by a bis(terpyridine)ruthenium complex (PS_{20}-[Ru]-PEO_y) were used to prepare aqueous micelles. The length of the PS block was kept constant, while two PEOs of different molecular weight were used. The resulting hydrated micelles and aggregates were characterized by a combination of cryogenic transmission electron microscopy (cryo-TEM) and dynamic light scattering measurements. The results were compared to those obtained for a covalent counterpart (PS_{22}-*b*-PEO_{70}). Cryogenic transmission electron microscopy allowed visualization of the PS core of the micelles. Moreover, the aggregates result from clustering of individual micelles.

Keywords Metallo-supramolecular chemistry · Block copolymers · Micelles · Cryogenic transmission electron microscopy · Dynamic light scattering

Introduction

Amphiphilic block copolymers self-aggregate into block copolymer micelles in aqueous media. They consist of a core formed by the insoluble blocks, surrounded by a corona formed by the water-soluble segments [1]. In a very recent set of papers, the synthesis and characterization of a new type of amphiphilic block copolymers, namely metallo-supramolecular amphiphilic block copolymers, has been described. These compounds are formed of a poly(ethylene oxide) (PEO) A block linked to various hydrophobic B blocks through a bis-2,2′:6′,2″-terpyridine-ruthenium(II) complex [2, 3, 4, 5, 6]. Moreover, it has been shown that these copolymers assemble into aqueous micelles with a hydrophobic core and a PEO corona [3, 4, 6]. The bis-2,2′:6′,2″-terpyridine-ruthenium(II) complexes are assumed to be at the core–corona interface. The high stability of the utilized complexes in various environments allowed the integrity of the amphiphilic copolymers to be kept. Previous room temperature transmission electron microscopy (TEM) of the dried solution indicated spherical entities and larger aggregates [3, 4, 5, 6]. However, the emergence of these structures could be a result of drying artifacts during sample preparation [7]. Moreover, the core could not be distinguished from the shell in these TEM observations.

In this contribution, a combination of cryogenic TEM (cryo-TEM) imaging and dynamic light scattering DLS was used in order to study the micelles formed by two different metallo-supramolecular block copolymers containing PS and PEO blocks (Fig. 1). DLS measurements were aimed at providing average values for the hydrodynamic diameter, D_h, and D_h distribution, while cryo-TEM is a direct, model-independent technique depicting the internal structure of the individual micelles and aggregates.

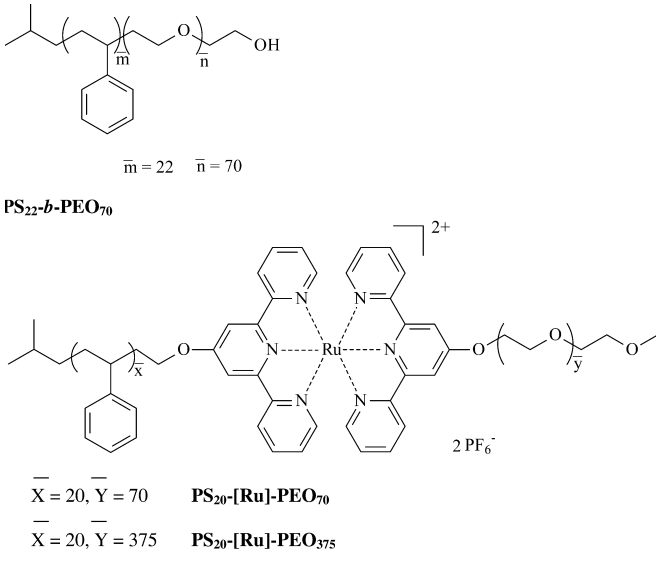

Fig. 1 Structures of the metallo-supramolecular and covalently bonded amphiphilic block copolymers investigated

Experimental

Block copolymer preparation

The two metallo-supramolecular block copolymers investigated were prepared by sequential self-assembly process of terpyridine-functionalized polymer blocks and are designated by the PS_{20}-[Ru]-PEO_{70} and PS_{20}-[Ru]-PEO_{375} acronyms (the numbers in the subscript being the average degree of polymerization of each block). The starting polymer blocks (PS_{20}, PEO_{70} and PEO_{375}) were synthesized by living anionic polymerization techniques and have a polydispersity below 1.2. The exclusive formation of the AB diblock copolymers was proven by ^{1}H NMR, size-exclusion chromatography, UV–vis spectroscopy and matrix-assisted laser desorption/ionization–time-of-flight spectrometry, as described in Ref. [2]. A covalent PS_{22}-*b*-PEO_{70} was synthesized by sequential living anionic polymerization of styrene and ethylene oxide, as described elsewhere [8]. The structures of the amphiphilic block copolymers investigated are shown in Fig. 1.

Micelle preparation

Micelles were obtained by direct dissolution of the PS_{20}-[Ru]-PEO_{375} sample in water. However, this was not the case for the PS_{20}-[Ru]-PEO_{70} one. Therefore, the PS_{20}-[Ru]-PEO_{70} was first dissolved in *N*,*N*-dimethylformamide (DMF) and water was added dropwise to induce aggregation [9]. Later, DMF was removed by dialysis against pure water, which was regularly replaced. This finally resulted in kinetically frozen PS_{20}-[Ru]-PEO_{70} micelles in water. PS_{20}-[Ru]-PEO_{375} micelles were also prepared according to this procedure. Aqueous micelles from a covalent PS_{22}-*b*-PEO_{70} amphiphilic diblock were also prepared by the water/DMF method [4].

Dynamic light scattering

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. The samples were prepared in bidistilled and filtered water and used without further filtering for the DLS measurements. The experimental intensity autocorrelation function was measured and analyzed by the CONTIN routine as described elsewhere [10]. Each DLS measurement was repeated at least ten times. Very good reproducibility was found between these independent measurements, the standard deviation being less than 2%. In the following, D_h refers to the mean hydrodynamic diameter calculated for each population in the CONTIN histogram.

Cryo-TEM

The samples were prepared using a vitrification robot—Vitrobot—in which the relative humidity was kept close to saturation to prevent water evaporation from the sample [11]. A 3-μl drop of the solution was placed on a carbon-coated lacey substrate supported by a TEM 300-mesh copper grid (Ted Pella). After automatic

blotting with filter paper, in order to create a thin liquid film over the grid, the grid was rapidly plunged into liquid ethane at its melting temperature. This resulted in a vitrified film. The vitrified specimen was then transferred under a liquid nitrogen environment to a cryogenic holder (model 626, Gatan, Warrendale, PA) into the electron microscope, Tecnai 20—Sphera (FEI), operating at 200 kV with a nominal underfocus of 2–4 μm. The working temperature was kept below −175 °C, and the images were recorded on a Gatan 794 MultiScan digital camera and processed with Digital Micrograph 3.6.

Results and discussion

Micelles from the PS_{20}-[Ru]-PEO_{70} sample were investigated by DLS (Fig. 2a) and showed a bimodal distribution. The mean D_h of the population attributed to individual micelles (65 nm) is not in agreement with the expected value for spherical micelles formed by such low molecular weight amphiphilic block copolymers [1]. In this respect, a previous investigation reported D_h in the 15–25-nm range for PS-*b*-PEO micelles with block lengths in the same range as the samples under consideration [12]. In complete agreement with these observations, one population of much smaller objects (18 nm) was obtained for the covalently bonded PS_{22}-*b*-PEO_{70} copolymer (Fig. 2b, Table 1).

A longer PEO block (PEO_{375}) was associated with the PS_{20} hydrophobic block, resulting in the PS_{20}-[Ru]-PEO_{375} amphiphilic copolymer (Fig. 1). Micelles were prepared by direct dissolution of this bulk sample in water, and were characterized by DLS as shown in Fig. 2c. Once again, the CONTIN size distribution histogram revealed a bimodal distribution of aggregates. For the sake of comparison, PS_{20}-[Ru]-PEO_{375} micelles were also prepared by the water/DMF method (see Experimental part). This resulted again in a bimodal distribution of objects, as shown in Fig. 2d. It is worth noting that the two different methods utilized for PS_{20}-[Ru]-PEO_{375} micelles did not yield exactly the same micelles/aggregates: smaller individual micelles were observed whenever PS_{20}-[Ru]-PEO_{375} was dissolved directly in water. This difference highlights the effect of the preparation method on the micellar characteristic

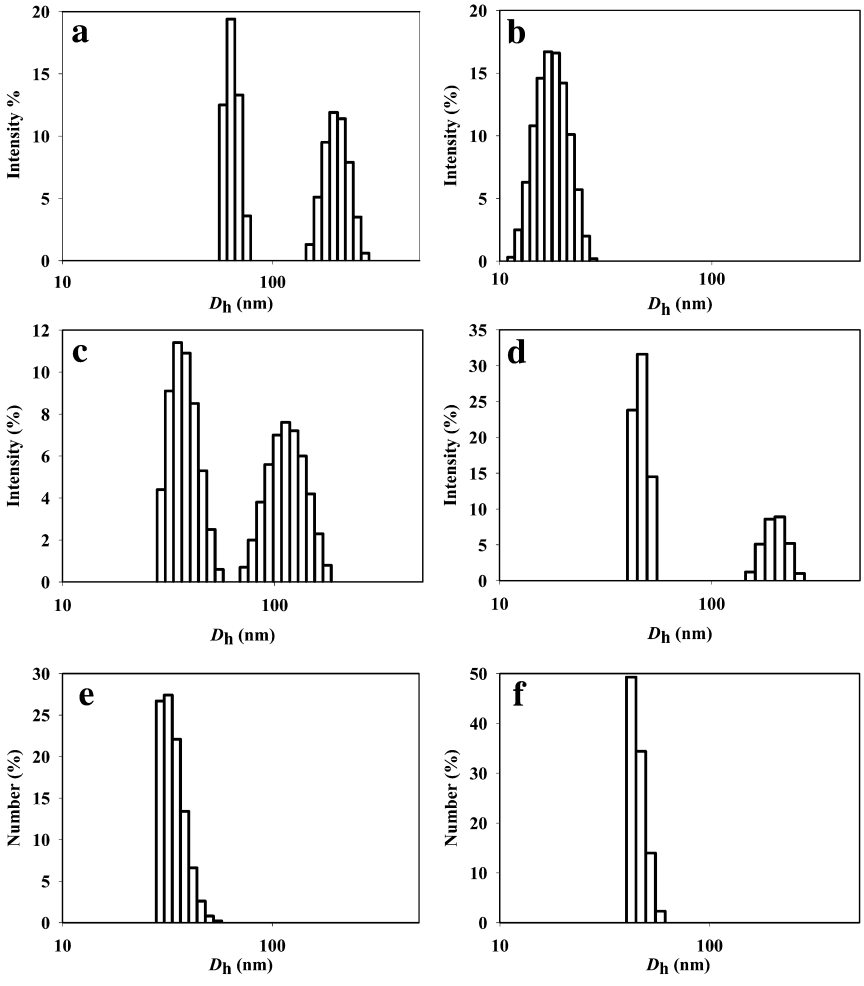

Fig. 2 CONTIN size distribution (intensity percentage) for the PS_{20}-[Ru]-PEO_{70} micelles (**a**), PS_{22}-*b*-PEO_{70} micelles (**b**), PS_{20}-[Ru]-PEO_{375} micelles initially solubilized in water (**c**) or in DMF and later dialyzed against pure water (**d**). Number percentages were calculated for the different populations of the samples represented in **c** and **d** and are shown in **e** and **f**, respectively. The concentration for all the samples was 0.5 wt%

Table 1 Characteristic sizes (nm) of metallo-supramolecular and covalent- polystyrene (*PS*)–poly(ethylene oxide) (*PEO*) copolymers as measured by dynamic light scattering (*DLS*), cryogenic transmission microscopy (*cryo-TEM*) and atomic force microscopy (*AFM*)

	PS_{20}-[Ru]-PEO_{70}	PS_{22}-*b*-PEO_{70}	PS_{20}-[Ru]-PEO_{375}	
			From *N*,*N*-dimethylformamide	From H_2O
DLS, D_h[a]	65, 202	18	47, 209	32, 113
Cryo-TEM (PS core)	10	10	~10 (broad)	10
AFM (PS core)	10[b]	–	–	–

[a]The mean D_h of the first and second population are indicated
[b]From Ref. [3]

features. In this respect, micelles close to the thermodynamic equilibrium are expected by the direct dissolution method, while kinetically frozen micelles are formed by the water/DMF method.

Previous TEM investigations on the dried PS_{20}-[Ru]-PEO_{70} micelles revealed polydisperse spherical entities without visible internal structure that were attributed either to micelles for the small objects or to aggregates for the large one. The cryo-TEM images of the metallo-supramolecular PS_{20}-[Ru]-PEO_{70} and covalent PS_{22}-*b*-PEO_{70} micelles are shown in Fig. 3. Individual micelles containing a PS core are imaged in both samples. The contrast essentially comes from the high electron density of the PS core with respect to the background. The core appears as a 10-nm diameter dark sphere for both samples. This diameter is in good agreement with previous atomic force microscopy investigations on the same sample [3] and molecular calculations (the mean end-to-end distance of the PS block is $0.067\times2{,}000^{1/2} = 3$ nm [13], the degree of stretching of the PS chains in the core is accordingly $5/3 = 1.6$). This indicates that the presence of the charged bis(terpyridine)ruthenium complexes has no influence on the size of the PS cores. The PEO corona could not be easily distinguished for both samples; however, clusters of approximately 20 individual micelles are clearly and reproducibly seen for the metallo-supramolecular PS_{20}-[Ru]-PEO_{70} micelles (Fig. 3a), while only individual micelles are observed for the covalent PS_{22}-*b*-PEO_{70} system (Fig. 3b), in line with the DLS results (Table 1). The cryo-TEM images strongly suggest that individual micelles aggregate into clusters. Clustering of micelles is also reported as a result of the thickness gradient of the biconcave vitrified film during cryo-TEM sample preparation (crowding) [14, 15]. This possibility is unlikely in the present case since the clustering occurs away from the thicker part of the film (Fig. 3a). For the covalent derivative (PS_{22}-*b*-PEO_{70}), no micellar clustering was imaged (Fig. 3b) as expected from individual, noninteracting micelles if proper sample preparation techniques are applied. Therefore, the clusters observed in the cryo-TEM image (Fig. 3a) could not be an artifact due to sample preparation. The short-range "crystalline" ordering in the cluster (Fig. 3a) is not sufficient to generate a crystalline signature in X-ray or electron diffraction.

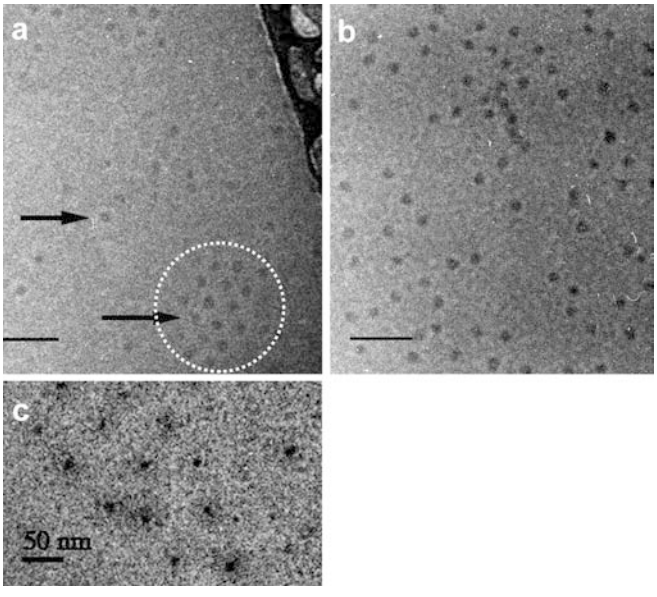

Fig. 3 Cryogenic transmission electron microscopy micrographs of **a** PS_{20}-[Ru]-PEO_{70} micelles (*arrows* indicate an individual micelle and a small cluster of micelle), **b** PS_{22}-*b*-PEO_{70} micelles and **c** PS_{20}-[Ru]-PEO_{375} micelles prepared by direct dissolution in water. Concentration 0.5 wt%

At this point, it should be recalled that cryo-TEM images directly show the numbers of micelles and aggregates. This is not true for the CONTIN size distribution histograms shown in Fig. 2a–d, which represent the percentage of scattered intensity for each population. Since larger aggregates scatter much more light than smaller ones, the histograms of Fig. 2a–d overestimate the number of aggregates [10]. In order to allow a correct comparison between cryo-TEM and DLS results, it is desirable to have number percentages of each population in the DLS histograms. This was performed by considering that the scattering intensity is proportional to the cubic root of the molar mass of the diffusing species. Taking this rough approximation into account and a density of 1 for both populations, number percentages were calculated for micelles and aggregates. This is illustrated in Fig. 2e and f, which are the

"number" counterparts of the "intensity" ones shown in Fig. 2c and d. It then appears that the number of large aggregates should be very low (zero after the rough calculation). Therefore, the smaller size population observed in the CONTIN size distribution histogram of the PS_{20}-[Ru]-PEO_{70} sample could integrate individual micelles together with the small clusters observed by cryo-TEM. The calculation performed by the CONTIN routine has a resolution too low to allow the visualization of individual micelles from small clusters. That could explain why the mean D_h associated with this population (65 nm) is much larger than that measured for the covalent PS_{22}-*b*-PEO_{70} sample (18 nm).

Cryo-TEM was also performed on both PS_{20}-[Ru]-PEO_{375} samples mentioned previously. Individual micelles were observed for both samples. The PS_{20}-[Ru]-PEO_{375} sample prepared via the water/DMF method gave similar DLS results (Fig. 2) to the PS_{20}-[Ru]-PEO_{70} micelles [3], although the individual micelles had a broad size polydispersity. Spherical micelles with a core–shell structure were imaged for the PS_{20}-[Ru]-PEO_{375} micelles prepared by direct dissolution in water (see individual micelles in Fig. 3c). The external diameter of the core–shell PS_{20}-[Ru]-PEO_{375} micelles (35 nm) is in good agreement with DLS results. A recent cryo-TEM investigation of micelles containing a PEO corona revealed that a certain minimal degree of polymerization of the PEO corona (around 113) was required to observe a core–shell structure [16]. For the PS_{20}-[Ru]-PEO_{70} micelles, it is not possible to observe a clear-cut core–shell structure using cryo-TEM, as already reported for other short block systems [17].

Conclusions

DLS and cryo-TEM were combined in order to obtain detailed information on the association behavior of amphiphilic PS_{20}-[Ru]-PEO_x metallo-supramolecular micelles in water. Two different species were identified by cryo-TEM for the metallo-supramolecular samples: individual monodisperse spherical micelles and small clusters of micelles. DLS data also revealed two populations: micelles and a very small proportion of large clusters. For DLS results, the population of the micelles is, however, believed to be formed by individual micelles and small clusters of micelles in line with the results obtained by cryo-TEM imaging. Comparison with the covalent PS_{22}-*b*-PEO_{70} counterpart showed that only individual micelles are formed in the covalent sample and that the diameter of the PS core is the same for the covalent and metallo-supramolecular samples. Increasing the length of the PEO block has no effect on the core dimension; nevertheless, it allows visualization of the PEO corona by cryo-TEM.

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