Discovering new block terpolymer micellar morphologies†

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The self-assembly of a new type of triblock terpoly(2-oxazoline) was investigated in water revealing vesicular and aggregated cylindrical micellar structures.

The self-assembly of amphiphilic block copolymers in a selective solvent has been intensely studied, since it may give rise to many potential applications in nanotechnology. Their basis would be the ability of block copolymers to form micellar nanostructures with predetermined sizes and well-defined shapes, including spherical, cylindrical and vesicular morphologies.[1] Most of the systems investigated up to now are based on amphiphilic AB diblock copolymers. However, the structures available from these copolymers only allow the partitioning of space into a soluble and an insoluble compartment. In contrast, much more complex structures are accessible by the micellization of ABC triblock terpolymers with two immiscible hydrophobic blocks.[2] Prominent examples using this approach are found in previous work by Wooley, Pochan *et al.*,[3] Lodge *et al.*,[4] Laschewsky *et al.*,[5] and Liu *et al.*,[6] among others. Such micelles have also been discussed in the context of bioinspired and biomimicking approaches since they may contain multicompartmentalized micellar cores: several miniaturized compartments with different properties eventually allowing selective functionalization, in analogy to multi-domain proteins and cell organelles.

In designing multicompartment micelles based on triblock terpolymers, it is most important to establish repulsion between each pair of blocks that should segregate into separate nanodomains. In this respect, the preferred method to create distinct compartments in polymeric micelles is the use of block copolymers containing both non-soluble hydrocarbon and fluorocarbon segments, associated with a hydrophilic sequence.[4,5]

In this context, poly(2-oxazoline)s are a promising class of polymers for the preparation of well-defined block copolymers. The living character of the cationic ring-opening polymerization of 2-oxazolines, under appropriate polymerization conditions,[7] allows the creation of tailor-made triblock terpolymers, which contain both fluorocarbon and hydrocarbon segments. Poly(2-oxazoline)s additionally possess other attracting features: (i) the ease of variation of the side group, enabling the formation of poly(2-oxazoline)s which exhibit different characters (lipophilic, hydrophilic, fluorophilic, hard, soft, crystallizable...),[8] and (ii) their biocompatibility. During the last few years, in particular poly(2-methyl-2-oxazoline)s as well as poly(2-ethyl-2-oxazoline)s and their copolymers emerged to be of significant interest for biological and pharmaceutical applications.[8,9] Furthermore, poly(2-ethyl-2-oxazoline) has been approved by the Food and Drug Administration as indirect food additive.[10] In the field of triblock copoly(2-oxazoline)s, Komenda and Jordan used a fluorinated 2-oxazoline besides 2-methyl-2-oxazoline and 2-nonyl-2-oxazoline to form triblock poly(2-oxazoline)s containing both lipophilic and fluorophilic blocks. The length of the units, however, did not exceed 10 repeating units, and their self-assembly behavior was not reported.[11]

To the best of our knowledge, the preparation of amphiphilic triblock terpoly(2-oxazoline)s with longer fluorine-containing blocks has not yet been reported. Recently we described the synthesis of 2-(fluoro-phenyl)-2-oxazolines and the successful polymerization of these monomers by using microwave irradiation as heating source.[12] In the present work, this experience has been used for the preparation of a new triblock terpolymer: we have synthesized a linear terpoly(2-oxazoline) containing fluorinated 2-(2,6-difluorophenyl)-2-oxazoline (ODFOx), lipophilic 2-(1-ethylheptyl)-2-oxazoline (EPOx), and hydrophilic 2-ethyl-2-oxazoline (EtOx) blocks (see Fig. 1 for the schematic representation of the chemical structure). The EPOx and ODFOx monomers are not commercially available and were synthesized according to a two-step procedure described in literature.[12,13]

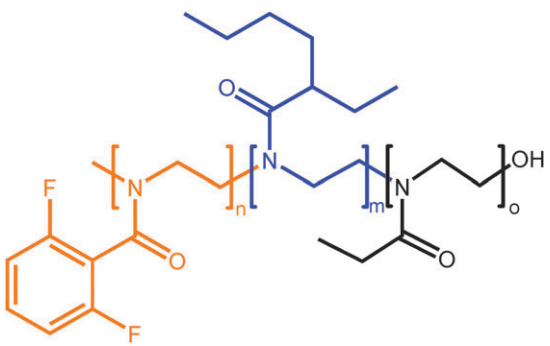

Fig. 1 Schematic representation of the chemical structure of the poly($ODFOx_{23}$-*b*-$EPOx_{28}$-*b*-$EtOx_{49}$) triblock terpolymer.

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The present triblock terpoly(2-oxazoline) was synthesized using the sequential monomer addition technique enabled by the living character of the cationic ring-opening polymerization of the 2-oxazolines.[8] The resulting terpolymer was purified by means of preparative size exclusion chromatography and characterized using ^{1}H NMR spectroscopy, size exclusion chromatography and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which evidenced the formation of a well-defined triblock terpolymer with a polydispersity index of 1.12.

The self-assembly of the poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) triblock terpolymer, in which the numbers in subscript refer to the calculated mole percentages of each block based on ^{1}H NMR spectroscopy, was studied in aqueous medium. Solutions obtained by direct dissolution of the triblock terpolymer in water were analyzed by dynamic light scattering (DLS) and cryo-transmission electron microscopy (cryo-TEM). DLS measurements were performed on the solutions at terpolymer concentrations ranging from 1 to 0.1 g L^{-1} and at angles varying from 50° to 140°. In all measurements, large and polydisperse objects were detected, and multimodal distributions were found in the CONTIN analysis of the DLS data. Moreover, a non-linear relationship was deduced from the plot of the mean decay rate *versus* the square of the scattering vector, which is evidence for complex translational motions not compatible with spherical micelles. These results indicate the formation of polydisperse and/or non-spherical micelles.

Cryo-TEM is the method of choice to unravel complex micellar structures and was therefore employed in this study. Fig. 2 shows typical images collected from the poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) sample. Obviously, several intricate mixed morphologies are present.

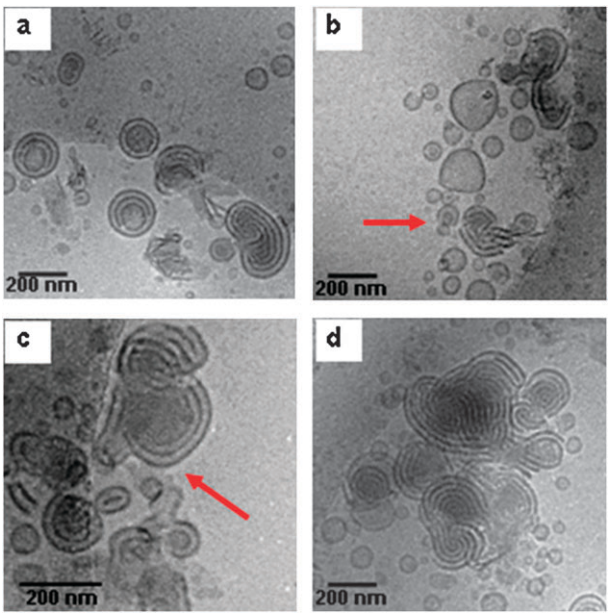

Fig. 2 Cryo-TEM pictures of micellar aggregates formed by the poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) triblock terpolymer in water. Red arrows highlight the appearance of vesicular sacks from rolled-up cylindrical micelles.

From a large number of pictures (more than 100) collected on this sample, the formation of two main morphologies can be deduced: vesicles with an average diameter ranging from 50 to 200 nm, and rolled-up structures with characteristic sizes in the range from 150 to 250 nm. In all of these structures, a darker layer is observed, which is attributed to ODFOx-rich micellar compartments since no staining agent was used. Thus, the differences in contrast reflect the (partial) segregation of the fluorine-containing polymer blocks yielding a higher electronic contrast in TEM due to the high electron density of the fluorine substituents.[4] Since the weight fraction of the hydrophilic EtOx block in the investigated triblock terpolymer is 33 wt%, the formation of vesicles in water could be expected.[14] The inner layer of the vesicles should be formed by both EPOx and ODFOx blocks; according to the block order in the terpolymer this layer should consist of an EPOx–ODFOx–EPOx alternating structure. The EtOx chains should point towards the inside and outside of the vesicles. Demixing of the EPOx and ODFOx blocks might be expected based on the existence of two separate T_g's in a mixture of both homopolymers (see ESI†), suggesting the formation of segregated microphases inside the hydrophobic layer and thus a core-shell-corona structure which might be compartmentalized as depicted in Fig. 3.

In order to obtain more information about the structure of the other observed morphology, *i.e.* rolled-up micellar aggregates, additional cryo-TEM investigations were performed by tilting the sample in the microscope. These experiments confirmed that the rolled-up aggregates are flat and thus non-spherical structures. Further evidence of the flatness of these structures can be seen in Fig. 2d where several of the aggregates are lying on top of each other. Superimposing spherical structures with a diameter of 200 nm would indeed not be possible in a cryo-TEM specimen for which the thickness of the frozen menisci is about 200 nm. These observations lead to the suggestion that the rolled-up structures are originating from cylindrical micelles which consist of an ODFOx central core surrounded by an EPOx water-insoluble shell and an EtOx corona (Fig. 4). Based on the coexistence of cylindrical micelles and vesicles, it was suggested by other authors that the

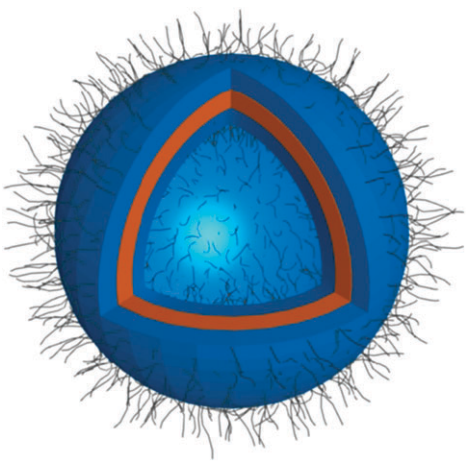

Fig. 3 Potential representation of a vesicle of poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) triblock terpolymer in water (EtOx in black, EPOx in blue and ODFOx in orange).

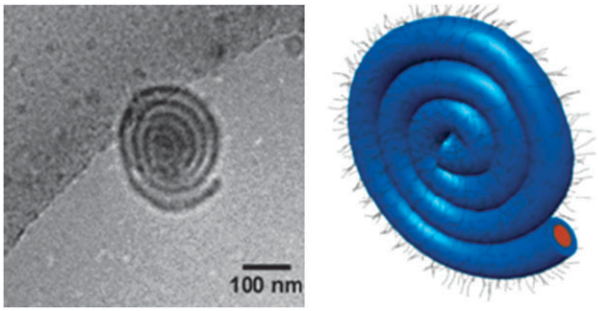

Fig. 4 Left: cryo-TEM picture of a spiral-like micellar aggregate from the poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) triblock terpolymer in water. Right: schematic representation of a potential spiral-like aggregate made by the poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) triblock terpolymer in water (EtOx in black, EPOx in blue and ODFOx in orange).

formation of vesicular structures can result from the merging of such cylindrical micelles.[15] The rolled-up cylindrical micelles could thus be proposed to be metastable intermediates towards the formation of vesicles. Indeed, this transition is suggested by some of the cryo-TEM pictures (see Fig. 2, red arrows) where vesicular sacks seem to be emerging from rolled-up cylindrical micelles. As far as we know, the formation of such self-assembled rolled-up morphologies has not been reported before.

The folding of individual cylindrical micelles into rolled-up aggregates could be related to the limited solubility of the EtOx block in water as a possible driving force for this process.[16] The high flexibility of the cylindrical micelles formed by short EPOx and ODFOx blocks of low T_g would allow the rolling-up of the cylindrical micelles. It is not clear whether only a single long cylindrical micelle rolls-up or whether one rolled-up aggregate is formed by merging of several cylindrical micelles. In a further step, these rolled-up aggregates could reorganize into vesicular structures, which were initially expected based on the polymer composition. It is remarkable that no isolated cylindrical micelles have been observed, indicating that the EtOx coronal blocks tend to minimize their contact with the aqueous phase. In the case of the spiral-like aggregates (see Fig. 4), it seems possible that they originate from one single long cylindrical micelle since no discontinuity can be visualized in their internal structure.

In conclusion, we have studied the aqueous association behavior of a new type of triblock terpolymer based on poly(2-oxazoline)s. For the investigated poly(ODFOx$_{23}$-*b*-EPOx$_{28}$-*b*-EtOx$_{49}$) containing a monomer with a branched hydrophobic side chain (EPOx) as well as a fluorinated hydrophobic monomer (ODFOx), two main morphologies were observed to coexist in water, namely vesicles and flat aggregates of rolled-up cylindrical micelles. The vesicles are proposed to consist of a segregated hydrophobic domain based on the contrast in cryoTEM and the demixing of a mixture of both hydrophobic homopolymers. Besides vesicles, rolled-up spiral-like micellar aggregates were observed, which are proposed to be metastable intermediates in between the transition from cylindrical micelles to vesicles. The limited solubility of the EtOx blocks in water is proposed to induce further aggregation of the initially formed cylindrical micelles while the low T_g of the EPOx block provides sufficient flexibility for rolling-up the cylinders. These two structural paramaters are believed to be the key factors driving the formation of the observed rolled-up hierarchical superstructures.

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