Polymers

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One-Pot Synthesis of Biomimetic Shell Cross-Linked Micelles and Nanocages by ATRP in Alcohol/Water Mixtures**

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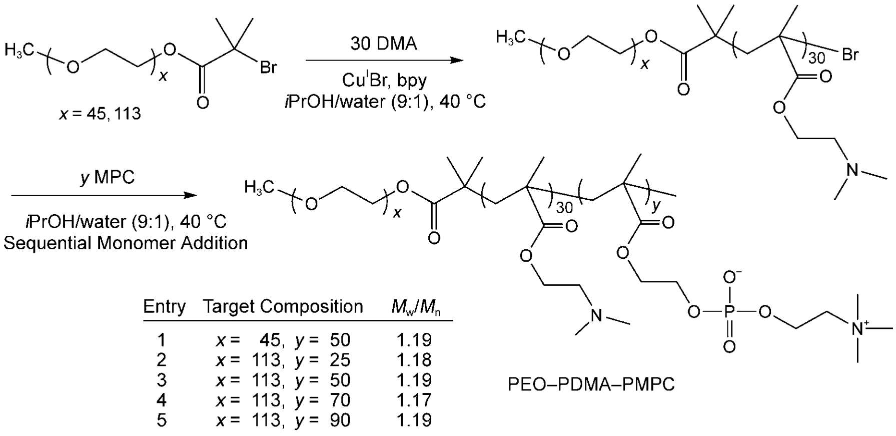
Recently, the rational design of well-defined macromolecular nanostructures with unique architectures and controlled dimensions has become increasingly important, with the self-assembly of block copolymers being of considerable scientific interest.[1] In particular, shell cross-linked (SCL) micelles are covalently stabilized supramolecular structures that combine the desirable properties of micelles, microgels, nanoparticles, and dendrimers.[2] However, to the best of our knowledge, there has been only a single report describing the one-pot synthesis of SCL micelles.[3] So-called "hollow nanocages" can be obtained from SCL micelles by selective degradation and extraction of the core-forming chains to leave a membrane-like shell.[4–7] Microencapsulation of biologically active components within such nanocages has been evaluated for the development of artificial cells.[8]

The phosphorylcholine motif is a significant component of cell membranes. It is well known that phosphorylcholine-based polymers can be used to produce surfaces which are remarkably resistant to protein adsorption and bacterial/cellular adhesion, therefore allowing many biomedical applications to be developed.[9] Over the last decade our research group has shown that atom transfer radical polymerization (ATRP)[10] can be used to prepare low polydispersity block copolymers based on a commercially important biomimetic monomer, namely 2-(methacryloyloxy)ethyl phosphorylcholine (MPC).[11]

In this study we exploit the unusual cononsolvency behavior exhibited by PMPC chains[12] so as to develop only the second example of a one-pot synthesis of SCL micelles. Furthermore, our synthetic strategy also allows the first ever one-pot synthesis of hollow nanocages and, moreover, does not require chemical degradation of the micelle cores.

The one-pot synthesis of SCL micelles based on a novel ABC triblock copolymer using ATRP in a 9:1 *i*PrOH/water mixture at 40 °C is described in Scheme 1 and Figure 1. The triblock copolymer was prepared using a poly(ethylene oxide) macroinitiator (PEO), which was used to polymerize first 2-(dimethylamino)ethyl methacrylate (DMA) and then MPC using the sequential monomer addition method. Controlled polymerization proceeded smoothly to reach almost complete conversion at each stage. The triblock copolymer composition was determined by ^{1}H NMR spectroscopy. The actual compositions are in good agreement with the target compositions



Entry	Target Composition	M_w/M_n
1	$x = 45$, $y = 50$	1.19
2	$x = 113$, $y = 25$	1.18
3	$x = 113$, $y = 50$	1.19
4	$x = 113$, $y = 70$	1.17
5	$x = 113$, $y = 90$	1.19

Scheme 1. ATRP synthesis of PEO$_x$–PDMA$_{30}$–PMPC$_y$ triblock copolymers using sequential monomer addition. bpy = 2,2′-bipyridine.

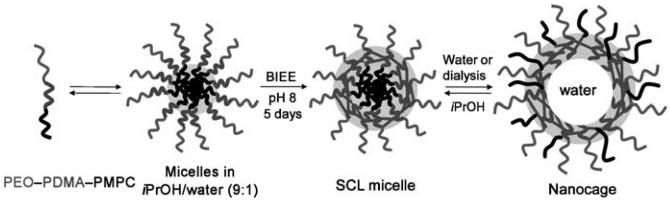


Figure 1. One-pot synthesis of shell cross-linked (SCL) micelles at 10 wt.% solids using a PEO–PDMA–PMPC triblock copolymer.

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and the final copolymer polydispersities were relatively narrow, as judged by size exclusion chromatography (see Scheme 1 and also Figures S1–S3 and Table S1 in the Supporting Information).

The resulting PEO–PDMA–PMPC triblock copolymers underwent spontaneous self-assembly to form PMPC-core micelles in situ as a result of the well-known cononsolvency behavior of PMPC chains in alcohol/water mixtures.[12] Thus in the ^{1}H NMR spectrum of a PEO_{113}–$PDMA_{30}$–$PMPC_{50}$ triblock copolymer (entry 3 of inset Table in Scheme 1; see also Table S1) recorded in D_2O, all the signals corresponding to each of the three blocks were well resolved (Figure 2). In a 3:7 mixture of $[D_8]$*i*PrOH/D_2O, all these copolymer signals were merely shifted upfield relative to HDO. However, on adding further $[D_8]$*i*PrOH to obtain a 9:1 $[D_8]$*i*PrOH/D_2O mixture, the signals corresponding to the PMPC chains broadened significantly, whereas the signals corresponding to PEO and PDMA remained relatively sharp. This indicates that PMPC-core micelles are formed at this particular solvent composition.

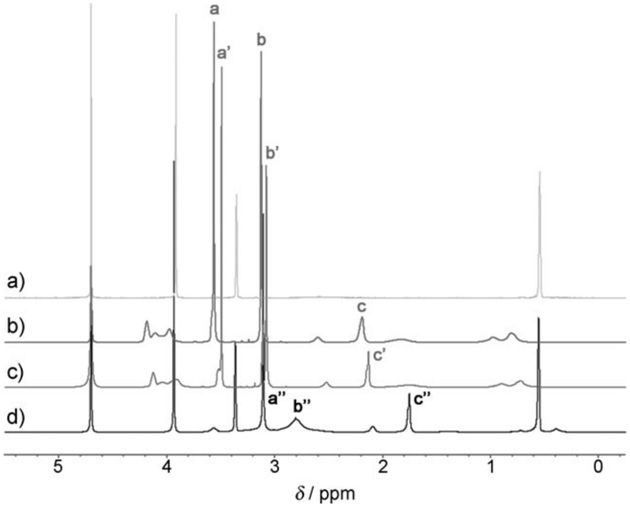

Figure 2. Typical ^{1}H NMR spectra recorded for PEO_{113}–$PDMA_{30}$–$PMPC_{50}$ triblock copolymer (entry 3 in Table S1) in various solvents: a) 9:1 $[D_8]$*i*PrOH/D_2O mixture only (no copolymer); b) D_2O; c) 3:7 $[D_8]$*i*PrOH/D_2O mixture; d) 9:1 $[D_8]$*i*PrOH/D_2O mixture. Signals a, b, and c are characteristic of the PEO, PMPC, and PDMA blocks, respectively. These are shifted by adding $[D_8]$*i*PrOH: a→a′→a″, b→b′→b″, and c→c′→c″.

Dynamic light scattering (DLS) was used to study a PEO_{113}–$PDMA_{30}$–$PMPC_{50}$ copolymer (entry 3 in inset Table of Scheme 1; see also Table S1) dissolved in various *i*PrOH/water mixtures at 0.20 wt.% (Figure 3a). Below an *i*PrOH weight fraction of 0.40, this copolymer is molecularly dissolved and shows an intensity-average hydrodynamic diameter (D_h) of around 8–10 nm, a relatively high polydispersity (μ_2/Γ^2), and a low scattering intensity. On gradual addition of *i*PrOH, micellization occurred at an *i*PrOH weight fraction of approximately 0.50, as indicated by the slightly opaque appearance that is characteristic of micellar solutions. The micelle diameter and polydispersity determined under these conditions were 40 nm and 0.039, respectively. As the mean degree of polymerization of the PMPC block was increased from 25 to 83 (Table S1, entries 2–5), the micelle diameter increased from 32 nm to 51 nm and the polydispersity decreased from 0.188 to 0.025, respectively (Figure S4). Such triblock copolymer micelles are expected to form a three-layer "onionlike" structure, with the PMPC block occupying the micelle core and the PDMA and PEO blocks forming the inner shell and micelle corona layers, respectively. This three-layer structure is consistent with our ^{1}H NMR studies (Figure 2).

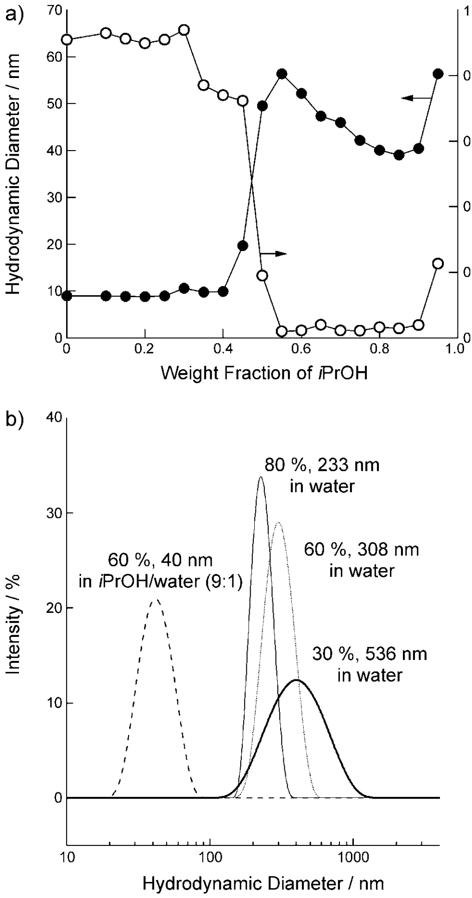

Figure 3. a) Variation of hydrodynamic diameter and polydispersity with solvent composition for a PEO_{113}–$PDMA_{30}$–$PMPC_{50}$ triblock copolymer (entry 3 in Table S1). b) Typical intensity-average particle diameters for SCL micelles in either water or a 9:1 mixture of *i*PrOH/water prepared at various target degrees of cross-linking (%).

SCL micelles were prepared by adding 1,2-bis(2-iodoethoxy)ethane (BIEE) to the micelles prepared directly in situ during the polymerization solution. The addition of BIEE led to quaternization of the DMA residues and hence cross-linking of the inner shell. Here the PEO block acts as a steric stabilizer and minimizes any intermicelle cross-linking.[2b,13] In practice, some undesirable intermicelle fusion occurs at 10 wt.% solids for PEO–PDMA–PMPC triblocks prepared using the PEO_{45} macroinitiator (entry 1 in inset Table in Scheme 1; see also Table S1). For example, DLS studies indicate the formation of SCL micellar aggregation in a 9:1 *i*PrOH/water mixture at a target cross-linking of 80%. Fortunately, using the longer PEO_{113} block as a steric stabilizer was much more effective in preventing intermicelle fusion at 10 wt.% solids. Thus we investigated cross-linking at this copolymer concentration. The actual degree of quaternization was about half of that actually targeted, as indicated by ^{1}H NMR spectroscopy[13] (Figures S5 and S6). Presumably, this lower degree of quaternization is either due to incomplete reaction or possibly due to partial hydrolysis of the iodoethyl groups of the BIEE. For these SCL micelles, zeta potentials (ζ) were also determined by aqueous electrophoresis. In each case the ζ value was positive because of the presence of quaternized nitrogen atoms in the inner shell (Figure S7). Figure 3b compares the effect of varying the target cross-linking on the micelle diameter in either a 9:1 *i*PrOH/water mixture or in water (after dialysis). Under the former conditions, non-swollen SCL micelles with a mean-intensity-average diameter of 40 nm were obtained, and no evidence for any intermicelle fusion was observed. After dialysis against water, these SCL micelles became highly swollen and their final dimensions depended on their target degrees of cross-linking (Figure S8). If shell cross-linking had been unsuccessful, no micelles should exist in pure water since the PMPC block is highly hydrophilic and micelle dissociation would inevitably occur. Figures 4a,b shows the corresponding AFM images. Spherical SCL micelles were obtained at 80% target cross-linking when deposited onto mica from water. This observation suggests that the PMPC chains remain within the cores of the micelles owing to their relatively high degree of cross-linking. Topography (height) measurements determined by AFM also indicated substantial flattening of the cationic SCL micelles on the highly anionic mica surface owing to the strong electrostatic interaction. TEM measurements were consistent with these AFM studies and indicated spherical SCL micelles (Figure S9) whose size depended on the PMPC block length (Figure S10). However, a target cross-linking of only 30% allows the PMPC chains sufficient mobility to migrate into the coronal shells. Thus these SCL micelles form “nanocages” in aqueous solution or toroids when deposited onto mica from water. This conclusion is confirmed by ^{1}H NMR studies (see Figure S5) and cross-sectional AFM profiles, which indicate that the SCL micelle cores become “hollowed out” under these conditions (Figures 4c,d). However, we have not yet analyzed a sufficiently large number of nanocages by AFM to obtain statistically meaningful nanocage size distributions.

In summary, well-defined PEO–PDMA–PMPC triblock copolymers were synthesized using ATRP in 9:1 *i*PrOH/water mixtures. Subsequent cross-linking of the PDMA chains in the inner shells was achieved using BIEE in the same reaction solution. The precise block compositions and target degrees of cross-linking have a significant influence on the swelling behavior of these SCL micelles in water. If the target degree of cross-linking is sufficiently low ($\approx 30\%$), shell cross-linked nanocages can be obtained as the hydrophilic PMPC chains can migrate to join the PEO chains in the coronal layer. However, for higher degrees of cross-linking, the PMPC chains cannot penetrate the inner shell ‘mesh’ and the micelles retain their original spherical morphology. The AFM images of the SCL micelles provided good evidence for both types of nanostructures. This method is an attractive new one-pot route to shell cross-linked nanocages, as it avoids chemical degradation of the core-forming blocks.

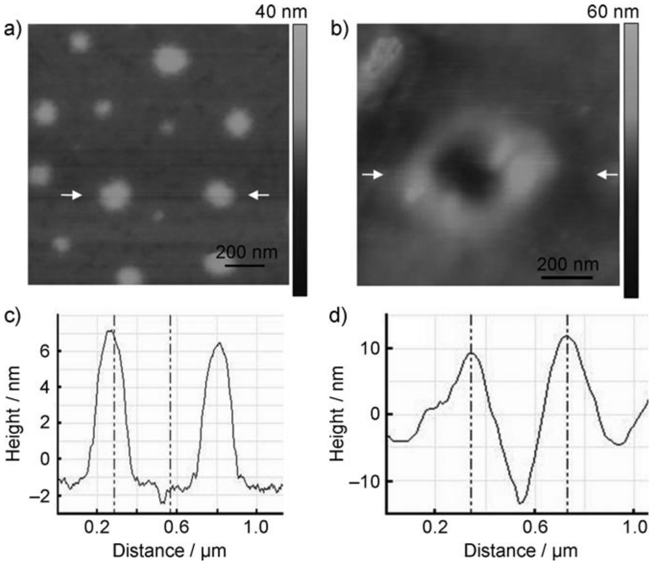

Figure 4. Tapping-mode AFM (height) images of a) the spherical SCL micelles at 80% target cross-linking in water and b) the hollow nanocages obtained at 30% target cross-linking in water deposited onto mica (entry 3 in Table S1). Cross-sectional profiles c) and d) were obtained from the images shown in a) and b), respectively. Other hollow nanocages on mica are shown in Figure S9.

Experimental Section

Typical protocols for the controlled polymerization of MPC using the PEO_{45}–Br and PEO_{113}–Br macroinitiators in *i*PrOH and a 9:1 *i*PrOH/water mixture, respectively: for PEO_x–$PDMA_{30}$–$PMPC_y$, PEO_x–Br (either 505 mg for $x=45$ or 1.26 g for $x=113$, 0.253 mmol, 1 equiv), bpy (79 mg, 0.505 mmol, 2 equiv), DMA (1.19 g, 7.58 mmol) was dissolved in either *i*PrOH (5.0 mL) or a 9:1 *i*PrOH/water mixture (5.0 mL). After purging with nitrogen for 20 min, the Cu^IBr catalyst (36.0 mg, 0.253 mmol, 1 equiv) were added to the stirred solution under nitrogen. The reaction mixture immediately became dark brown. The polymerization was monitored by ^{1}H NMR spectroscopy. After high DMA conversion had been achieved (> 97%), MPC (1.86–6.71 g; 6.32- 22.74 mmol) in *i*PrOH/water (9:1; 7.5 mL) was added at 40°C. In the case of polymerization using PEO_{45}–Br, the solvent composition of the reaction mixture was adjusted to a 9:1 *i*PrOH/water mixture at this stage. The reaction mixture became progressively more viscous. After a certain period, ^{1}H NMR analysis indicated that more than 99% of the MPC had polymerized (disappearance of vinyl signals). On exposure to air, the reaction solution turned blue, indicating aerial oxidation of the Cu^I catalyst. The solution was diluted to 10 wt.% solids using a 9:1 mixture of *i*PrOH/water and the pH was adjusted to around pH 8. Shell cross-linking was achieved by adding BIEE to the same reaction mixture without purification and stirring the solution for at least 5 days at 20°C. Subsequently, purification by chromatography on silica gel removed the spent ATRP catalyst. Target degrees of quaternization were calculated from the $[BIEE]_0/[DMA]_0$ molar ratio, allowing for the bifunctionality of the BIEE reagent. The degree of quaternization, which is related to the actual degree of cross-linking, was estimated using ^{1}H NMR spectroscopy.[13]

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