

Synthesis of Zwitterionic Shell Cross-Linked Micelles

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Wooley and co-workers^{1–4} and also Ding and Liu⁵ have recently reported the synthesis of shell cross-linked “knedel” (SCK) micelles. Various applications, in areas as diverse as solubilization, catalysis, fillers, coatings, and delivery, have been proposed for these remarkable new nanoparticles.¹ However, in all of these studies the micelle cores were based on polystyrene or polyisoprene and are therefore permanently hydrophobic. Very recently, we described the synthesis of SCK micelles with temperature-variable core hydrophilicity.⁶ This was achieved by reacting partially quaternized 2-(dimethylamino)ethyl methacrylate-*N*-(morpholino)ethyl methacrylate (DMAEMA–MEMA) diblock copolymer micelles with a bifunctional cross-linker, 1,2-bis-(2-iodoethoxy)ethane (BIEE), in aqueous solution at 60 °C. Under these conditions the MEMA block is above its cloud-point and therefore forms the micelle core. On being cooled to 25 °C, the hydrophobic MEMA micelle cores become hydrated. Thus, the cores of these SCK micelles can be reversibly hydrated or dehydrated, depending on the solution temperature.

Herein we report the first synthesis of zwitterionic SCK micelles. These new nanoparticles also have hydrophilic micelle cores. Depending on the reaction conditions used, two classes of zwitterionic SCK micelles can be obtained: **Type I** micelles, which have anionic cores and cationic coronas, and **Type II** micelles, which have cationic cores and anionic coronas (see Figure 1). Both micelle types exhibit isoelectric points (iep's) and thus can be precipitated from aqueous solution by judicious adjustment of the solution pH.

The two 2-(dimethylamino)ethyl methacrylate-2-tetrahydropyranyl methacrylate (DMAEMA–THPMA) diblock copolymer precursors were synthesized as described previously.⁷ One had a DMAEMA content of 51 mol % and an overall M_n of 34 000 [determined by GPC using poly(methyl methacrylate) standards], and the other had a DMAEMA content of 43 mol % and an overall M_n of 42 400. These linear copolymers are readily converted into zwitterionic 2-(dimethylamino)ethyl methacrylate-methacrylic acid (DMAEMA–MAA) blocks by acid hydrolysis.⁷

Depending on the reaction *sequence*, two types of zwitterionic SCK micelles could be prepared from the DMAEMA–THPMA block copolymer precursors using the BIEE cross-linker (see Figure 1). For the synthesis of the **Type I** SCK micelles, which have anionic MAA cores and cationic DMAEMA coronas, conventional micelles of the DMAEMA–THPMA precursor were initially formed in aqueous solution using 5 vol % THF. This cosolvent ensured that the hydrophilic–hydrophobic precursor

block copolymer forms well-defined micelles with THPMA cores.⁸ Shell cross-linking of the DMAEMA residues in the micelle coronas was achieved at 25 °C and pH 10 by quaternization using BIEE. Finally, the THP groups were removed by acid hydrolysis⁷ to obtain the zwitterionic SCK micelles. Initially, the methacrylic acid residues were obtained in their unionized form, but addition of base leads to carboxylate formation. **Type II** SCK micelles (cationic cores and anionic coronas) were synthesized by first removing the THP groups from the DMAEMA–THPMA precursor by acid hydrolysis to give a DMAEMA–MAA block copolymer. This zwitterionic block copolymer forms micelles with DMAEMA cores and MAA coronas in aqueous solution at elevated temperature.⁷ Shell cross-linking of these micelles was achieved at 60 °C using BIEE. It is noteworthy that this shell cross-linking chemistry involves *esterification* of the MAA residues, rather than quaternization.⁹ Control experiments with MAA and DMAEMA homopolymers confirmed that the MAA residues are much more reactive toward BIEE than the DMAEMA residues (MAA homopolymer reacted near-quantitatively within 5–10 min, whereas only a low degree of quaternization was obtained for DMAEMA homopolymer after several days). Relatively high degrees of shell cross-linking (typically 40–50%) were sought in order to ensure that stable zwitterionic SCK micelles were obtained. However, given the pronounced difference in reactivities observed, substoichiometric quantities of BIEE (based on the concentration of MAA residues present) should nevertheless ensure that any cross-linking between the DMAEMA residues in the cores of the **Type II** micelles is negligible.

Proton NMR spectroscopy¹⁰ was used to determine the compositions of the precursor copolymers and also to verify that the THPMA block initially formed the nonsolvated micelle cores during the synthesis of the **Type I** SCK micelles. In addition, complete deprotection of the THPMA residues was confirmed, since a commensurate increase in the methacrylate backbone signal due to the now-solvated MAA residues was observed after acid hydrolysis. Finally, variable temperature NMR studies during the synthesis of the **Type II** SCK micelles verified that the DMAEMA blocks were substantially de-solvated at 60 °C prior to shell cross-linking. On cooling to 25 °C, the DMAEMA signals reappeared as the SCK micelle cores became hydrated, as expected.⁷ FTIR spectroscopy studies¹¹ of the dried **Type I** SCK micelles proved useful for assessing the conversion of the THPMA residues into MAA residues. After acid hydrolysis, the micelles were isolated from *alkaline* solution. Two bands, which were absent in the IR spectrum of the DMAEMA–THPMA micelles prior to hydrolysis, were observed at 1555 and 1450 cm⁻¹. These new features are characteristic of carboxylate anion and indicate that deprotonation of the acidic micelle cores can be easily achieved.

Dilute suspensions of the two zwitterionic SCK micelles (**Types I and II**) were dried onto TEM grids and examined directly (without staining) using an Hitachi 7100 instrument. Image analysis of a **Type I** SCK micelle indicated a mean number-average diameter of approximately 18 nm. Allowing for dehydration and polydispersity effects, this value is in reasonable

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(10) NMR spectra were acquired using a 250-MHz spectrometer. Block compositions were determined using CDCl₃, the extent of deprotection of the THPMA residues was assessed using a 95:5 D₂O:THF-*d*₆ mixture, and variable temperature NMR studies were conducted between 25 and 60 °C in D₂O at pH 10.

(11) FTIR spectra (64 scans; 4 cm⁻¹ resolution) of the dried SCK micelles were recorded using a Nicolet Magna 550 spectrometer equipped with a diamond ATR Golden Gate accessory.

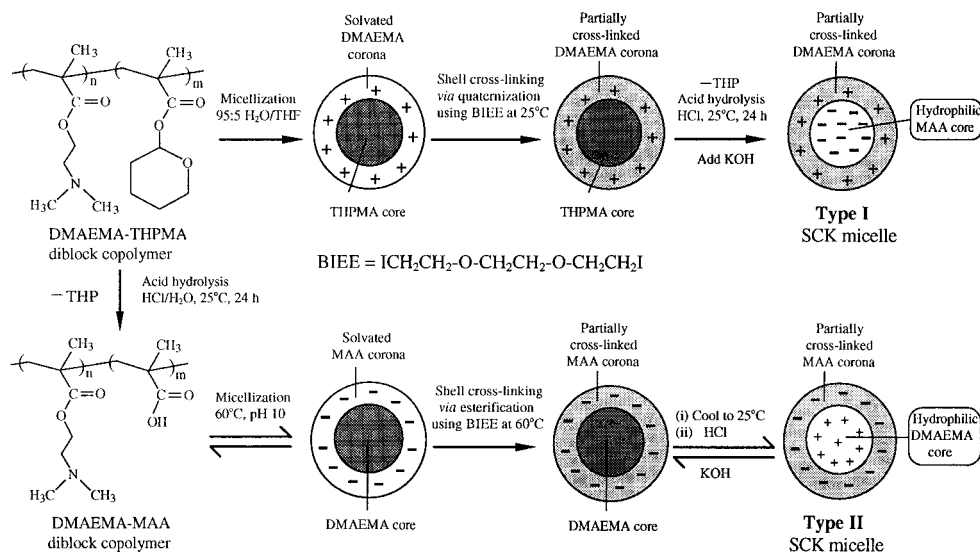


Figure 1. Reaction scheme for the synthesis of **Type I** and **Type II** zwitterionic SCK micelles. Note that the same block copolymer precursor and cross-linking reagent (BIEE) is used in each route.

agreement with the intensity-average diameter of 33 nm obtained from dynamic light-scattering measurements.¹² TEM studies of the **Type II** zwitterionic SCK micelles suggested somewhat larger micelles of around 25 nm diameter, which is reasonably consistent with a diameter of 40 nm obtained from dynamic light scattering. The larger size of the **Type II** micelles is probably related to the relatively hydrated nature of the DMAEMA cores prior to shell cross-linking.⁷

One fascinating aspect of these zwitterionic SCK micelles is that they exhibit iep's. Thus, at a certain critical pH (the iep) the micelles become electrically neutral and are precipitated quantitatively from aqueous solution. Addition of acid or base leads to complete redissolution of the micelles. This precipitation–dissolution behavior is well-known for conventional proteins and their synthetic analogues.¹³ However, as far as we are aware, it has not been reported for micelles, SCK micelles, or nanoparticles. The precise iep depends mainly on the relative block composition (i.e., the DMAEMA/MAA molar ratio). A secondary factor is the degree of shell cross-linking: quaternization of the DMAEMA residues with BIEE leads to a permanent increase in the cationic charge density of the **Type I** micelles, whereas esterification of the MAA residues with BIEE leads to an irreversible reduction in the anionic charge density of the **Type II** micelles. Aqueous electrophoresis measurements¹⁴ are a useful method for determining the iep of an SCK micelle. Three zeta potential vs pH curves are shown in Figure 2. The first curve (●) represents a **Type I** SCK micelle synthesized using a 51:49 DMAEMA–THPMA copolymer prior to removal of the THP groups from the micelle cores. As expected, no iep is observed, and the surface charge remains cationic across the entire pH range since the only ionizable groups are the DMAEMA residues in the micelle coronas. The reduction in cationic surface charge observed at around pH 8.5 corresponds approximately to the pK_a reported for DMAEMA homopolymer by Hoogveen et al.¹⁵ After deprotection of the THPMA residues in the micelle core, the resulting zwitterionic micelles (○) have an iep at approximately pH 7.60, as expected. Given the relatively high degree of shell

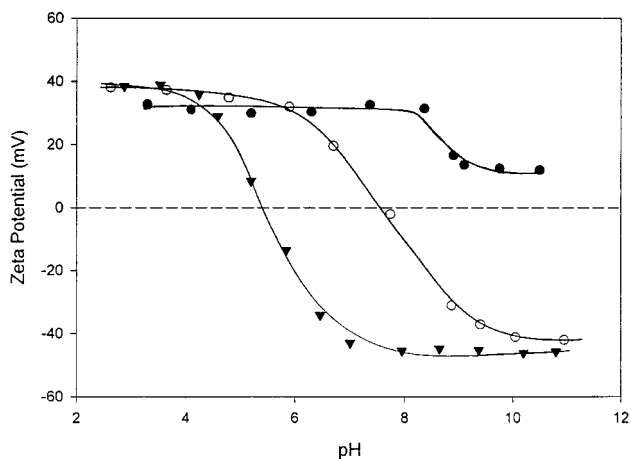


Figure 2. Zeta potential vs pH curves obtained for: (a) **Type I** SCK micelles prepared using a 51:49 DMAEMA–THPMA copolymer prior to acid hydrolysis (●); (b) **Type I** zwitterionic SCK micelles prepared using the same copolymer after acid hydrolysis (○); (c) **Type II** zwitterionic SCK micelles prepared using a 43:57 DMAEMA–MAA copolymer (▲).

cross-linking of the DMAEMA residues, this iep is quite close to the value of 6.74 reported earlier for the identical *linear* DMAEMA–MAA block copolymer.⁷ Similarly, **Type II** SCK micelles synthesized using a 43:57 DMAEMA–MAA block copolymer have an iep at pH 5.50 (▲). A lower iep is expected in this case due to the increased proportion of MAA residues in the copolymer.

Clearly this unusual aqueous solution behavior offers considerable scope for the isolation, purification, and harvesting of zwitterionic SCK micelles. Since these nanoparticles contain both acidic (MAA) and basic (DMAEMA) binding sites they are expected to be novel delivery vehicles for a wide range of ionic “actives” (e.g., drugs or pesticides). They may also prove to be useful as novel DNA vectors for gene therapy.¹⁶

Supporting Information Available: TEM of **Type I** zwitterionic SCK micelles prepared using a 51:49 DMAEMA–THPMA block copolymer (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(12) Malvern 4700 photon correlation spectrometer equipped with an 80-mW argon ion laser. All experiments were conducted at 25 °C on dilute aqueous solutions. The scattered light was detected at 90° and analyzed using CONTIN software.

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