Block Copolymers

Preparation of Shell Cross-Linked Micelles by Polyelectrolyte Complexation**

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Covalent stabilization of micelles and vesicles has attracted increasing attention in recent years.[1–4] In particular, shell cross-linked (SCL) micelles are potentially useful nanosized vehicles for the delivery of various actives (e.g., drugs, fragrances, pesticides).[1–3] The first example of SCL micelles was described by Wooley and co-workers, who oligomerized pendent styrene groups by using radical chemistry.[1a] Our group has reported the use of 1,2-bis(2-iodoethoxy)ethane (BIEE) as a bifunctional cross-linker for the covalent stabilization of SCL micelles.[2] However, in view of its cost, toxicity, limited water solubility, and likely mutagenicity, BIEE is unlikely to be employed in commercial applications of SCL micelles, particularly in the biomedical field. The

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cross-linking strategies described by other workers are also rather unsatisfactory. For example, Wooley's group[1e] now favour the use of carbodiimide coupling chemistry to link carboxylic acid groups through diamines but this method suffers from expensive reagents and requires purification to remove small molecule by-products. Liu and co-workers[3a] prefer the UV-induced coupling of cinnamoyl groups; this is relatively "clean" chemistry but cinnamoyl groups are probably too hydrophobic to be suitable for use in aqueous media. It is clear that there is considerable scope for the development of new, improved cross-linking strategies.

Complexation of oppositely charged polyelectrolytes is a well-known phenomenon, which has been recently exploited in the context of layer-by-layer deposition[5] and also DNA condensation.[6] Moreover, Kabanov et al. have demonstrated that micellization can be induced by adding either a cationic homopolyelectrolyte (quaternized poly(4-vinylpyridine)) or a cationic surfactant (hexadecyltrimethylammonium bromide) to an aqueous solution of a molecularly dissolved neutral–anionic AB diblock copolymer, for example, poly(ethylene oxide-block-sodium methacrylate).[7] Both Harada and Kataoka and also Jerome's group have reported similar observations,[8] and very recently Schlaad and co-workers have exploited polyelectrolyte complexation in non-aqueous media to prepare vesicles.[9] In principle, polyelectrolyte complexation offers four advantages over conventional "small-molecule" cross-linking strategies: (1) polyelectrolytes are generally regarded as having low toxicity; (2) physical cross-linking means that there is no possibility of unwanted chemical modification of the active compound to be encapsulated; (3) there are no small-molecule by-products, hence purification is straightforward; (4) ionic cross-linking is, in principle, reversible in the presence of added salt. Furthermore, it might be reasonably expected that the glassy, rigid nature of the inter-polyelectrolyte complex layer would lead to efficient encapsulation (or at least retarded release) of the active hydrophobic compound within the micelle cores. Herein, we explore the feasibility of synthesizing SCL micelles by using either an anionic homopolymer or diblock copolymer as an ionic cross-linker for a cationic ABC triblock copolymer in which the cationic charge density resides in the central B block (see Figure 1).

The anionic cross-linkers, PEO_{113}–$NaStS_{34}$ diblock copolymer and $NaStS_{32}$ homopolymer (NaStS = sodium 4-styrenesulfonate), were each prepared by atom transfer radical polymerization (ATRP) in mixed aqueous media at 20 °C by using similar protocols to those described by Choi and Kim.[10] The $NaStS_{32}$ homopolymer had an $\bar{M}_n$ of 6900 and an $\bar{M}_w/\bar{M}_n$ of 1.26 as judged by aqueous gel-permeation chromatography (GPC) with near-monodisperse NaStS homopolymer calibration standards ($\bar{M}_n$ is the number-average molar mass, $\bar{M}_w$ is the weight-average molar mass). The PEO_{113}–$NaStS_{34}$ diblock copolymer was prepared by using a PEO-based macroinitiator[2d] ($DP_n = 113$, $\bar{M}_n = 5{,}100$, $\bar{M}_w/\bar{M}_n = 1.17$ with aqueous GPC and PEO standards; PEO = poly(ethylene oxide), DP_n is the degree of polymerization) and had an $\bar{M}_n$ of 12,000 as determined by ^{1}H NMR; aqueous GPC studies indicated an $\bar{M}_w/\bar{M}_n$ of 1.24 (with respect to NaStS homopolymer standards). The cationic ABC triblock copolymer was prepared by ATRP with the same PEO-based macroinitiator as that described above. This macroinitiator was used to polymerize 2-(dimethylamino)ethyl methacrylate (DMA) at ambient temperature in an 2:1 v/v isopropanol/water mixture. After 98% conversion, 2-(diethylamino)ethyl methacrylate (DEA) monomer was added to the reaction solution. The final $\bar{M}_n$ and polydispersity of the PEO_{113}–DMA_{38}–DEA_{54} triblock were determined to be 33100 g mol^{-1} and 1.20, respectively, as judged by THF GPC with poly(methyl methacrylate) standards. Assuming 100% macro-initiator efficiency and perfect blocking efficiency for the DEA polymerization, we esti-

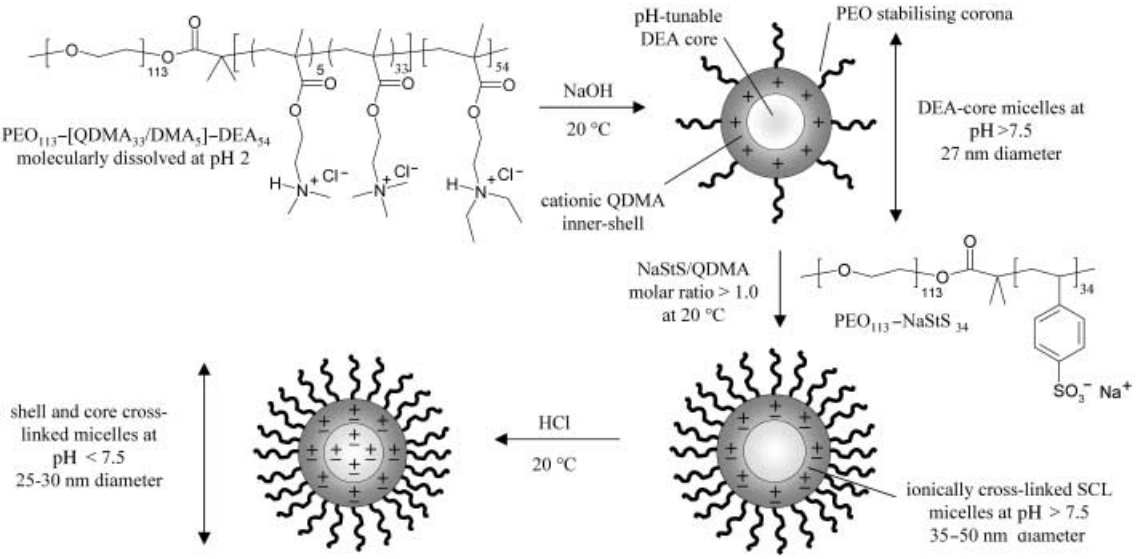

Figure 1. Schematic representation of the formation of shell cross-linked micelles by using an ionic cross-linking strategy. Conventional micelles formed by a PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{50} triblock copolymer (see text for details) are stabilized by the addition of an anionic PEO_{113}–$NaStS_{34}$ diblock copolymer. At low pH the conventional micelles dissociate to form individual chains but the ionically cross-linked micelles remain intact, provided that an excess of the anionic diblock copolymer cross-linker is employed.

mated the overall $\bar{M}_n$ of the triblock copolymer to be 21 000 g mol^{-1} by ^{1}H NMR by using the PEO as an end-group. The DMA residues were selectively quaternized in the presence of the more sterically congested DEA residues by using methyl iodide in THF at 20 °C[11] so as to create a permanently cationic central block (the degree of quaternization of the DMA block was determined to be approximately 88 % after 48 h by ^{1}H NMR). Thus the composition of the final quaternized triblock copolymer is given by PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54}, QDMA is methyl iodide-quaternized DMA residues. As expected, this quaternized triblock copolymer dissolved molecularly in acidic solution (the DEA block becomes protonated and hence hydrophilic at low pH)[11,12] but formed DEA-core micelles with an intensity-average diameter of around 26–28 nm above pH 7, as judged by both dynamic light scattering (DLS) studies (Figure 2a) and ^{1}H NMR spectroscopy (Figure 3). Static light scattering studies (multiangle Zimm plot analysis by using a DAWN DSP light scattering instrument) of the noncross-linked micelles at pH 9 indicated an $\bar{M}_w$ of 3.67×10^5 g mol^{-1}, which corresponds to a mean micelle aggregation number of approximately 10. In our initial experiments we attempted to ionically cross-link the QDMA residues of these DEA-core micelles by using the NaStS homopolymer. However, this shell cross-linking was not successful: on addition of the $NaStS_{32}$ homopolymer to the ABC triblock copolymer micelles at pH 10, a large increase in particle diameter from 26 nm to 56 nm was observed on increasing the NaStS/QDMA molar ratio from 0.4 to 0.8 (Figure 2a). This observation suggests that bridging flocculation of the micelles occurs, despite the relatively low molecular weight of the anionic cross-linker. On decreasing the solution pH at this NaStS/QDMA molar ratio, micellar dissociation occurred at around pH 6. This indicates that these ionically cross-linked micelles have insufficient stability to maintain their structural integrity once the core-forming DEA block becomes hydrophilic. Similar results were obtained with various other anionic homopolyelectrolytes, including poly(acrylic acid), poly(methacrylic acid) and poly(ammonium 2-sulfatoethyl methacrylate). However, ionic cross-linking was much more effective when the PEO_{113}–$NaStS_{34}$ diblock copolymer was used. Moreover, addition of this anionic diblock cross-linker led to a relatively small increase in the micelle diameter (Figure 2a); presumably the PEO chains of the anionic cross-linker reinforce the PEO corona of the PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54} micelles and hence suppress intermicelle bridging flocculation through enhanced steric stabilization. ^{1}H NMR spectroscopy studies confirmed that inter-polyelectrolyte complexation occurred as expected, since all the signals assigned to the QDMA residues and the NaStS

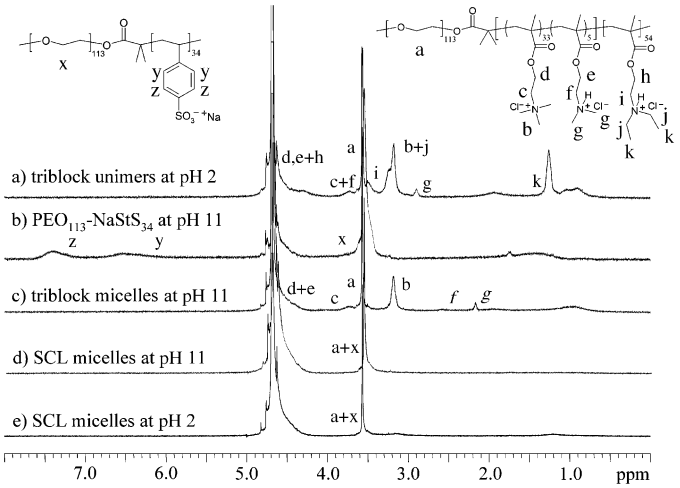

Figure 3. ^{1}H NMR spectra (D_2O) obtained for a) the PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54} triblock precursor at pH 2; b) the PEO_{113}–$NaStS_{34}$ diblock copolymer cross-linker; c) the PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54} micelles alone at pH 11; d) a binary mixture of these two block copolymers at pH 11 (NaStS/QDMA molar ratio = 2); e) the same binary mixture as that of (d) at pH 2 after addition of DCl. The italicized labels represent proton resonances due to deprotonated DMA residues not shown in the chemical structures.

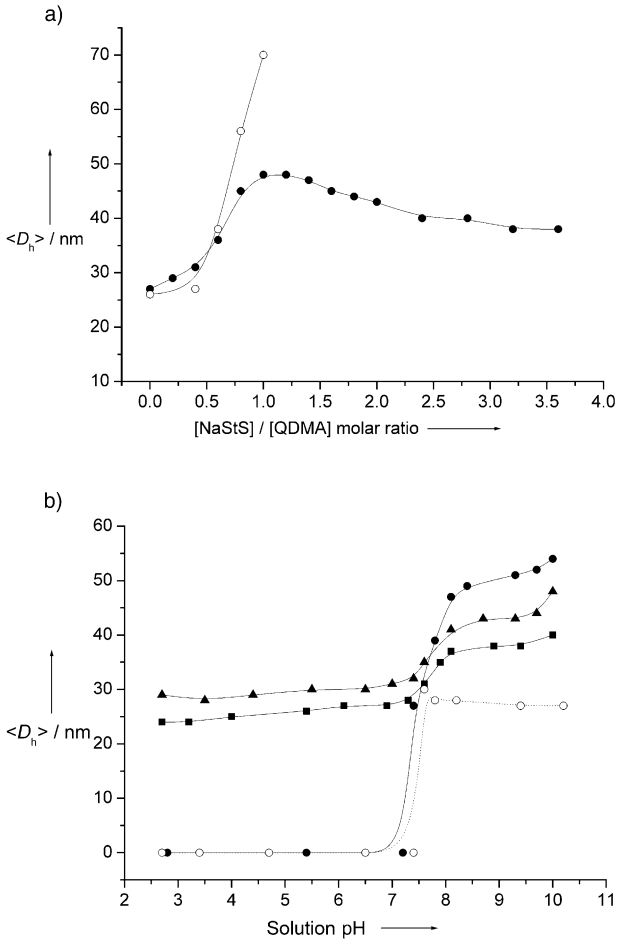

Figure 2. a) Variation of micelle diameter ($< D_h >$) as a function of NaStS/QDMA molar ratio at pH 10 and 20 °C for 5.0 g L^{-1} solutions containing the PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54} triblock copolymer and either the PEO_{113}–NaStS diblock copolymer (●) or a PNaStS homopolymer (○) as the ionic cross-linker. The lines serve as a guide to the eye. b) Variation of micelle diameter with solution pH for the conventional PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{50} triblock copolymer micelles and the PEO_{113}–$NaStS_{34}$/PEO_{113}–[$QDMA_{33}$/DMA_5]-DEA_{54} shell cross-linked (SCL) micelles at NaStS/QDMA molar ratios of 2.0 (▲), 1.5 (■), 1.0 (●) and 0.0 (○).

residues were suppressed on addition of the PEO_{113}–$NaStS_{34}$ cross-linker to the PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54} triblock copolymer micelles (see Figure 3). Again, the structural integrity of the SCL micelles was assessed by switching the solution pH from pH 10 to around pH 2, which leads to protonation of the DEA residues in the micelle cores. If ionic cross-linking had been unsuccessful, micellar dissociation would have occurred spontaneously below pH 7 as the protonated, cationic DEA chains are no longer hydrophobic. Instead, the micelles remain intact at pH 2. However, some micellar disintegration is observed if insufficient PEO_{113}–$NaStS_{34}$ cross-linker is present: the minimum NaStS/QDMA molar ratio required to preserve the micelles at low pH lies somewhere between 1.0 and 1.5 (see Figure 2b), which means that the PEO_{113}–$NaStS_{34}$ cross-linker must be present in reasonable excess. However, since the aromatic signals completely disappear in the ^{1}H NMR spectra recorded for the ionically cross-linked micelles at neutral pH (Figure 3), it seems that essentially all of the PEO_{113}-$NaStS_{34}$ diblock copolymer chains interact with the PEO_{113}–[$QDMA_{33}$/DMA_5]–DEA_{54} micelles. Previously, we observed that the ^{1}H NMR signals assigned to DEA residues in DEA-core SCL micelles were invisible at neutral pH but reappeared at low pH.[2d] However, in the present study, no signals due to protonated DEA residues were observed on addition of DCl. Presumably this is because the noncomplexed "excess" NaStS residues that are in the micelles at neutral pH are available to interact with the protonated DEA residues that are formed at low pH. Thus, we believe that the protonated micelle cores become ionically cross-linked in acidic solution, as well as the QDMA residues in the inner shell.[12]

Remarkably, DLS studies indicate that these ionically cross-linked SCL micelles (5.0 g L^{-1} triblock copolymer solution prepared by using a NaStS/QDMA molar ratio of either 1.5 or 2.0) retain their structural integrity at pH 3.3 in the presence of up to 1.0 M NaCl (see Supporting Information). Furthermore, the SCL micelles appear to be more stable when prepared with higher levels of the anionic diblock cross-linker. At higher levels of added electrolyte micellar dissociation occurs, as expected. Finally, DLS studies suggest that ionically cross-linked SCL micelles prepared at a NaStS/QDMA molar ratio of 2.0 in the absence of any electrolyte are stable to high dilution (< 0.08 g L^{-1}) at pH 3.3.

In summary, SCL micelles can be conveniently prepared simply by inter-polyelectrolyte complexation. The polymeric "reagent" forms physical complexes rapidly in aqueous solution at ambient temperature; ionic cross-linking is readily reversible as the SCL micelles dissociate in the presence of sufficient added electrolyte. However, a simple homopolyelectrolyte cross-linker only leads to flocculated micelles; it is necessary to use excess diblock copolymer for effective ionic cross-linking.

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[12] Clearly there are two ways in which the protonated DEA residues could become charge-compensated. Either this core-forming block migrates to the inner shell to form a polyion complex or, alternatively, the "excess" anionic NaStS residues enter the cationic micelle cores. At this stage in our investigations we are not able to distinguish between these two possibilities.

