Synthesis of pH-Responsive Shell Cross-Linked Micelles and Their Use as Nanoreactors for the Preparation of Gold Nanoparticles

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Poly[(ethylene oxide)-*block*-glycerol monomethacrylate-*block*-2-(diethylamino)ethyl methacrylate] (PEO−GMA−DEA) and poly[(ethylene oxide)-*block*-2-hydroxyethyl methacrylate-*block*-2-(diethylamino)ethyl methacrylate] (PEO−HEMA−DEA) triblock copolymers were synthesized directly, without recourse to protecting group chemistry, via atom transfer radical polymerization by successive polymerization of GMA (or HEMA) and DEA monomers using a PEO-based macroinitiator. These triblock copolymers dissolved molecularly in aqueous solution at low pH; on addition of NaOH, micellization occurred above pH 7−8 to form three-layer "onionlike" micelles comprising DEA cores, GMA (or HEMA) inner shells, and PEO outer coronas. Selective cross-linking of the GMA (or HEMA) inner shell was successfully achieved by adding divinyl sulfone [DVS] to the alkaline micellar solution at room temperature. Unexpectedly, the PEO−HEMA−DEA triblock proved to be much less reactive toward DVS than the two PEO−GMA−DEA triblocks, and an excess of DVS was required to prepare shell cross-linked (SCL) micelles using the former triblock. The resulting SCL micelles exhibited reversible swelling behavior on varying the solution pH. At low pH, the DEA cores became protonated and hence hydrophilic. The effect of varying the block composition and the [DVS]/[GMA] molar ratio on the structural stability and pH-dependent (de)swelling of the SCL micelles was studied. Longer DEA blocks and lower [DVS]/[GMA] molar ratios led to increased swellability, as expected. Finally, these SCL micelles can serve as nanoreactors for the synthesis of gold nanoparticles. The basic DEA residues in the cores of the SCL micelles were first protonated using $HAuCl_4$, and then the electrostatically bound $AuCl_4^-$ anions were reduced to nanoparticles of elemental gold using $NaBH_4$ at neutral pH. The gold-loaded SCL micelles exhibited excellent long-term colloid stability.

Introduction

Shell cross-linked (SCL) micelles combine the properties of micelles, microgels, nanoparticles, and dendrimers, and various applications such as targeted drug delivery, sequestration of metabolites, and entrapment of environmental pollutants have been suggested.[1−4] In particular, recent efforts have focused on the synthesis of SCL micelles that have either hollow cores[5,6] or tunably hydrophilic cores.[7−9] Both Wooley's group[5] and Liu and co-workers[6] have prepared hollow SCL micelles by selective chemical degradation of the core-forming block, whereas SCL micelles with tunably hydrophilic cores have been synthesized either by in situ deprotection of a hydrophobic core-forming block[7,8] or by use of core-forming blocks which exhibit dual hydrophilic/hydrophobic character.[9] In principle, hollow SCL micelles offer larger loading capacities, but tunable hydrophilic cores are also attractive since no core removal step is required and there is the potential for the triggered release of encapsulated actives via various chemical stimuli (pH, temperature, ionic strength, etc.).

In 1998, our group described the first example of synthesis of SCL micelles with tunable core hydrophilicity.[9a] An aqueous micellar solution of partially quaternized poly[2-(dimethylamino)ethyl methacrylate-*block*-2-(*N*-morpholino)ethyl methacrylate] (DMA−MEMA) was reacted 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 forms the micelle cores. On cooling to 20 °C, the hydrophobic MEMA micelle cores become hydrated. Thus the SCL micelle cores could be reversibly (de)hydrated depending on the solution temperature.

One major drawback in early syntheses of SCL micelles was that shell cross-linking had to be carried out at high dilution (typically <0.50% solids) in order to avoid extensive intermicellar cross-linking. Clearly, unless this problem was addressed, the synthesis of SCL micelles was unlikely to be commercially viable, even for specialty applications. Fortunately, we recently demonstrated that ABC triblock copolymers offer great advantages over conventional AB diblock copolymers, since the former allow shell cross-linking to be carried out at high solids with

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little or no intermicellar cross-linking. In a proof-of-concept study, a poly(ethylene oxide) (PEO)−DMA−MEMA triblock copolymer was cross-linked at a copolymer concentration of 10% w/v with negligible intermicellar cross-linking.[10] This is because the coronal PEO chains prevented interpenetration of the micelles due to a steric stabilization mechanism which in turn ensured that only localized, intramicellar cross-linking occurred. In view of this success, all of our current synthetic effort is now devoted to the synthesis of ABC triblock copolymers, rather than AB diblocks.

The synthesis of well-defined block copolymers requires either living or pseudo-living polymerization chemistry. Given the stringent purification required for ionic polymerizations, increasing attention is now being given to the use of atom transfer radical polymerization (ATRP)[11] for the preparation of the block copolymer precursors that are required for SCL micelle syntheses.[2d,2e,7,8,12] At Sussex, we have recently developed ATRP for the efficient polymerization of hydrophilic monomers in either water or alcoholic media at room temperature.[12−17]

To date, we have used BIEE as a bifunctional cross-linking agent in all of our SCL micelle studies.[9,16] Generally, BIEE has been used to cross-link DMA residues via quaternization, but it can also react efficiently with methacrylic acid residues, forming ester linkages.[9] However, in view of its cost, toxicity, limited water solubility, and likely mutagenicity, BIEE is highly unlikely to be employed in commercial applications of SCL micelles, particularly for use in the biomedical field. The alternative cross-linking strategies described in the literature are equally unsatisfactory. For example, Wooley's group[1,2] use carbodiimide coupling chemistry to link carboxylic acid groups via diamines (obvious disadvantages here are the cost of the reagents and the cleanup requirements when dealing with several molecular species), whereas Liu and co-workers[4,6] prefer the UV-induced coupling of cinnamoyl groups, which are too hydrophobic to be suitable for use in aqueous media. Thus it is indisputable that new, improved cross-linking strategies are urgently required. The ideal cross-linker should be water-soluble, nontoxic, and cost-effective. The cross-linking chemistry should be facile under mild conditions (e.g., aqueous solution and ambient temperature), produce no small molecule byproducts, and preferably be potentially reversible. We have examined several new strategies, and in this work we describe the use of divinyl sulfone (DVS) to selectively cross-link hydroxylated blocks (Figure 1a). This reagent has been previously used for the preparation of gels,[18] microgels,[19] and nanoparticle networks[20] from hydroxyl-containing precursors[21,22] but never, as far as we are aware, employed for the synthesis of SCL micelles.

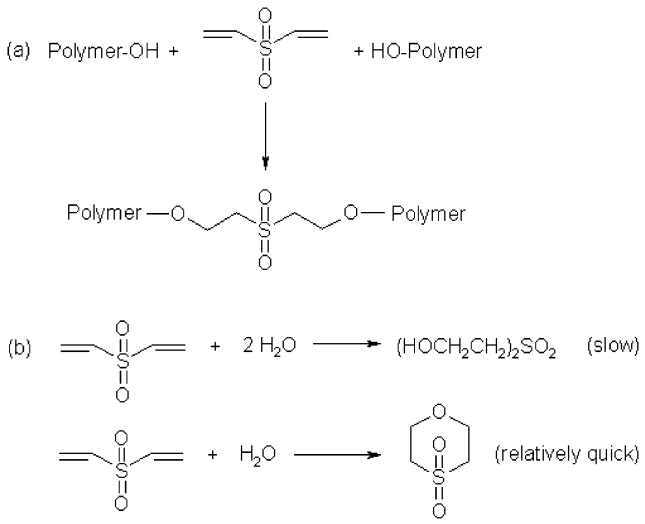

Figure 1. (a) Reaction scheme for cross-linking of the hydroxy-functional triblock copolymers with DVS; (b) side reaction of DVS in aqueous solution at neutral and alkaline pH.

In the present study, we describe the efficient synthesis of SCL micelles with pH-responsive cores from novel ABC triblock copolymers prepared via ATRP. The triblock copolymers are based on either poly[(ethylene oxide)-*block*-glycerol monomethacrylate-*block*-2-(diethylamino)-ethyl methacrylate] (PEO−GMA−DEA) or poly[(ethylene oxide)-*block*-2-hydroxyethyl methacrylate-*block*-2-(diethylamino)ethyl methacrylate] (PEO−HEMA−DEA). The pH-induced micellization of these copolymers and the subsequent shell cross-linking of the GMA (or HEMA) inner shell with DVS were studied in detail. The effect of varying the relative block compositions and the [DVS]/[GMA] (or [DVS]/[HEMA]) molar ratio on the pH-induced (de)swelling of the SCL micelles was also investigated. Figure 2 shows the reaction scheme for the preparation of SCL micelles from the hydroxy-functionalized triblock copolymers.

Experimental Section

Materials. GMA monomer was kindly donated by Röhm (Germany). HEMA monomer and monohydroxy-capped poly(ethylene oxide) (PEO_{45}−OH) [mean degree of polymerization = 45, M_w/M_n = 1.10] were donated by Laporte Performance Chemicals (Hythe, U.K). DEA was purchased from Aldrich. These monomers were treated with basic alumina and then vacuum-distilled from CaH_2 and stored at −20 °C prior to use. $Cu^{I}Cl$, 2,2′-bipyridine (bpy), 2-bromoisobutyryl bromide, triethylamine, and DVS were purchased from Aldrich and used without further purification.

Preparation of PEO Macroinitiator (1). In a typical example, PEO_{45}−OH (50.0 g, 0.025 mol) was dissolved in 300 mL of toluene in a 500 mL three-neck flask. After azeotropic distillation of 30−40 mL of toluene at reduced pressure to remove traces of water, triethylamine (4.17 mL, 0.03 mol) was added and the solution mixture was cooled to 0 °C. Then 2-bromoisobutyryl bromide (3.7 mL, 0.03 mol) was added dropwise via syringe over 1 h, and the reaction mixture was stirred overnight at room temperature. The stirred solution was treated with charcoal, which was subsequently removed by filtration, and most of the toluene was removed by rotary evaporation prior to precipitation

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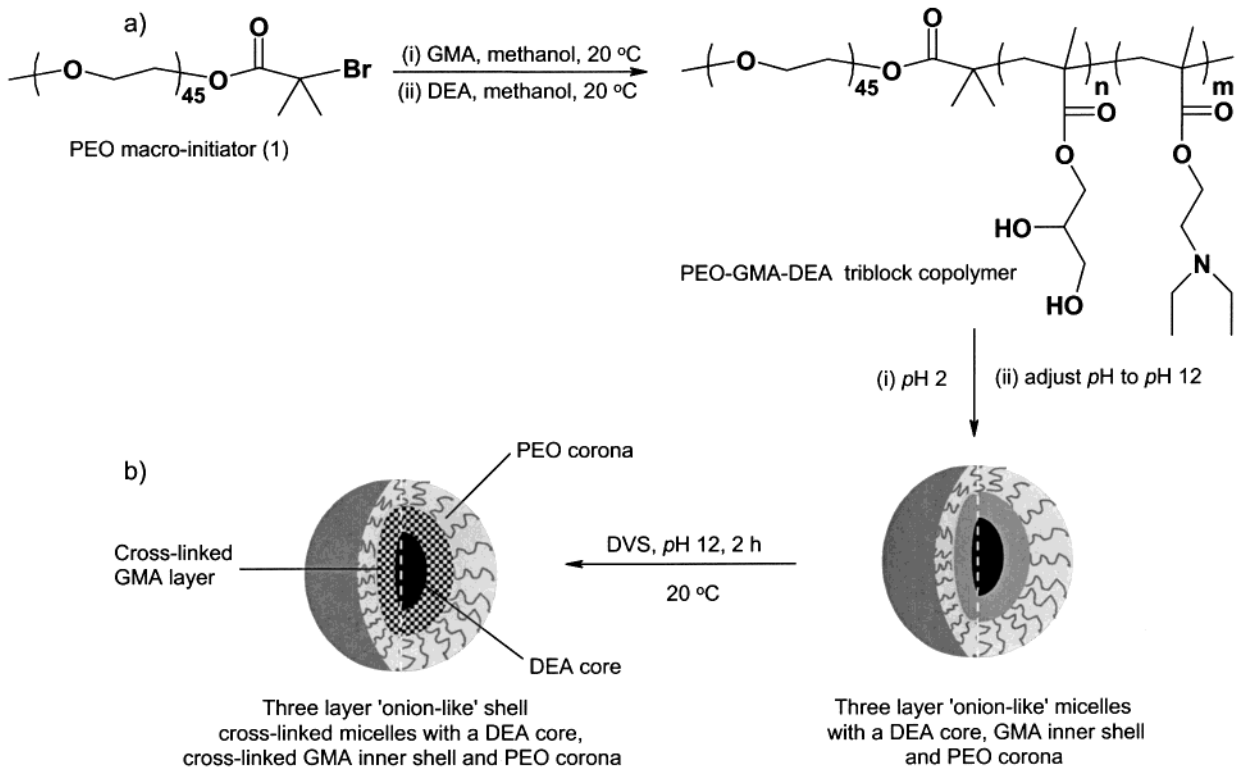

Figure 2. (a) Reaction scheme for the synthesis of the PEO−GMA−DEA triblock copolymers; (b) schematic illustration of the formation of three-layer onionlike micelles and shell cross-linked micelles from PEO−GMA−DEA triblock copolymers.

Table 1. Molecular Parameters of the Three Triblock Copolymers Used in This Study[a]

sample code	DP of PEO block	DP of second block	DP of DEA block	$M_{n,cal}$	$M_{n,GPC}$	M_w/M_n
$PEO_{45}-GMA_{40}-DEA_{55}$	45	40	55	25 200[b]	19 400[c]	1.23
$PEO_{45}-GMA_{25}-DEA_{70}$	45	25	70	23 300[b]	25 500[c]	1.26
$PEO_{45}-HEMA_{30}-DEA_{50}$	45	30	50	15 200	15 700[d]	1.17

[a] DP refers to the number-average degree of polymerization. [b] Theoretical calculated molecular weight assuming that all GMA residues are derivatized with benzoic anhydride. [c] THF GPC for the two derivatized GMA-based triblock copolymers. [d] THF GPC for the underivatized HEMA-based triblock copolymer.

into a 10-fold excess of ether. The crude polymer was dried under vacuum, dissolved in water at pH 8−9, and then extracted with dichloromethane. The organic layers were collected and dried over $MgSO_4$, and removal of the solvent under vacuum led to isolation of the purified macroinitiator ($PEO_{45}-Br$).

Preparation of PEO−GMA−DEA and PEO−HEMA−DEA Triblock Copolymers. The typical procedure was as follows. The $PEO_{45}-Br$ macroinitiator and GMA or HEMA monomer were added to one reaction flask and were degassed under nitrogen purge. Methanol was degassed separately and added to the monomer/initiator mixture via a double-tipped needle, followed by a freeze−pump−thaw cycle. The Cu^IBr and bpy catalysts were introduced into the reaction flask to start the polymerization at room temperature. The monomer concentration was 37% w/v. After the conversion of GMA (or HEMA) had reached more than 95%,[23] degassed DEA monomer diluted with methanol (1/1 v/v) was transferred to the reaction flask via a double-tipped needle. After another 12−15 h, the dark brown reaction solution was exposed to air and diluted with methanol; termination occurred rapidly, as indicated by the color change from brown to blue due to the aerial oxidation of Cu(I) to Cu(II). The triblock copolymer was purified by passing through a silica gel column to remove the copper catalyst. After evaporating all the solvent, drying in a vacuum oven at room temperature yielded colorless polymer. For gel permeation chromatography (GPC) analysis using tetrahydrofuran (THF) as the eluent, the GMA residues in the PEO−GMA−DEA triblock copolymers were derivatized by reacting with a 4-fold excess of benzoic anhydride in pyridine, as previously described.[14] The modified triblock copolymers exhibit symmetrical, unimodal GPC traces, and there is no evidence for any residual $PEO_{45}-Br$ macroinitiator, suggesting high initiation efficiency. Moreover, compared to the GPC traces obtained for the PEO−GMA or PEO−HEMA diblock precursors, there is a clear shift to higher molecular weights for the triblock copolymers, which had relatively narrow final polydispersities. All of these data indicated that the syntheses of the triblock copolymers had been successful. Table 1 summarizes the molecular weight data for the three PEO−GMA−DEA and PEO−HEMA−DEA triblock copolymers examined in this study.

Preparation of Micelles and SCL Micelles. The PEO−GMA−DEA triblock copolymers were molecularly dissolved in water at pH 2 at various copolymer concentrations, and the solution pH was adjusted to pH 12 so as to induce micelle formation. Shell cross-linking was achieved by adding DVS, and the reaction solutions were stirred for 3−4 h at room temperature. For the HEMA-based triblock copolymer, the target degree of cross-linking is given by $y = 2[DVS]/[HEMA] \times 100\%$. Assuming that the secondary hydroxy groups in the GMA residues are much less reactive than the primary hydroxy groups, the analogous equation for the GMA-based triblock is $y = 2[DVS]/[GMA] \times 100\%$. However, we do not know whether this assumption of differential reactivity is valid. Moreover, it is quite likely that some (unknown) fraction of the DVS may react with hydroxy groups on the *same* GMA or HEMA chain and will not therefore contribute to shell cross-linking. Furthermore, the DVS is prone to hydrolysis under the cross-linking conditions (see Figure 1), which is likely to reduce the actual degree of cross-linking achieved. In view of these uncertainties, we have chosen to cite the initial molar ratio of reacting groups ([DVS]/[HEMA]

(23) We have previously studied the kinetics of polymerization of both GMA and HEMA by ATRP in some detail; see refs 13 and 14.

Table 2. Hydrodynamic Diameter, $\langle D_h \rangle$, and Polydispersity, μ_2/Γ^2, of Micelles and SCL Micelles Prepared from PEO−GMA−DEA and PEO−GMA−DEA Triblock Copolymers at 1.0% w/v

sample code	micelle diameter at pH 12 (nm)	μ_2/Γ^2	diameter of SCL micelles at pH 12 (nm)	μ_2/Γ^2	diameter of SCL micelles at pH 2 (nm)	μ_2/Γ^2
$PEO_{45}-GMA_{40}-DEA_{55}$[a]	21	0.05	21	0.10	31	0.20
$PEO_{45}-GMA_{25}-DEA_{70}$[a]	35	0.15	36	0.17	62	0.26
$PEO_{45}-HEMA_{30}-DEA_{50}$[b]	30	0.09	31	0.04	42	0.24

[a] At a [DVS]/[GMA] molar ratio of 0.50. [b] At a [DVS]/[HEMA] molar ratio of 1.00.

or [DVS]/[GMA]) used to synthesize the SCL micelles, rather than the target degree of shell cross-linking.

In preliminary experiments, it was found that the PEO−HEMA−DEA triblock did not dissolve molecularly in aqueous acidic solution (pH 2) at 20 °C; dynamic light scattering studies indicated some degree of weak aggregation. The origin of this aggregation is not known, but it may involve hydrogen bonding between the PEO block and the HEMA residues. Alternatively, the relatively low cloud point of the HEMA block with a Dp of 30 (approximately 36 °C[24]) may be important. Fortunately, further studies confirmed that molecular dissolution could be achieved in *cold* aqueous acidic solution (around 5 °C and pH 2). Addition of base at 5 °C led to the formation of colloidally stable DEA-core micelles in alkaline media; these micelles were allowed to warm to ambient temperature (20 °C) prior to shell cross-linking with the DVS reagent at pH 12 for 5−6 h.

For all three triblock copolymers, the pH of the SCL micellar solutions was adjusted from pH 12 to approximately pH 9 after DVS cross-linking in order to minimize any unwanted alkaline hydrolysis of the triblock copolymer.

Synthesis of Gold Nanoparticles Using SCL Micelles as Nanoreactors. Aqueous SCL micellar solutions of PEO−GMA−DEA triblocks (0.1−5.0% w/v) were mixed with aqueous solutions of $HAuCl_4$ at various $HAuCl_4$/DEA stoichiometries. After stirring these light yellow solutions for 1 h, an aqueous solution of excess sodium borohydride was added. The solutions immediately turned wine red, indicating the formation of colloidal gold.

Characterization. Molecular weights and molecular weight distributions were determined by THF GPC using a Viscotek instrument. The setup comprised a PLgel 3 μm MIXED-E 300 × 7.5 mm column, poly(methyl methacrylate) (PMMA) calibration standards, and a refractive index detector. Transmission electron microscopy (TEM) images were recorded using a Hitachi 7100 microscope. Samples were prepared by dipping a Formvar-coated copper grid into an aqueous solution of SCL micelles, followed by air-drying at ambient temperature. All ^{1}H NMR spectra were recorded on 1.0% w/v copolymer solutions in D_2O using a Bruker Avance DPX 300 MHz spectrometer.

Dynamic light scattering (DLS) studies were performed on a Brookhaven Instruments Corp. BI-200SM goniometer equipped with a BI-9000AT digital correlator using a solid-state laser (125 mW, $\lambda = 532$ nm) at a fixed scattering angle of 90°. The intensity-average hydrodynamic diameter, $\langle D_h \rangle$, and polydispersity (μ_2/Γ^2) were calculated for each micellar solution before and after cross-linking by cumulants analysis of the experimental correlation function.[25,26]

The particle size distributions of the SCL micelles were also assessed using a Polymer Laboratories Particle Size Distribution Analyzer (PL-PSDA). This instrument uses the principle of packed column hydrodynamic chromatography (HDC) to fractionate particles according to their hydrodynamic volume. This technique is similar in some respects to GPC, except that the packed bed comprises nonporous beads and separation takes place in the channels between the beads. HDC is a relative technique, and the conversion from elution time to particle size involves calibration using a series of near-monodisperse polystyrene latexes (ex. Duke Scientific) as standards. A type I cartridge, with a nominal operating range of 5−300 nm, was selected, and the eluent flow rate was 2.0 mL min^{-1}. Shell cross-linked micellar solutions of 2.0−2.5% w/v at either pH 3 or 10 were filtered through a 0.45 μm Whatman filter prior to analysis, and the sample injection volume was 20 μL.

Results and Discussion

Background. DEA homopolymer is a weak polybase with a pK_a of about 7.3.[27] It is water-insoluble at neutral or alkaline pH. Below pH 7.0, it is soluble as a weak cationic polyelectrolyte due to protonation of its tertiary amine groups. In contrast, both PEO and GMA homopolymers are water-soluble over a wide pH range. HEMA homopolymer is usually considered to be a water-swellable polymer, rather than water-soluble polymer.[13] However, we have recently shown that, for a sufficiently low mean degree of polymerization (Dp < 50), HEMA homopolymers are water-soluble and exhibit inverse temperature solubility behavior.[24]

pH-Induced Formation of Three-Layer "Onion" Micelles. Both PEO−GMA−DEA triblock copolymers could be molecularly dissolved at acidic pH; on addition of NaOH, micellization occurred above pH 7−8, as indicated by the bluish color that is characteristic of micellar solutions. Above pH 8, DLS studies revealed a unimodal population corresponding to near-monodisperse micelles. On the basis of chemical intuition, these micelles are expected to have a three-layer onion structure, with the DEA block occupying the micelle core and the GMA and PEO blocks forming the inner shell and corona, respectively. Table 2 lists the intensity-average hydrodynamic diameters, $\langle D_h \rangle$, and polydispersities (μ_2/Γ^2) of the micelles. The $PEO_{45}-GMA_{40}-DEA_{55}$ triblock formed micelles with a $\langle D_h \rangle$ of 21 nm, while the $PEO_{45}-GMA_{25}-DEA_{70}$ triblock formed somewhat larger micelles with a $\langle D_h \rangle$ of 35 nm. As described in the Experimental Section, molecular dissolution of the PEO−HEMA−DEA triblock could be achieved only at 5 °C, and thus a "low temperature" route was adopted to prepare SCL micelles using this particular triblock copolymer.

Figure 3a,b depicts the NMR spectra recorded for the $PEO_{45}-GMA_{40}-DEA_{55}$ triblock copolymer at different solution pHs. At pH 2, the copolymer chains are fully solvated and all the signals expected for each block are visible. At pH 8.5, the signals due to the deprotonated DEA block at δ 1.3 and δ 4.2 completely disappeared, while the signals from PEO and GMA residues are still evident, indicating the formation of DEA-core micelles with an inner layer comprising hydrophilic GMA residues and an outer corona of PEO chains.

Shell Cross-Linked Micelles with pH-Responsive Cores. DVS has been widely used to cross-link hydroxy-containing polymers to prepare gels, microgels, and nanoparticle networks in alkaline solutions (around pH 12 or higher) according to Figure 1a.[18−22] This is an example of Michael addition chemistry, so in principle no byproducts should be generated. However, in practice DVS is hydrolyzed by water (slowly at neutral pH and more rapidly in alkaline solution; see Figure 1b). Fortunately,

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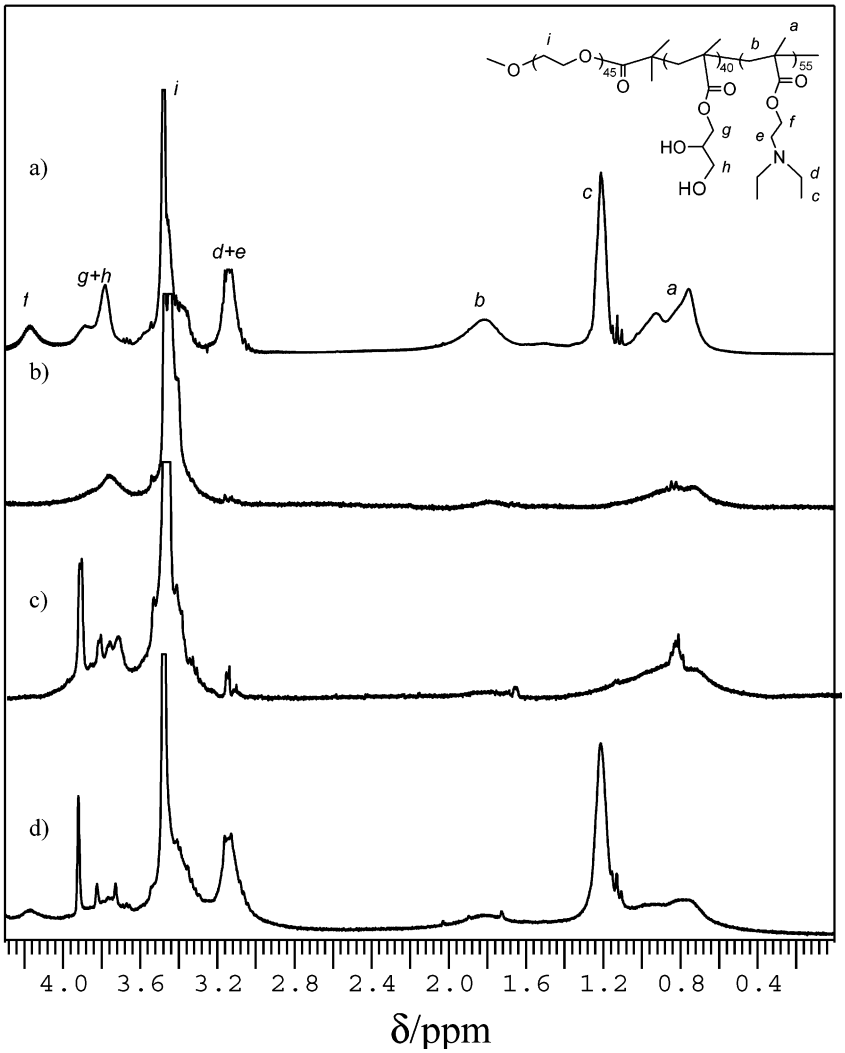

Figure 3. ^{1}H NMR spectra of the PEO_{45}-*b*-GMA_{40}-*b*-DEA_{55} triblock copolymer: (a) at pH 2; (b) at pH 8.5; (c) after cross-linking at a [DVS]/[GMA] molar ratio of 0.50 at pH 12; (d) at pH 2 after shell cross-linking.

DVS reacts more readily with the hydroxy groups on the GMA (or HEMA) residues. It is also likely that the relatively high *local* concentration of hydroxy groups within the inner shell of the micelles is beneficial in aiding the reaction with the DVS. One advantage of using DVS as the cross-linker is that, in principle, the extent of incorporation of the DVS can be assessed by sulfur microanalyses.

SCL micelles were readily obtained by adding DVS to aqueous solutions of the triblock copolymer micelles at pH 12 and ambient temperature. For SCL micelles prepared from a 1.0% w/v aqueous solution of PEO_{45}−GMA_{40}−DEA_{55} at a [DVS]/[GMA] molar ratio of 0.50 relative to the primary hydroxy groups ([DVS]/[GMA] = 0.5), DLS studies indicated a $\langle D_h \rangle$ of 21 nm at pH 12, which is very similar to the micelle diameter prior to cross-linking. This confirmed the expected intramicellar cross-linking mechanism. The solution pH was then adjusted to pH 2 using HCl. If no shell cross-linking had occurred, micellar dissociation into individual triblock copolymer chains would be expected, since the DEA core block becomes soluble under these conditions. DLS studies of the SCL micellar solutions at pH 2 revealed a $\langle D_h \rangle$ of 31 nm with comparable scattering intensity to that observed at pH 12, indicating that cross-linking had been successful. The increase in $\langle D_h \rangle$ for the SCL micelles at pH 2 compared to pH 12 corresponded to a 3-fold increase in volume. This is due to protonation of the DEA chains in the micellar cores at low pH. The cationic charge density makes the DEA chains hydrophilic and mutually repulsive, leading to micellar swelling. For SCL micelles prepared from the PEO_{45}−GMA_{25}−DEA_{70} triblock copolymer under similar conditions, the $\langle D_h \rangle$ increased from 36 to 62 nm, corresponding to a 5-fold increase in hydrodynamic volume. This increased degree of swelling can be attributed to the longer core-forming DEA block. Returning to Figure 3, spectra c and d show the ^{1}H NMR spectra recorded for PEO_{45}−GMA_{40}−DEA_{55} SCL micelles at pH 12 and at pH 2, respectively. The reappearance of signals assigned to the (protonated) DEA residues at pH 2 at δ 1.3 and δ 4.2 confirms that the SCL micelle cores become hydrophilic, as expected.

For SCL micelles prepared from PEO−HEMA−DEA triblock copolymers, it was found that the [DVS]/[HEMA] molar ratio should be at least 1.00 to ensure covalent stabilization of the micellar structure. In contrast, it was much easier to cross-link the PEO−GMA−DEA triblock copolymer micelles. This is perhaps due to the higher local density of hydroxy groups on the GMA blocks. Moreover, some difference between GMA and HEMA residues might be expected in their reactivity toward DVS, that is, the hydroxy groups on GMA are more nucleophilic due to the electron-withdrawing effect of the adjacent hydroxy groups. The results obtained for the PEO_{45}−$HEMA_{30}$−

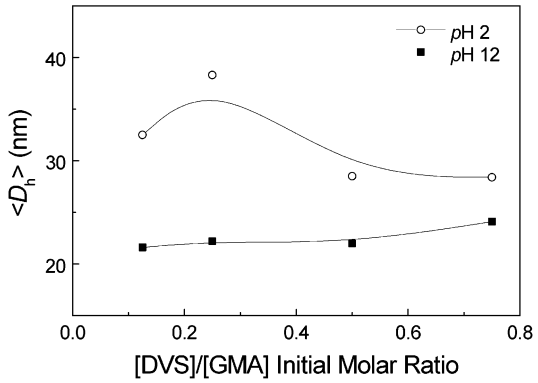

Figure 4. Relationship between $\langle D_h \rangle$ and [DVS]/[GMA] initial molar ratio for SCL micelles prepared from the $PEO_{45}-GMA_{40}-DEA_{55}$ copolymer at 2.5% w/v at both pH 2 (hydrated micelle cores) and pH 12 (dehydrated micelle cores).

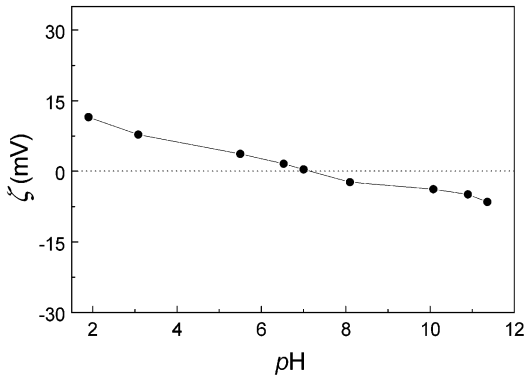

Figure 5. Zeta potential versus solution pH for SCL micelles prepared from the $PEO_{45}-GMA_{40}-DEA_{55}$ copolymer at 2.5% w/v at a [DVS]/[GMA] molar ratio of 0.25.

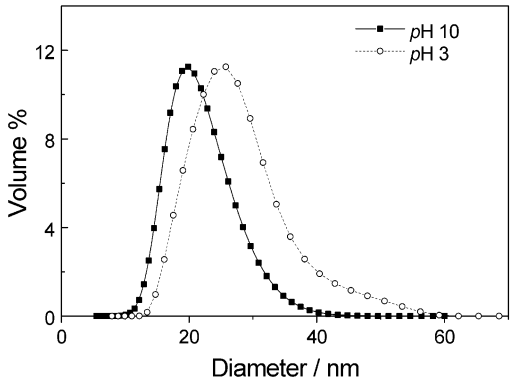

Figure 6. Typical volume-average particle size distribution curves at pH 10 and pH 3 obtained using the PL-PSDA instrument for SCL micelles prepared from the $PEO_{45}-GMA_{40}-DEA_{55}$ triblock at 2.5% w/v at a [DVS]/[GMA] molar ratio of 0.25.

DEA_{50} triblock are summarized in Table 2. Before shell cross-linking, these micelles had a mean diameter of 30 nm with a μ_2/Γ^2 of 0.09. After cross-linking at a [DVS]/[HEMA] molar ratio of 1.00, the resulting SCL micelles had a mean diameter of 31 nm at pH 12. On addition of acid (pH 2), the micelles swell considerably to 42 nm, which corresponds to a 2.5-fold increase in hydrodynamic volume.

Since the side products of the hydrolysis of DVS in alkaline media are water-soluble (see Figure 1b), the SCL micelles were purified by dialysis after the cross-linking reaction. Sulfur microanalyses were then utilized to assess the DVS content of the SCL micelles, which is related to the degree of cross-linking. After purification by dialysis, the sulfur content (normalized to nitrogen) of SCL micelles prepared from the $PEO_{45}-GMA_{25}-DEA_{70}$ triblock indicated a DVS/GMA molar ratio of 0.75, as compared to an initial molar ratio of 1.00. This reduction is attributed to the loss of physically occluded DVS (and/or its water-soluble side products) from the SCL micelles. Dialysis also results in the disappearance of the sharp signal at δ 3.9 observed in the NMR spectrum (see Figure 3d), which indicates that this feature is due to hydrolyzed side products of DVS. Similar results were obtained for the PEO−HEMA−DEA triblock, except that a much lower DVS/HEMA molar ratio of 0.125 was obtained, compared to an initial molar ratio of 1.00. This is believed to be related to the relatively low reactivity of DVS with the HEMA residues compared to the GMA residues.

Figure 4 shows the relationship between $\langle D_h \rangle$ and the [DVS]/[GMA] molar ratio for selected SCL micelles at pH 12 and pH 2. Before cross-linking, the $PEO_{45}-GMA_{40}-DEA_{55}$ micelles prepared at 2.5% w/v had a hydrodynamic diameter of 22 nm. The $\langle D_h \rangle$ of SCL micelles at pH 12 is little changed from that of the non-cross-linked precursor micelles and remains almost constant as the [DVS]/[GMA] molar ratio is varied from 0.125 to 0.75. Within this range of molar ratios, the $\langle D_h \rangle$ of the SCL micelles at pH 2 is always larger than that at pH 12, as expected. A [DVS]/[GMA] molar ratio as low as 0.125 is enough to covalently stabilize the micellar structure. The most marked pH-induced swelling behavior for SCL micelles was observed at a [DVS]/[GMA] molar ratio of 0.25. At higher [DVS]/[GMA] molar ratios, less swelling is observed, because the DEA chains in the micelle core are more constrained by the more heavily cross-linked GMA residues in the inner shell.

Further DLS studies of SCL micelles indicated that most of the pH-induced swelling occurred at around pH 7−8, which is consistent with the known pK_a value for the DEA block.[27] This augurs well for the potential application of such SCL micelles as nanosized drug delivery vehicles, since the abrupt change in hydrophilicity of the DEA cores (and concomitant increased permeability of the GMA cross-linked layer) is expected to allow "triggered release" of hydrophobic drugs.

Figure 5 shows the aqueous electrophoresis data obtained for SCL micelles prepared from the $PEO_{45}-GMA_{40}-DEA_{55}$ triblock copolymer at a [DVS]/[GMA] molar ratio of 0.25. Only relatively small changes in zeta potential (from −6 to +10 mV) were observed when the solution pH was decreased from pH 12 to pH 2, with an isoelectric point occurring at around pH 7.0−7.5. This relatively weak electrophoretic response is consistent with the neutral PEO chains being located in the micelle corona. It seems that most of the hydrophilic, protonated DEA chains remain inside the SCL micelle cores even at pH 2, although we cannot rule out (partial) migration of the cationic DEA chains through the cross-linked GMA layer to form a "mixed" corona with the neutral PEO chains.

A new commercial particle size analyzer, the PL-PSDA (ex. Polymer Laboratories), was used to assess the effect of pH on the particle size distributions of SCL micelles prepared from the $PEO_{45}-GMA_{40}-DEA_{55}$ triblock copolymer at a concentration of 2.5% w/v (see Figure 6). At pH 10, the particle size distribution ranged from 10 to 40 nm. The mean volume-average diameter was calculated to be 21 nm, which is very close to the intensity-average diameter $\langle D_h \rangle$ of 22 nm obtained from DLS. Figure 5 also shows the particle size distribution of the same SCL micelles at pH 3. The shift in the particle size distribution is evident, and the volume-average diameter of SCL micelles at pH 3 is 26 nm. Bearing in mind the different

moments of the size distribution measured by DLS and the PSDA, this value compares well to the $\langle D_h \rangle$ of 29 nm obtained from DLS. The increase in volume-average particle diameter relative to that obtained at pH 10 again clearly indicates significant swelling of the SCL micelles. As far as we are aware, this is the first time that the PL-PSDA technique has been applied to SCL micelles. Advantages of this sizing technique include the following: (i) peak-to-peak resolution is much better compared to that for DLS; (ii) typical analysis times are less than 10 min; (iii) many samples can be run sequentially using the auto-sampler mode; (iv) the reported volume-average particle diameter is likely to be more comparable to that observed by electron microscopy; (v) no knowledge of the particle density is required. On the other hand, careful calibration is required and, as in DLS, aqueous solutions should be ultrafiltered prior to use. On balance, we believe that the PL-PSDA instrument is a useful new characterization tool for SCL micelles, and we intend to exploit this technique further in future studies.

Preparation of Gold Colloids Using SCL Micelles as Nanoreactors. Recently there has been a great deal of interest in the synthesis of metal or semiconductor nanoparticles because of their unique size-dependent chemical and physical properties, which makes them ideal candidates for electronic and optical nanodevices.[28−32] In particular, polymer-coated metal or semiconductor nanoparticles exhibit increased colloid stability, enhanced protection against oxidation, and much higher catalytic activity.[33,34]

In the present study, we have found that the cores of the SCL micelles can be selectively loaded with $AuCl_4^-$ ions simply by using $HAuCl_4$ to protonate the basic DEA residues. In situ chemical reduction of the Au(III) with $NaBH_4$ leads to elemental gold nanoparticles confined within the SCL micelles, which act as nanoreactors. The reduction of Au(III) to Au(0) can be conveniently followed by UV−visible absorption spectroscopy.[35] Figure 7 depicts UV−visible spectra recorded for (a) dilute aqueous solutions of SCL micelles ($\langle D_h \rangle$) prepared from the PEO_{45}−GMA_{25}−DEA_{70} triblock copolymer, (b) the same SCL micelles loaded with $HAuCl_4$, and (c,d) two gold-loaded SCL micelles obtained after $NaBH_4$ reduction at different $HAuCl_4$ /DEA molar ratios. The precursor SCL micelles exhibited an absorption peak at 278 nm. After loading with $HAuCl_4$, the micellar solution became light yellow and an absorption band was observed at 316 nm, as expected.[36] After $NaBH_4$ reduction, this solution turned wine red. The absorption peak at 316 nm was replaced with a broad absorption envelope in the 400−600 nm range, which was assigned to the surface plasmon resonance band of gold nanoparticles.[36] The λ_{max} of this plasmon band increased from 499 to 508 nm as the $HAuCl_4$/DEA molar ratio increased from 0.2 to 0.4. This indicates that the mean size of the gold nanoparticles is around 1−5 nm and increased at higher $HAuCl_4$ loadings. No changes in the visible absorption spectra were observed after storage for 4 months, suggesting high chemical and colloidal stability for these gold-loaded SCL micelles.

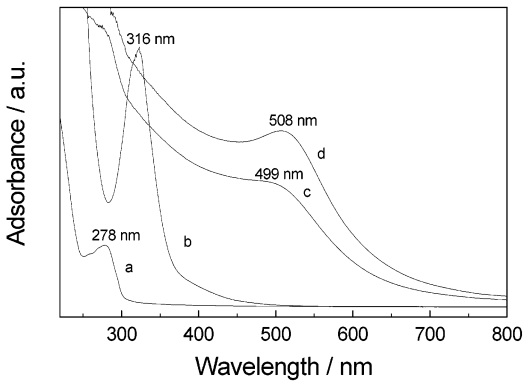

Figure 7. UV−visible spectra of SCL micelles prepared from PEO_{45}−GMA_{25}−DEA_{70} triblock copolymers at 2.5% w/v and pH 12 at a [DVS]/[GMA] molar ratio of 0.50: (a) at pH 7.5 prior to loading with $HAuCl_4$; (b) at pH 3 after loading with $HAuCl_4$ at an $HAuCl_4$/DEA molar ratio of 0.20; (c) after reduction with $NaBH_4$ at the same $HAuCl_4$/DEA molar ratio; (d) after reduction at an $HAuCl_4$/DEA molar ratio of 0.40.

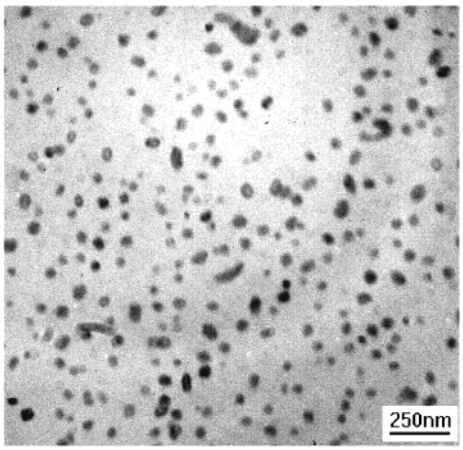

Figure 8. Typical TEM image of SCL micelles prepared from the PEO_{45}−GMA_{25}−DEA_{70} triblock at 2.5% w/v at a [DVS]/[GMA] molar ratio of 0.50.

Figure 8 shows a typical TEM image obtained for SCL micelles at pH 4. Approximately spherical micelles with a mean diameter of around 45 nm are observed; this is in reasonable agreement with the DLS diameter of 62 nm if solvation and polydispersity effects are taken into account. For gold-loaded SCL micelles synthesized at a $HAuCl_4$/DEA molar ratio of 0.40, further TEM studies (not shown) suggest that gold nanoparticles are mainly located inside the SCL micelles. Each SCL micelle appears to act as a nanoreactor for the synthesis of gold colloids, and there are many gold nanoparticles inside each SCL micelle. This is understandable given the very fast rate of reduction achieved using $NaBH_4$ at neutral pH.[37] The mean TEM diameter of the gold nanoparticles is of the order of several nanometers, which is consistent with the plasmon absorption peak observed at 508 nm.[36]

There are several advantages to using SCL micelles prepared from the PEO−GMA−DEA triblock copolymer for the synthesis of gold nanoparticles. First, this hydrophilic, functional triblock is readily synthesized using ATRP without requiring protecting group chemistry. Second, in this particular case the shell cross-linking is

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essential to preserve the nanoreactor dimensions, since the conventional non-cross-linked micelles would dissociate immediately on addition of the acidic $HAuCl_4$ solution (due to protonation of the DEA block). Third, in our most recent experiments we have shown that the shell cross-linking chemistry, the $HAuCl_4$ loading, and the in situ reduction can be carried out at relatively high solids (5% w/v) with the resulting gold-loaded SCL micelles retaining good colloid stability. In principle, much higher concentrations could be employed without risking deformation of the spherical morphology of the SCL micelles.

Conclusions

Novel well-defined PEO−GMA−DEA and PEO−HEMA−DEA triblock copolymers were successfully synthesized by ATRP using a PEO-based macroinitiator. These triblock copolymers dissolved molecularly in aqueous solution at low pH; on addition of NaOH, combined DLS and ^{1}H NMR studies confirmed that micellization occurred at around pH 7−8 to form three-layer onionlike micelles comprising DEA cores, GMA (or HEMA) inner shells, and PEO coronas. Selective cross-linking of the GMA (or HEMA) residues was achieved by adding divinyl sulfone to alkaline micellar solutions at room temperature. The resulting SCL micelles exhibited reversible swelling behavior on varying the solution pH. At low pH, the DEA cores become protonated and hence hydrophilic. The effect of varying the block compositions and the [DVS]/[GMA] molar ratio on the structural stability and pH-dependent (de)swelling of the SCL micelles was studied in some detail. These SCL micelles can act as nanoreactors: loading the DEA cores with $HAuCl_4$, followed by reduction with $NaBH_4$, produced gold nanoparticles within the interior of the micelles.

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