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In Situ Formation of Gold-“Decorated” Vesicles from a RAFT-Synthesized, Thermally Responsive Block Copolymer[§]

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Vesicles self-assembled from amphiphilic block copolymers, often referred to as polymersomes, have recently been the focus of research due to their increased stability compared to conventional liposomes.[1−3] Most traditional methods of vesicle formation from amphiphilic block copolymers involve the use of organic solvents such as tetrahydrofuran, *N*,*N*-dimethylformamide, or dioxane[1,2,4] and require extensive purification processes which can be time-consuming and problematic. Recently, pH-responsive, synthetic or bioconjugate block copolymers that spontaneously self-assemble into vesicles in aqueous or mixed solvents have been reported.[5,6] Armes’ group demonstrated that such vesicles can also be decorated with gold nanoparticles using an external reducing agent.[6] Gold nanoparticles, including those stabilized by (co)polymers, have been of particular interest because of their potential applications as biomedical, optic, and electronic materials.[7] Thiol chemistry is frequently employed for surface modification of gold nanoparticles, often via in situ reduction with NaBH$_4$ in the presence of thiol-functional molecules as stabilizers.[8,9] For example, we previously reported NaBH$_4$ reduction of dithioester-terminated, water-soluble polymers directly in water in the presence of noble metal salts including NaAuCl$_4$ to yield sterically and electrostatically stabilized zerovalent metal nanoparticles.[10] Recent reports have demonstrated that some (co)polymers without thiol functionality can act as both reducing agent and stabilizer simultaneously in the formation of gold nanoparticles.[11,12]

In our continuing research efforts related to the construction of well-defined block copolymers, we recently reported the first example (to our knowledge) of thermally responsive vesicles that could be stabilized by interpolyelectrolyte complexation.[13] Herein we report a similar thermally responsive vesicle system that is easily decorated with gold nanoparticles. These vesicles are formed (as illustrated in Scheme 1) by the self-assembly of the thermally responsive block copolymer poly[2-(dimethylamino)ethyl methacrylate-*block*-(*N*-isopropylacrylamide)] (PDMAEMA-*b*-PNIPAM) in aqueous solution. By simply mixing the polymer solution with a NaAuCl$_4$ solution at 50 °C under specified conditions, gold nanoparticle-decorated vesicles can be obtained. This procedure does not require the addition of an external reducing agent and results in stabilized vesicles which remain dispersed in aqueous solution upon cooling to room temperature.

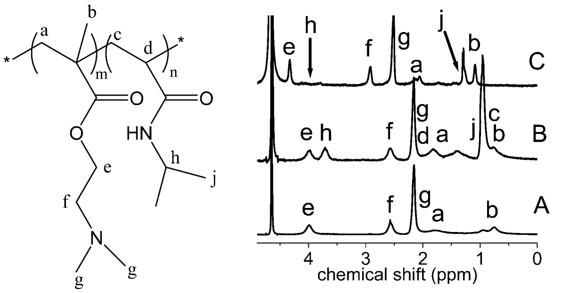

Figure 1. ^{1}H NMR spectra of the homo and block copolymers in D$_2$O at selected temperatures. Key: (A) PDMAEMA$_{73}$ homopolymer, 25 °C; (B) PDMAEMA$_{73}$-*b*-PNIPAM$_{99}$ block copolymer, 25 °C; (C) 0.01 wt % PDMAEMA$_{73}$-*b*-NIPAM$_{99}$ block copolymer solution, 50 °C.

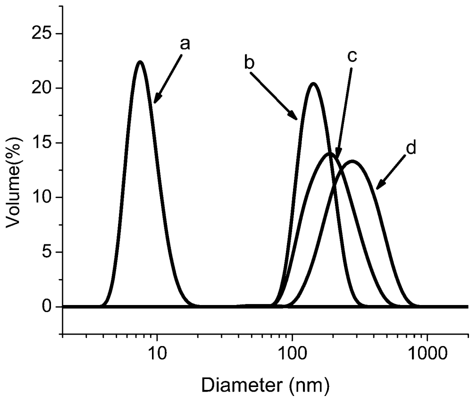

Figure 2. Dynamic light scattering size distribution of a 0.01 wt % PDMAEMA$_{73}$-*b*-PNIPAM$_{99}$ diblock copolymer solution: (a) 25 °C; (b) 50 °C; (c) 50 °C after in situ reduction of NaAuCl$_4$; (d) after in situ reduction of NaAuCl$_4$ upon lowering temperature to 25 °C.

Reversible addition−fragmentation chain-transfer (RAFT) polymerization was utilized in the synthesis of the diblock copolymer composed of pH-responsive DMAEMA and the thermally responsive NIPAM segments. In order to design diblocks with low polydispersity indices (PDIs) and compositions for (a) vesicle formation[1−3] above the lower critical solution temperature (LCST) and (b) maintenance of electrosteric stabilization of the resulting gold-decorated vesicles, it was necessary to optimize reaction conditions, monomer concentration, and blocking order. It was determined the DMAEMA should be polymerized first using 4-cyanopentanoic acid dithiobenzoate as the RAFT chain transfer agent (CTA). The resulting macroCTA could then be utilized for efficient polymerization of NIPAM. Considering these aforementioned design criteria, we first synthesized the PDMAEMA macroCTA, stopping conversion at 69% to maintain end group fidelity and molecular weight control; the number-average molecular weight (M_n) and PDI were determined to be 11 400 and 1.08, respectively. This macroCTA was then chain extended with NIPAM yielding a well-defined PDMAEMA$_{73}$-*b*-PNIPAM$_{99}$ diblock copolymer with M_n and PDI values of 22 900 and 1.14, respectively. (See the Experimental Section in Supporting Informaiton for details of synthesis and characterization (Figure S1) by SEC/MALLS).

A 0.01 wt % solution of this diblock copolymer at pH 7.4 was then prepared and the aggregation behavior studied as a function of temperature (Figure S2) utilizing dynamic light scattering (DLS). A sharp transition at 38 °C is observed from unimers with hydrodynamic diameter (D_h) below 8 nm to vesicles with average D_h of 140 nm. This process is completely

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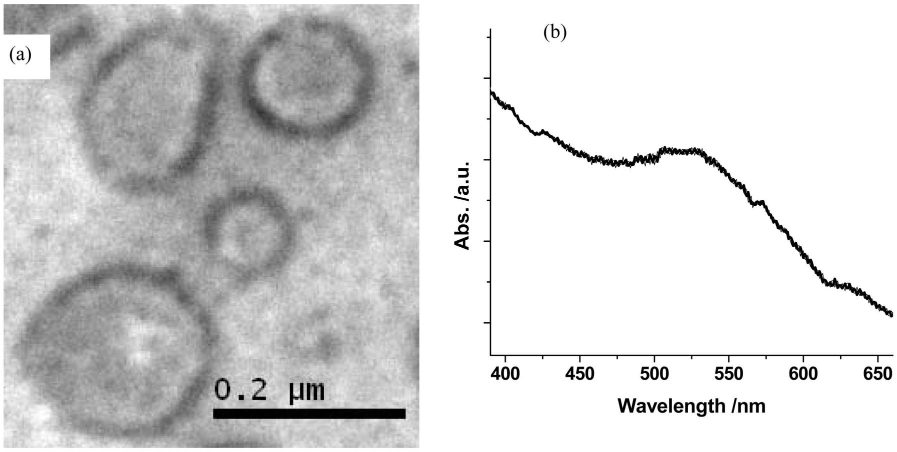
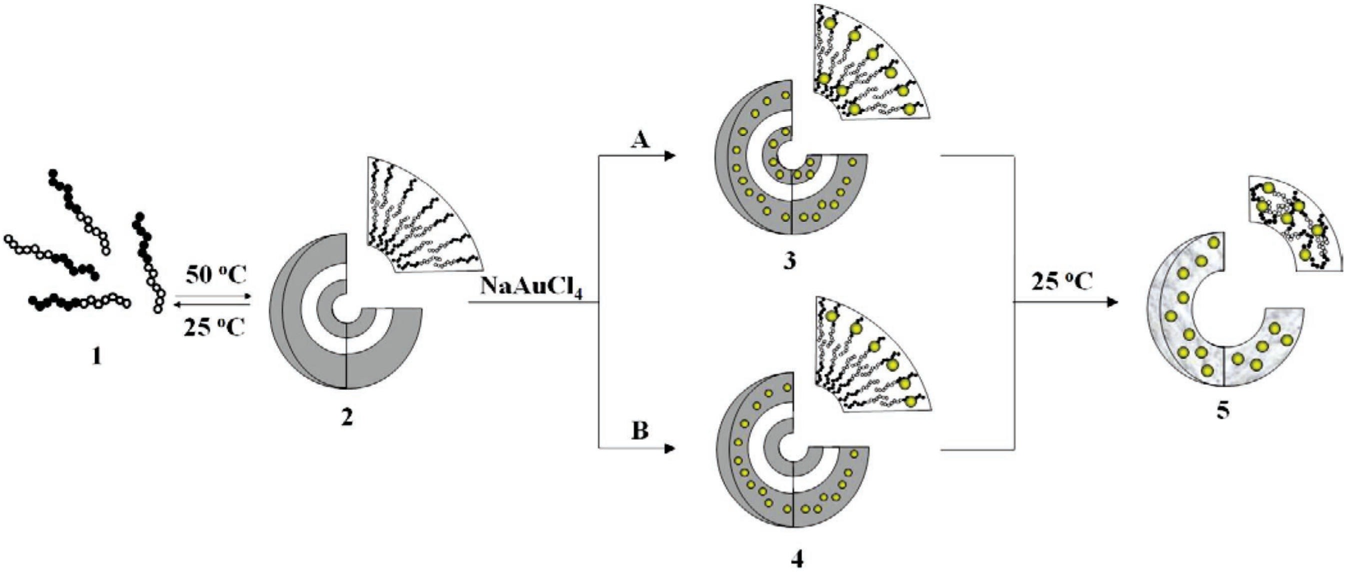


Figure 3. (a) Transmission electron microscopy images and (b) UV−vis absorption spectrum of vesicles decorated with gold nanoparticles prepared from PDMAEMA$_{73}$-*b*-PNIPAM$_{99}$ as described in the text.

Scheme 1. Formation of Thermally Responsive Vesicles Decorated with Gold Nanoparticles from PDMAEMA$_{73}$-*b*-PNIPAM$_{99}$



reversible. Also shown are the ^{1}H NMR spectra (Figure 1) of the homo and block copolymers at selected temperatures. At 25 °C, the diblock copolymer is fully solvated, and signals associated with each block are visible. An increase in the solution temperature to 50 °C causes the NIPAM signal to become broadened and significantly suppressed while the PDMAEMA signal remains, for the most part, unattenuated. This, in addition to zeta potential measurements (Figure S3, Supporting Information), reflects the presence of the positively charged PDMAEMA blocks located on the surface of the particles at pH 7.

Recently, the use of small molecule amines as reducing agents in the formation of gold nanoparticles was reported.[14] In addition, Armes et al. showed that PDMAEMA polymers can be utilized to reduce AuCl$_4^-$ counterions to zero-valent gold, and, at the same time, stabilize the resulting gold nanoparticles.[12] In our experiments, after dissolving PDMAEMA$_{73}$-*b*-PNIPAM$_{99}$ at 0.01 wt %, we first allowed vesicle formation to occur at 50 °C; the resulting solution was then mixed with the NaAuCl$_4$ solution in a 10:1 molar ratio of DMAEMA units:NaAuCl$_4$. The pH of the initial polymer solution was 7.4, reaching an equilibrated pH value of 6.4 after the addition of NaAuCl$_4$. DLS analysis (Figure 2) shows that vesicle size and size distribution increased slightly with this reaction (b to c), which is attributed to increased protonation of the PDMAEMA segments during equilibration and gold complex reduction. The mixed solution was kept at 50 °C for 2 days, after which time the solution temperature was lowered to 25 °C. DLS analysis detected no dissociation into unimers. It appears that the vesicle structure is permanently “fixed” since the thermally responsive vesicles do not dissociate into unimers at 25 °C. The vesicle size is increased at 25 °C (d) relative to that at 50 °C (c) due to the swelling behavior of the vesicles as the PNIPAM block becomes more hydrophilic at 25 °C. Compared to chemical cross-linking of vesicles,[15] this method is quite attractive since it allows for simultaneous gold nanoparticle formation, “locking” of the resulting structure, and still permits long-term stability in aqueous media.

It should be noted that the molar ratio of the PDMAEMA and NaAuCl$_4$ is critical for the formation of the gold nanoparticles-decorated vesicles. In our experiment, as previously mentioned, PDMAEMA:NaAuCl$_4$ was kept at 10:1. When the ratio was lowered to 5:1, the decrease in the hydrophilicity of the PDMAEMA/NaAuCl$_4$ block results in precipitation as manifested by the onset of turbidity.

Shown in Figure 3a is a transmission electron microscopy image of gold-decorated structures. The structures are spherical and possess morphology consistent with that of vesicles. The bound gold nanoparticles function to “stain” these structures, enhancing the TEM image. The formation of the gold nanoparticles decorating the vesicles is also confirmed by a gradual change in solution color after mixing the polymer solution with the $NaAuCl_4$ solution. Figure 3b shows the UV−vis absorbance spectrum that indicates a maximum absorbance at 525 nm, which corresponds to reported surface plasmon resonance of gold nanoparticles.[16]

In order to demonstrate that the morphology observed in Figure 3a could not be attributed to association induced by mere reduction of $AuCl_4^-$ counterion associated with the protonated PDMAEMA segments, a control experiment was conducted under identical reaction conditions and block copolymer concentration; however the temperature was maintained at 25 °C—well below the experimentally determined LCST. Within 48 h, the solution turned the characteristic red color, indicating successful reduction; however, the stabilized gold nanoparticles, roughly 20 nm in diameter, had no resemblance to the vesicles formed at 50 °C. (Figure S4 of Supporting Information).[17]

In summary, thermally responsive vesicles have been prepared from the self-assembly of a $PDMAEMA_{73}$-*b*-$PNIPAM_{99}$ block copolymer. Simply mixing the vesicle solution with a solution of $NaAuCl_4$, without the necessity of an external reducing agent, leads to the formation of gold nanoparticle decorated vesicles. On the basis of our studies thus far, we postulate a sequence of events (Scheme 1) which may account for formation and "locking" of gold nanoparticle-decorated vesicles reported in this manuscript. Thermally driven vesicle formation from unimers occurs above the LCST of responsive NIPAM block. Mixing the polymer solution with $NaAuCl_4$ allows counterion exchange with the protonated DMAEMA polyelectrolyte segments. Subsequent in situ reduction to zero-valent gold occurs, perhaps induced by the small number of unprotonated amines present at the reaction pH.[11,12,14] The conversion of complexed $AuCl_4^-$ to zero-valent gold nanoparticles is confirmed by the observed surface plasmon resonance.

Structure **5** depicted in Scheme 1 is an idealized representation gleaned from TEM (Figure 3a). At this level of resolution a single ring of gold nanoparticles is observed. In principle the rate and extent of $AuCl_4^-$ ion exchange with the Cl^- counterions along the DMAEMA segments and the ability of the former to pass through the phase separated NIPAM segments at 50 °C should determine whether both outside and inside surfaces would be decorated, hence giving one or two gold shells. We are currently unable to distinguish between the proposed pathways A and B leading to idealized structures **3** and **4**, at least utilizing our current TEM equipment.

Regardless of the precise location of the gold nanoparticles during formation at 50 °C relative to the constituent block copolymer segments, it is anticipated, based on DLS studies (Figure 2) and incipient stabilization, that morphological rearrangements will occur in the "locked" vesicles on lowering temperature to 25 °C in water. The nature of such rearrangements and possible remixing of phase separated "cross-linked" structures as suggested recently by Armes[18] are issues that need further study. We are currently in the process of investigating the effects of overall molecular weight and segmental sequence length, parameters critical to vesicle formation. As well, if the model we propose in Scheme 1 is correct, there will likely be a critical extent of interparticle bridging of the gold nanoclusters in order to maintain vesicle stability and solubility/dispersion in aqueous media—factors key to potential diagnostic and targeting applications of these systems.

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Supporting Information Available: Text giving experimental details and details of the GPC, DLS, and TEM measurements and figures showing DMF SEC traces, variation of hydrodynamic diameter with temperature, ζ potential vs pH curves, and a TEM of the control experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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