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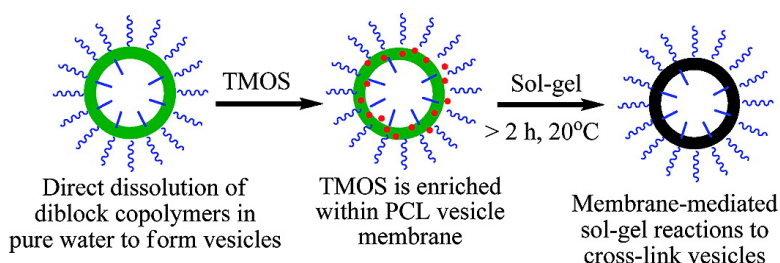
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Preparation of Primary Amine-Based Block Copolymer Vesicles by Direct Dissolution in Water and Subsequent Stabilization by Sol–Gel Chemistry

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A new amphiphilic biocompatible diblock copolymer, poly(ϵ -caprolactone)-*block*-poly(2-aminoethyl methacrylate), PCL-*b*-PAMA, was synthesized in three steps by (i) ring-opening polymerization of ϵ -caprolactone, (ii) end-group modification by esterification, and (iii) atom transfer radical polymerization (ATRP) of 2-aminoethyl methacrylate hydrochloride (AMA) in its hydrochloride salt form. This copolymer forms block copolymer vesicles with the hydrophobic PCL block forming the vesicle membrane. Unusually, these vesicles are easily prepared by direct dissolution in water without using organic co-solvents, pH adjustment, or even stirring. These vesicles can be stabilized by aqueous sol–gel chemistry using tetramethyl orthosilicate (TMOS) as the silica precursor. It is well-known that cationic polymers can catalyze silica formation, but in this particular case, it seems that the TMOS precursor is solubilized within the hydrophobic PCL membrane. Thus, the neutral membrane actually directs silica formation, rather than the cationic PAMA chains. The final vesicle morphology and the silica content depend on the silicification conditions. Provided that the TMOS/AMA molar ratio does not exceed 10:1, silicification is solely confined within the PCL membrane. At higher ratios, silica nanoparticles (5–12 nm) are also observed on the outer surface of the silicified vesicles. However, these nanoparticles appear to be only weakly adsorbed, since they can be easily removed by dialysis. The mean hydrodynamic diameter of the silicified vesicles varies from 175 to 205 nm with solution pH due to (de)protonation of the externally expressed PAMA chains. Calcination of the silicified vesicles at 800 °C leads to the formation of hollow silica particles. ^1H NMR, transmission electron microscopy (TEM), dynamic light scattering (DLS), aqueous electrophoresis, and thermogravimetric analysis (TGA) were employed to characterize the vesicles, both before and after silicification.

Recently, there has been substantial research activity directed toward the preparation of block copolymer vesicles using self-assembly techniques.^{1–9} Compared to conventional surfactant-based liposomes, polymer-based vesicles are usually much more robust, provide a greater barrier toward diffusion, and offer the intriguing possibility of tunable physicochemical and biological properties. Poly(ϵ -caprolactone) [PCL] is a biodegradable polymer that has been used to prepare PCL-based block copolymer vesicles for drug release applications.^{10–12} However, water-

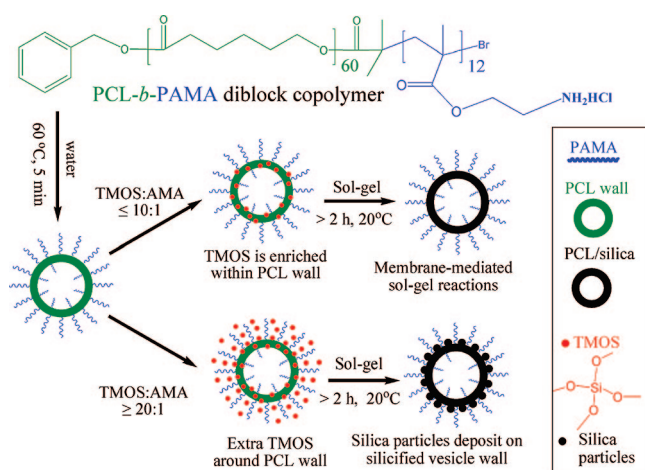
miscible organic co-solvents such as THF are typically required to prepare such vesicles. Removal of such co-solvents is essential for biomedical applications, but this is rather time-consuming using purification methods such as dialysis. Thus, processing techniques that avoid co-solvents have attracted increasing attention.^{13–18} Recently, it has been reported that direct dissolution of poly(ethylene oxide)-*b*-PCL-*b*-poly(acrylic acid) triblock copolymers in water can form vesicles. However, self-assembly required relatively long time scales (four days) and vigorous agitation,¹² and the resulting vesicles were always contaminated with other colloidal aggregates such as large compound micelles. In related work, Du et al. reported that pH-sensitive vesicles could be prepared directly in water by pH adjustment,⁷ while McCormick's group reported that thermoresponsive AB diblock copolymer vesicles could be formed in pure water by simply increasing the temperature above the LCST of the poly(*N*-isopropyl acrylamide) B block.¹⁸ Compared to pure organic polymer vesicles, organic/inorganic hybrid vesicles have more stability. Organosiloxane-based block copolymer vesicles can be silicified by *in situ* sol–gel chemistry that is spatially restricted within the vesicle walls to give water-soluble polymer/silica hybrid vesicles.^{4–6,19,20} Alternatively, tetramethyl orthosilicate (TMOS) has been used as a soluble silica precursor to coat either surfactant vesicles^{21–23} or Pluronic-based polymer vesicles.²⁴

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Scheme 1. Formation of PCL-*b*-PAMA Diblock Copolymer Vesicles by Direct Dissolution and Their Subsequent Silicification Using TMOS According to Our Proposed New Vesicle Membrane-Mediated Sol-Gel Reaction Mechanism



However, these templating methods are not always easy to control and can often lead to water-insoluble products. It is widely believed that cationic polymers or surfactants catalyze silica deposition from aqueous solution, particularly if the silica precursor(s) has *anionic* character. For example, recent papers describe the formation of ordered silica structures directed by amines or polyamines,²⁵ by cationic poly(L-lysine) in block copolypeptides,²⁶ and the cationic coronas of block copolymer micelles.²⁷ In contrast, PEO-*b*-PPO-*b*-PEO triblock copolymers can direct mesoporous silica deposition at pH 1, apparently because the protonated *cationic* silica precursor species preferentially interact with the hydrophilic PEO chains.²⁸ In most reports of block copolymer-mediated silica deposition, either the hydrophilic cationic blocks served as both a scaffold and a catalyst template, or else a “cooperative” mechanism was used to explain the complex structures that were obtained. Generally, the importance of having appropriate *hydrophilic* blocks has been emphasized, while the precise role (if any) played by the hydrophobic blocks is usually less clear.

Herein, we report a facile method to prepare well-defined block copolymer vesicles within 5 min simply by direct dissolution of the PCL-*b*-PAMA diblock copolymer in pure water at 60 °C (see Scheme 1). Moreover, these vesicles can be subsequently stabilized by aqueous sol-gel chemistry without any concomitant precipitation. In principle, the cationic PAMA chains expressed on the vesicle exterior could catalyze *in situ* silica formation at the interface between the PCL membrane and the aqueous solution. However, in practice silica formation only occurs within the membrane itself. This is because the silica precursor (TMOS) has relatively poor water solubility and is therefore preferentially solubilized within the hydrophobic PCL membrane. To the best of our knowledge, this is the first time that such a “template” role has been suggested for hydrophobic vesicle membranes during silicification: this new mechanism is termed “vesicle membrane-mediated sol-gel reaction”.

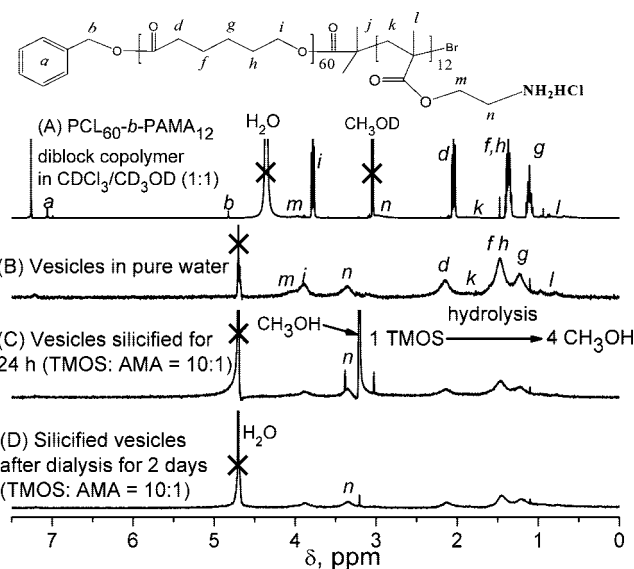


Figure 1. ^1H NMR spectra recorded at 20 °C for (A) the PCL₆₀-*b*-PAMA₁₂ diblock copolymer in a good solvent mixture (1:1 CDCl₃/CD₃OD); (B) the vesicles formed after direct dissolution of this block copolymer in water; (C) the same vesicles after silicification for 24 h using a TMOS/AMA molar ratio of 10:1; (D) the same silicified vesicles after dialysis against water for 48 h.

Results and Discussion

The particular diblock copolymer used in this study is PCL₆₀-*b*-PAMA₁₂, where the subscripts denote the number-average degrees of polymerization of each block. The synthetic route to this copolymer is shown in Scheme S1 (see Supporting Information). First, a monohydroxy-functional PCL precursor (PCL-OH; $M_n = 14\,000$; $M_w/M_n = 1.16$; THF GPC) was synthesized by ring-opening polymerization of ϵ -caprolactone in anhydrous THF. Once this polymerization had ceased, excess 2-bromoisobutyl bromide (relative to PCL-OH) was added to the reaction solution, and the resulting esterification produced the desired ATRP macroinitiator (PCL₆₀-Br after purification, as judged by ^1H NMR spectroscopy). The synthesis of the target block copolymer was achieved by ATRP of 2-aminoethyl methacrylate hydrochloride (AMA) monomer in a 5:2 THF/water mixture at 50 °C for 24 h. The ^1H NMR spectra shown in Figure S1 (see Supporting Information) and Figure 1 confirmed the successful synthesis of the PCL₆₀-Br macroinitiator and the final PCL₆₀-*b*-PAMA₁₂ diblock copolymer, respectively. The degree of esterification of the PCL₆₀-Br macroinitiator is estimated to be 99% by comparing the integrated areas of signals *b* and *j* (see Supporting Information Figure S1A). The mean block composition of PCL₆₀-*b*-PAMA₁₂ was determined by comparing the integrated areas of signals *b* and *d* (see Figure 1). By inspecting Supporting Information Figure S1, one finds it noteworthy that peak *j* at $\delta 1.95$ ppm [Br-C(CH₃)₂-COO] in the PCL₆₀-Br macroinitiator spectrum is shifted to 1.45 ppm [CH₂-C(CH₃)₂-COO] in the spectrum obtained for PCL₆₀-*b*-PAMA₁₂; this confirmed that there was no significant PCL₆₀-Br impurity in the diblock copolymer. Thus, the ^1H NMR and GPC data (see Experimental Section) confirmed successful synthesis and purification of a novel PCL₆₀-*b*-PAMA₁₂ diblock copolymer.

Vesicles were formed spontaneously on direct dissolution of the PCL₆₀-*b*-PAMA₁₂ diblock copolymer in pure water at 60 °C. The PCL chains form the hydrophobic membrane, and the hydrophilic PAMA chains are expressed at both the interior and exterior walls, with the latter chains providing colloidal stability by a steric stabilization mechanism. Despite significant problems

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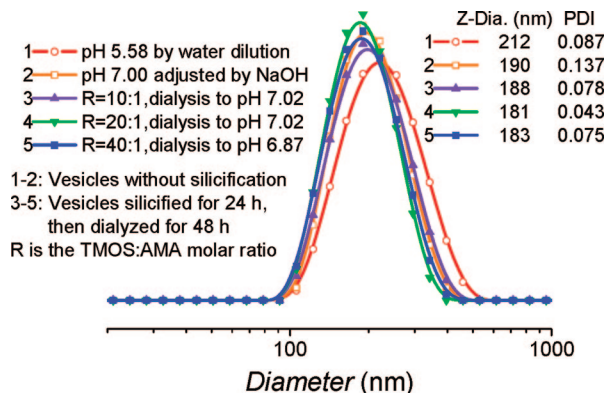


Figure 2. DLS studies of PCL₆₀-*b*-PAMA₁₂ diblock copolymer vesicles prepared by direct dissolution of the copolymer in water at 60 °C (the corresponding correlation functions are shown in Supporting Information Figure S3 and a better comparison between curves 1 and 2 is provided in Supporting Information Figure S4).

due to *in situ* beam damage, TEM studies conducted without any staining protocol provided some evidence for vesicle formation and yielded an estimated number-average vesicle diameter of 184 ± 28 nm (see Supporting Information Figure S2). Staining with ammonium heptamolybdate tetrahydrate allowed better-quality TEM images to be obtained (see Supporting Information Figure S5). Particle size analysis of more than 50 stained vesicles indicated a number-average diameter of approximately 190 ± 49 nm and allowed estimation of the mean PCL membrane thickness (12.4 ± 0.8 nm). The correlation functions obtained from DLS studies of dilute aqueous vesicle solutions gave good fits to the data (see Supporting Information Figure S3). The corresponding vesicle size distribution (see Supporting Information Figure S4) revealed that the hydrodynamic diameter (D_h) for the same vesicles ranged from 100 to 550 nm, with an intensity-average diameter of 212 nm. Given that DLS tends to oversize relative to TEM, these results are in reasonably good agreement and confirm successful vesicle preparation by the direct dissolution method.

Figure 3 shows TEM images of vesicles decorated with gold nanoparticles. A reaction scheme for this functionalization is shown in Supporting Information Scheme S2. First, dilute aqueous HAuCl₄ solution was added to an aqueous vesicle solution (see Supporting Information Figure S2) in order to protonate the PAMA chains and hence incorporate AuCl₄[−] as a counterion. Subsequent *in situ* reduction of this anionic Au(III) species by NaBH₄ produced zerovalent gold nanoparticles of 1–8 nm. This protocol selectively stains the vesicles and confirms the location of the PAMA chains within the vesicle coronas.

Analysis of the ¹H NMR spectrum recorded for block copolymer vesicles in D₂O indicates that the PCL signals are only partially attenuated: their apparent degree of solvation is approximately 34% (see Figure 1, plus Supporting Information Figure S6 for calculation details). This suggests that the moderately hydrophobic PCL blocks form partially hydrated vesicle membranes, whereas more hydrophobic membranes are known to be highly dehydrated, as judged by ¹H NMR.^{4,6}

Despite the partially hydrated nature of the PCL chains, the vesicle membranes formed by the PCL₆₀-*b*-PAMA₁₂ copolymer can solubilize the poorly water soluble TMOS rather efficiently. For example, up to 200 μL of TMOS can be dissolved in 1.00 mL of a 0.10 wt % aqueous copolymer vesicle solution within a few minutes, whereas only 5 μL of TMOS was sufficient to form visible oil droplets when added to the same volume of pure water. In a further control experiment, PCL₆₀ homopolymer was

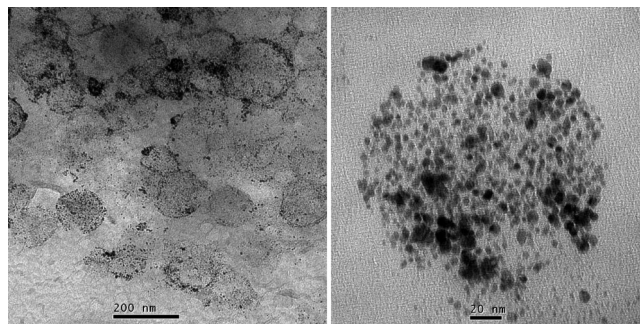


Figure 3. PCL₆₀-*b*-PAMA₁₂ diblock copolymer vesicles decorated with gold nanoparticles after *in situ* reduction of HAuCl₄ using NaBH₄. The same vesicles prior to staining are shown in Supporting Information Figure S2.

found to be fully soluble in pure TMOS. Thus, when added to an aqueous vesicle solution, TMOS should be preferentially solubilized within the PCL membranes. ¹H NMR spectra recorded for TMOS added to pure D₂O and a vesicle solution in D₂O are shown in Supporting Information Figure S7 and Supporting Information Figure S8, respectively. TMOS was fully hydrolyzed to afford Si(OH)₄ after 80 min in pure D₂O and hence became water-miscible on this time scale. However, some TMOS (and also partially hydrolyzed TMOS) was still detected by ¹H NMR 80 min after its addition to the vesicle solution due to solubilization (and hence protection against hydrolysis) within the hydrophobic PCL membrane. Similar encapsulation effects have also been reported for hydrophobic dyes within PEO-*b*-PCL vesicle membranes.¹⁰ In addition to its solubilization, TMOS is subject to concomitant sol–gel reactions within the PCL membrane, especially in the presence of acid (the pH of the aqueous vesicle solution is around 5). Moreover, polycondensation of the TMOS should be catalyzed by the cationic PAMA chains, particularly at the membrane/solution interface. Although acid is a better catalyst for hydrolysis than base (whereas base is a more effective catalyst for polycondensation), the former can also catalyze polycondensation.¹⁹ Thus, the solubilized, hydrolyzed TMOS within the PCL membrane will eventually be converted into silica. However, some hydrolyzed TMOS may escape into the aqueous phase during dialysis against water, since Si(OH)₄ is water-miscible. This point is discussed further below.

At a 10:1 TMOS/AMA molar ratio (which corresponds to a 2:1 mass ratio of TMOS to diblock copolymer), TEM studies suggest that the sol–gel reaction occurs exclusively within the PCL membrane walls, instead of within the PAMA coronas (see Figure 4). The mean membrane thickness increased slightly from 12.4 ± 0.8 nm (stained vesicle membrane) to 15.1 ± 0.7 nm (silicified vesicle membrane) due to incorporation of the silica component. In addition, ¹H NMR studies confirmed that the PAMA signals remained visible after silicification and the PCL signals were further attenuated from 34% in pure water to 20% after silicification for 24 h (see Supporting Information Figure S6). This is reasonable because, if the sol–gel reaction is confined solely within the membrane, the PCL chains will necessarily become less mobile while the PAMA chains will not be adversely affected. This interpretation is consistent with just a slight reduction in zeta potential (compare curves *a* and *b* in Figure 5), since this parameter is mainly affected by the solvated PAMA chains, rather than the vesicle walls. DLS studies on the silicified vesicles conducted at pH 7 revealed a relatively narrow size distribution and essentially the same intensity-average diameter as that observed before silicification (compare curves 2 and 3 in Figure 2). These observations are also consistent with

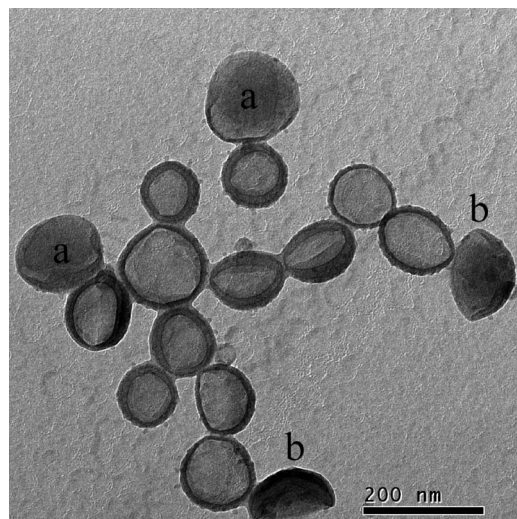


Figure 4. TEM images obtained for vesicles after membrane-mediated silicification. The TMOS/AMA molar ratio was 10:1, and the reaction time for silicification was 24 h. TEM samples were prepared by allowing 10 μ L of aqueous vesicle solution to dry onto TEM grids. The number-average diameter is 140 ± 13 nm, and the estimated wall thickness is 15.1 ± 0.7 nm. Some vesicles were partially collapsed (a) vertically and (b) laterally. The same vesicles prior to silicification are shown in Supporting Information Figures S2 and S5.

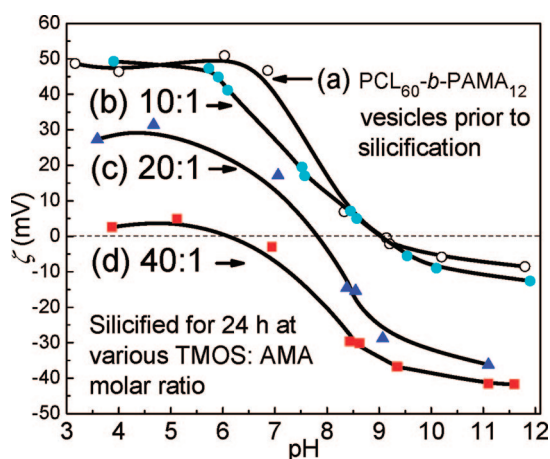


Figure 5. Zeta potential vs pH curves for various silicified PCL₆₀-b-PAMA₁₂ diblock copolymer vesicles prepared at TMOS:AMA molar ratios of 10:1, 20:1, and 40:1 for 24 h at 20 °C. The initial nonsilicified copolymer vesicles are included as a comparison.

silicification being confined within the PCL membrane, since this should minimize vesicle-vesicle interactions.

When the TMOS/AMA molar ratio was increased to 20:1 (TMOS/copolymer mass ratio = 4:1), both the PAMA and PCL signals remain visible as judged by ¹H NMR (see Supporting Information Figure S6), but the latter are further attenuated (to only 9% of the integrated intensity that is observed in a good solvent environment). This change is accompanied by a significant reduction in zeta potential (see curve c in Figure 5). The additional TMOS forms silica nanoparticles of 4–12 nm that are deposited onto the exterior of the silicified membrane among the PAMA chains, as shown in Figure 6. We suggest that the sol-gel reaction originates within the PCL membrane. As this PCL membrane is fully silicified, some of the excess TMOS is converted into water-soluble Si(OH)₄, which then diffuses out of the membrane and forms silica nanoparticles that adhere to the membrane exterior. According to the ¹H NMR spectra, the cationic PAMA chains remain well-solvated and mobile, which suggests that

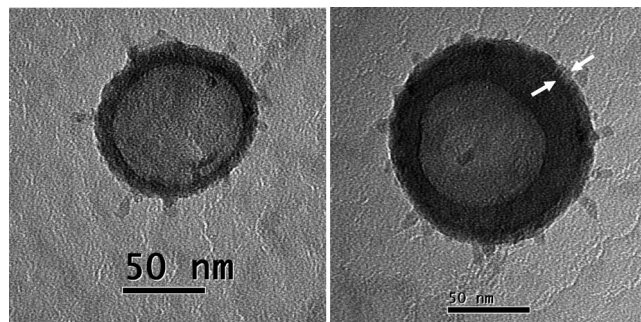


Figure 6. TEM images recorded for the nondialyzed PCL₆₀-b-PAMA₁₂ diblock copolymer vesicles after silicification for 24 h at 20 °C using a TMOS/AMA molar ratio of 20:1. Silica nanoparticles of 4–12 nm are deposited onto the silicified PCL membrane. In contrast, relatively few silica nanoparticles were observed at a TMOS/AMA molar ratio of 10:1 (see Figure 4). The vesicle shown on the right is vertically collapsed with a ruptured membrane at the bottom. This suggests that the particle labeled 'a' shown in Figure 4 is actually a vesicle, rather than a lamella-type structure. The arrows indicate the mean vesicle membrane thickness and the circular area with brighter contrast is due to the partial loss of the underlying membrane.

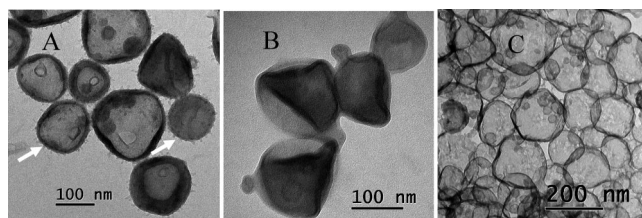


Figure 7. TEM images obtained for PCL₆₀-b-PAMA₁₂ diblock copolymer vesicles after silicification for 18 h at a TMOS/AMA molar ratio of 40:1. (A) before dialysis (white arrows indicate the silica nanoparticles located on the vesicle periphery); (B) after dialysis (silica nanoparticles are no longer present); (C) vesicles shown in B after calcination at 800 °C (TGA curve a in Figure 10 after redispersion in water). Note that silica nanoparticles deposited onto the surface of the silicified PCL membrane are removed by dialysis (compare images A and B) and the vesicular membrane is more electron-transparent after calcination (C).

they do not act as the locus for silica deposition. DLS studies indicate a relatively narrow vesicle size distribution and a slight decrease in D_h (from 190 to 181 nm) at pH 7.0 (see Figure 2).

When the TMOS/AMA molar ratio was further increased to 40:1 (TMOS/copolymer mass ratio = 8:1), the PCL membrane was also silicified, and additional silica nanoparticles were deposited onto the external surface of the vesicles, as confirmed by the TEM image shown in Figure 7A. This is consistent with the observed further reduction in zeta potential (see curve d in Figure 5). The relative degrees of solvation of the PAMA and PCL signals estimated from ¹H NMR remain unchanged (see Supporting Information Figure S6), suggesting that no further silicification within the PCL membranes occurs (and the additional TMOS is simply converted into silica nanoparticles adsorbed onto the silicified PCL vesicle walls). DLS studies indicated a relatively narrow vesicle size distribution: the D_h at pH 7.0 was close to that obtained for vesicles silicified at a TMOS/AMA molar ratio of 20:1 (compare curves 4 and 5 in Figure 2).

The silica nanoparticles observed on the vesicle surface can be easily removed by dialysis, suggesting only weak physical adsorption. Presumably, this is an electrostatic interaction between the cationic PAMA chains and the anionic silica nanoparticles. The electrophoretic behavior of silicified vesicles after dialysis is similar to that found for nonsilicified vesicles (see Supporting Information Figure S9). This suggests that the weakly adsorbed silica nanoparticles significantly affect the zeta potential of the

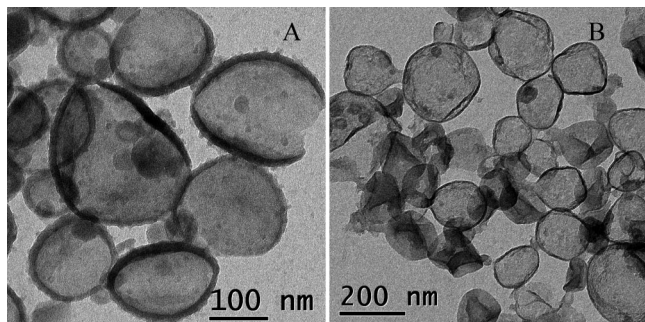


Figure 8. TEM images obtained for silicified vesicles after THF extraction for 16 h: (A) undialyzed vesicles silicified at a TMOS/AMA molar ratio of 10:1 for eight days (mean wall thickness = 14.4 ± 1.2 nm and the number-average vesicle diameter = 171 ± 42 nm, based on more vesicles shown in Supporting Information Figure S10); (B) vesicles silicified at a TMOS/AMA molar ratio of 40:1 for two days, followed by dialysis against water and THF extraction (mean wall thickness and diameter are 10.5 ± 0.5 nm and 168 ± 41 nm, respectively). The mean wall thickness of nondialyzed vesicles silicified under the same conditions is ~ 15 nm after THF extraction.

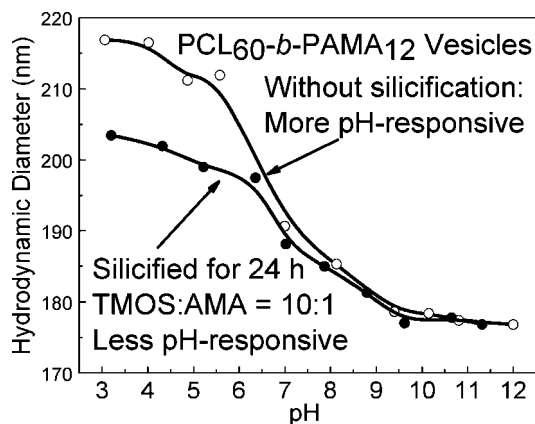


Figure 9. Effect of varying the solution pH on the dimensions of PCL₆₀-*b*-PAMA₁₂ copolymer vesicles before and after silicification, as judged by DLS. The vesicles were silicified using a TMOS/AMA molar ratio of 10:1 at 20 °C and dialyzed prior to DLS studies.

vesicles. A typical TEM image of silicified vesicles after dialysis is shown in Figure 7B. The sample shown in Figure 7B was freeze-dried and then calcined at 800 °C. The TEM image of the calcined vesicles shown in Figure 7C suggests reasonable mechanical integrity, despite some heat-induced shrinkage of the vesicle membrane.

After silicification, the vesicles were expected to have improved chemical resistance when exposed to good solvents for the PCL membrane such as THF. To test the above hypothesis, 200 μ L of a 0.10 w/w % aqueous solution of silicified vesicles (see Figure 4) were added to THF (1.0 mL) for 16 h. TEM studies (see Figure 8A and Supporting Information Figure S10) confirm that THF extraction causes no significant vesicle deformation or change in membrane thickness. The number-average vesicle diameter increased to 171 ± 42 nm after THF extraction compared to 140 ± 13 nm before THF extraction, as judged by TEM. This is reasonable since the vesicles were swollen in the presence of THF. Silicified vesicles after dialysis were also subjected to THF extraction (see Figure 8B and Supporting Information Figure S11). There was no appreciable vesicle deformation (number-average diameter = 168 ± 41 nm) but a thinner mean vesicle membrane was observed (10.5 ± 0.5 nm). However, the membrane thickness of nondialyzed vesicles remained unchanged prior to THF extraction. Thus, the membrane thinning observed

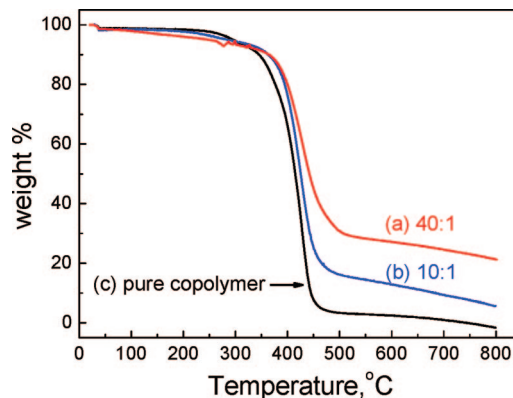


Figure 10. Thermogravimetric curves obtained for the vesicle copolymer precursor and two silicified vesicles (after purification by dialysis). Silicification was conducted using varying TMOS/AMA molar ratios for 48 h (see curve labels).

in Figure 8B is due to dialysis, rather than THF extraction. Presumably, this is because hydrolyzed TMOS precursor [i.e., water-soluble Si(OH)₄] diffuses from the PCL membrane during dialysis. On the other hand, PCL signals were not detected in ¹H NMR studies of silicified vesicles in *d*₈-THF, which is a good solvent for PCL homopolymer. This indicates that no diblock copolymer chains were extracted into this solvent (see Supporting Information Figure S12).

The strong attenuation of the PCL signal observed after silicification suggests that its chain mobility within the membrane is significantly reduced. This sol–gel chemistry also produces methanol. This small molecule byproduct is not completely removed by dialysis, possibly due to its encapsulation inside the silicified vesicle walls. No TMOS signals were observed after 5 h (see Supporting Information Figure S8), indicating its complete hydrolysis. However, under weakly acidic conditions its rate of polycondensation is much slower than that of hydrolysis, so effective silicification requires rather longer time scales.

It is clear from our results that the moderately hydrophobic PCL chains play an important role in silicification by solubilizing the poorly water soluble TMOS precursor. It is emphasized that the silicified vesicles are not simply *silica-coated* vesicles with the cationic PAMA chains acting as the template; otherwise, the maximum silica overlayer thickness would not exceed the contour length of PAMA₁₂ (~ 3 nm). The actual membrane thickness is 15.1 nm, which is consistent with silicification throughout the vesicle walls.

Although the cationic nature of the PAMA chains may aid silicification, this is not essential for efficient vesicle formation. Recently, we have found that nanorods formed by self-assembly of *nonionic* PCL-*b*-PEO block copolymers can also be silicified using TMOS in pure water under similar conditions to those described herein (see Supporting Information Figure S13).

The hydrodynamic diameter (D_h) of PCL₆₀-*b*-PAMA₁₂ vesicles in aqueous solution depends on the solution pH (see Figure 9). Pristine and silicified vesicles both have a larger D_h at lower pH due to protonation of the PAMA chains ($pK_a \sim 7.6$). However, the vesicles remain colloidally stable up to pH 12, which indicates that the PAMA chains are significantly more hydrophilic in their neutral, uncharged state than other polybases such as poly[2-(dimethylamino)ethyl methacrylate].²⁹ It is perhaps also noteworthy that the pH-induced change in dimensions is smaller for the silicified vesicles, presumably due to the reduced chain mobility and membrane flexibility in this case.

(29) Dupin, D.; Armes, S. P.; Connan, C.; Reeve, P.; Baxter, S. M. *Langmuir* 2007, 23, 6903–6910.

Assuming that the TMOS is fully converted into silica within the PCL membrane, a TMOS/AMA molar ratio of 10:1 should correspond to a maximum theoretical silica content of approximately 45 wt %. However, TGA studies (curve b in Figure 10) revealed that a sol–gel reaction time of 48 h produced vesicles with an incombustible residue of only 16 wt % at 500 °C. This residue was further reduced to just 6 wt % at 800 °C, indicating that further polycondensation of the partially reacted silica occurs at elevated temperature. This was further confirmed by the TGA results of silica prepared from pure TMOS in the absence of vesicles (Supporting Information Figure S14). Similar results were obtained for sol–gel reactions conducted under other conditions. For example, a TMOS/AMA molar ratio of 40:1 corresponds to a maximum theoretical silica content of 76 wt %, whereas only around 32 wt % residues remained at 500 °C, and the final residue was 22 wt % at 800 °C (curve a in Figure 10).

In summary, a new class of copolymer vesicles has been obtained by the spontaneous self-assembly of a new primary amine based block copolymer in purely aqueous solution without using cosolvents or mechanical stirring. Due to their primary amine functionalized exterior, these vesicles can be readily surface functionalized: this has demonstrated by gold nanoparticle decoration. Moreover, the hydrophobic PCL vesicle membrane can be readily silicified using TMOS to form colloiddally stable organic/inorganic hybrid vesicles. Unusually, the hydrophobic PCL membrane acts as a scaffold by solubilizing the poorly water soluble TMOS precursor, while the cationic PAMA chains act as an efficient catalyst for silicification at the vesicle/aqueous solution interface. This new mechanism is termed “vesicle membrane-mediated sol–gel reaction” and appears to be quite generic: we have already extended this new concept in silicification to include other PCL-based water-soluble block copolymers. In principle, the hydrophobic PCL chains should be prone to slow hydrolytic degradation, which suggests the possibility of intracellular delivery of water-soluble drugs and proteins. Thus, these new vesicles may offer biomedical applications as nanosized delivery vehicles.

Experimental Section

Materials. ϵ -Caprolactone (Aldrich) was dried azeotropically using anhydrous toluene to remove traces of water. Stannous 2-ethylhexanoate (SnOct₂, approximately 95%), tetrahydrofuran (THF), triethylamine, 2-bromoisobutyl bromide, copper(I) bromide (CuBr; 99.999%), 2,2'-bipyridine (bpy), sodium 2-acrylamido-2-methyl-1-propanesulfonate (50 wt % solution in water), and other reagents were purchased from Aldrich and used as received. Dialysis tubing with a molecular weight cutoff of 1000 was purchased from Medicell International Ltd. AMA monomer was synthesized according to a previously reported literature protocol.³⁰

Characterization. *DMF GPC.* The molecular weight distributions of the PCL₆₀-*b*-PAMA₁₂ diblock copolymer and PCL₆₀-Br macroinitiator were assessed at 70 °C using a Polymer Laboratories PL-GPC50 Integrated GPC system equipped with a Polymer Laboratories pump, three Polymer Laboratories PLgel 10 μ m MIXED-B columns in series, a Viscotek TriSEC model 302 refractive index detector. Six poly(methyl methacrylate) standards with M_p values ranging from 1310 to 211 400 were used for calibration purposes. The DMF eluent contained 10 mmol LiBr, and the flow rate was 1.0 mL min⁻¹. The data were processed using Viscotek TriSEC 3.0 software. The copolymers were dissolved in DMF and filtered prior to analysis. The PCL₆₀-Br macroinitiator had an M_n of 9000 and an M_w/M_n of 1.19, while the PCL₆₀-*b*-PAMA₁₂ diblock copolymer had an M_n of 11 400 and an M_w/M_n of 1.33 (derivatized with methyl acrylate in the presence of triethylamine in DMF).

¹H NMR Spectra. Spectra were recorded using a Bruker AV 400 MHz spectrometer at ambient temperature using either CDCl₃,

CD₃OD/CDCl₃, *d*₈-THF/H₂O, D₂O/H₂O, or D₂O as solvents. When D₂O/H₂O or *d*₈-THF/H₂O solvent mixtures were used, the “water suppression” software option was selected.

TEM. Images were obtained using a Philips CM100 electron microscope operating at 100 kV equipped with a LaB₆ gun and a Gatan 1K \times 1K digital camera. To prepare TEM samples, 10 μ L of a diluted aqueous vesicle solution was placed on a carbon-coated copper grid, and the water droplet was allowed to evaporate under ambient conditions. Aqueous ammonium heptamolybdate tetrahydrate solution (1%) was used as a positive stain for the vesicles, as shown in Supporting Information Figure S5.

Zeta Potential and DLS Studies. Studies were conducted at 25 °C using a Zetasizer Nano series instrument (Malvern Instruments) equipped with a multipurpose autotitrator (MPT-2). DLS studies of aqueous vesicles were conducted over a range of solution pH at a fixed scattering angle of 173°. The data were processed by cumulants analysis of the experimental correlation function, and vesicle diameters were calculated from the computed diffusion coefficients using the Stokes–Einstein equation. Each reported measurement was the average of three runs. A Hanna 9024 HI microcomputer pH meter (Aldrich) was calibrated before measurements.

Thermogravimetric Analyses (TGA). Analyses were conducted using a Perkin-Elmer Pyris 1 TGA instrument. Dried samples were heated in air to 800 °C at a heating rate of 20 °C min⁻¹. The observed mass loss was attributed to quantitative pyrolysis of the PCL₆₀-*b*-PAMA₁₂ diblock copolymer, with the remaining incombustible residues assumed to be pure silica (SiO₂).

Synthesis of the PCL₆₀-Br Macroinitiator. This was readily achieved using a one-pot method. A three-neck flask charged with a magnetic flea, ϵ -caprolactone (76.30 g, 0.6620 mol), and dry toluene (162.0 g) was placed into an oil bath at 144 °C. Approximately 100 g of toluene was removed by azeotropic distillation under nitrogen so as to remove traces of water from the flask. The reaction temperature was set at 110 °C, and the solution was further degassed using a N₂ sparge for 30 min. Benzyl alcohol (1.193 g, 0.0110 mol) was then added via syringe. SnOct₂ (18.8 μ L, 5.52 \times 10⁻⁵ mol) was added by micropipette under a positive nitrogen pressure. The monomer conversion reached 92% after 48 h at 110 °C, as judged by ¹H NMR. Aliquots of \sim 1.0 mL were withdrawn periodically for GPC and ¹H NMR analyses. The reaction flask was cooled to room temperature, toluene (400 mL) was added, and the flask was placed in an ice–water bath. Triethylamine (7.76 mL, 0.055 mol) was added, and then 2-bromoisobutyl bromide (6.77 mL, 0.055 mol) was added at a rate of approximately one drop every 10 s. Precipitation of insoluble hydrobromide salt was immediately observed, as expected. After 60 h, excess 2-bromoisobutyl bromide was reacted with methanol, and the insoluble salt was removed by filtration. The filtrate was concentrated to \sim 200 mL and then added slowly to excess methanol so as to obtain a fine precipitate. The precipitate was isolated by filtration and redissolved in dichloromethane (\sim 300 mL). This purification protocol was repeated at least five times to obtain a pure white polymer (85% yield). The ¹H NMR spectrum of this macroinitiator is shown in Figure S1 of the Supporting Information. Its mean degree of polymerization was estimated to be approximately 60 by comparing the integrated intensities of peaks b and g. Similar results were obtained by comparing peak b with other peaks (e.g., i or d).

ATRP Synthesis of PCL₆₀-*b*-PAMA₁₂ Diblock Copolymer. In a typical ATRP protocol, a flask with a magnetic flea and a rubber septum was charged with PCL₆₀-Br macroinitiator (2.000 g, 0.2860 mmol) and THF (30 mL). After complete dissolution, a solution of AMA monomer (1.037 g, 6.29 mmol), and bpy ligand (17.85 mg, 1.140 mmol) in water (12 mL) was added. This solution was deoxygenated using a N₂ sparge for 30 min before adding Cu(I)Br (0.0820 g, 0.5710 mmol). The [AMA]:[PCL-Br]:[CuBr]:[bpy] relative molar ratios were 22:1:2:4. The AMA polymerization was conducted under a nitrogen atmosphere at 50 °C. After 24 h, the reaction solution was diluted with water and purified by dialysis against water. A fine white powder (1.74 g) was obtained after freeze–drying. A ¹H NMR spectrum of the purified block copolymer was recorded using a 1:1 CDCl₃/CD₃OD solvent mixture.

Preparation of Vesicles. The following protocol was adopted. Block copolymer (typically 100.0 mg, but varied to obtain different copolymer concentrations) was dissolved in water (100 mL). Most of copolymer dissolved immediately at room temperature: full dissolution to form a near-transparent bluish aqueous vesicle solution was achieved after heating to 60 °C for 5 min. At higher copolymer concentrations (e.g., 1.0 wt %), the aqueous solution was turbid (milky). However, a near-transparent bluish solution was obtained immediately on dilution.

In Situ Formation of Gold Nanoparticles within the Vesicle Coronas. An aqueous vesicle solution prepared using the PCL₆₀-*b*-PAMA₁₂ copolymer (1.0 g of a 0.10 wt % solution) was mixed with an aqueous HAuCl₄ solution at a 1:1 HAuCl₄/AMA molar ratio (13.3 μL of 0.1 mmol HAuCl₄ stock solution). After stirring at 20 °C for 0.5 h, an aqueous solution of NaBH₄ (1:1 NaBH₄/HAuCl₄ molar ratio; 13.3 μL of 0.1 mmol NaBH₄ stock solution) was added. The solutions immediately became yellow after adding HAuCl₄. The solution became brown after adding NaBH₄.

Solubility of PCL₆₀-Br Macroinitiator and PCL₆₀-*b*-PAMA₁₂ Diblock Copolymer in TMOS. A 10 mL glass vial was charged with PCL₆₀-Br macroinitiator (4.0 mg) and TMOS (400 mg). The macroinitiator was only partly soluble at 21 °C, but dissolved immediately at 30 °C. The solubility of the PCL₆₀-*b*-PAMA₁₂ diblock copolymer increased with temperature, but full dissolution did not occur.

Solubility of TMOS in Aqueous PCL₆₀-*b*-PAMA₁₂ Vesicle Solution. The solubility of TMOS in the aqueous vesicle solution was evaluated according to the following protocol: Ten 20 μL aliquots of TMOS were added sequentially to a PCL₆₀-*b*-PAMA₁₂ aqueous vesicle solution (0.10 wt %; 1.0 mL), with full solubilization being observed within 10 s in each case. In contrast, just 5 μL of TMOS formed visible oil droplets when added to the same volume of pure water (these droplets disappeared after 10 min due to hydrolysis of the TMOS).

THF Extraction of Silicified Vesicles. 200 μL of aqueous silicified vesicles (0.10 wt %) were added to THF (1.0 mL) and stirred for 16 h prior to TEM examination.

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Supporting Information Available: Schemes for copolymer synthesis and gold-decorated vesicle preparation, additional ¹H NMR spectra (plus summary table), DLS data and TEM images. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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