# Preparation of Biocompatible Zwitterionic Block Copolymer Vesicles by Direct Dissolution in Water and Subsequent Silicification within Their Membranes

**ARTICLE** *in* LANGMUIR · AUGUST 2009

Impact Factor: 4.46 · DOI: 10.1021/la900834z · Source: PubMed



**2 AUTHORS:**

Jianzhong Du
Tongji University
**68** PUBLICATIONS **2,867** CITATIONS
SEE PROFILE

Steven P Armes
The University of Sheffield
**615** PUBLICATIONS **27,721** CITATIONS
SEE PROFILE

Langmuir Article

pubs.acs.org/Langmuir


# Preparation of Biocompatible Zwitterionic Block Copolymer Vesicles by Direct Dissolution in Water and Subsequent Silicification within Their Membranes

Jianzhong Du* and Steven P. Armes

*Department of Chemistry, The University of Sheffield, Brook Hill, Sheffield S3 7HF, United Kingdom*

*Received March 8, 2009. Revised Manuscript Received April 20, 2009*

The facile preparation of block copolymer vesicles in pure water and their subsequent stabilization by sol−gel chemistry within the vesicle membrane is described. An amphiphilic biocompatible zwitterionic diblock copolymer, poly(ε-caprolactone)-*block*-poly[2-(methacryloyloxy)ethyl phosphorylcholine], PCL-*b*-PMPC, was synthesized by (i) ring-opening polymerization of ε-caprolactone, (ii) end-group modification by esterification, and (iii) atom transfer radical polymerization (ATRP) of 2-(methacryloyloxy)ethyl phosphorylcholine (MPC). Unusually, block copolymer vesicles were formed *instantly* upon adding dried copolymer powder into hot water without using organic cosolvents, pH adjustment, or even stirring. This protocol is much more convenient than previously reported methods such as solvent-switching and film rehydration. The PCL vesicle membrane is moderately hydrophobic and fully biodegradable. The highly biocompatible PMPC chains are expressed on both the exterior and interior surface of the membrane. These vesicles can be stabilized by aqueous sol−gel chemistry within the hydrophobic PCL vesicle membrane by using tetramethyl orthosilicate (TMOS) as the silica precursor in the absence of any external catalyst. The water-immiscible TMOS precursor is initially solubilized within the hydrophobic membrane prior to its in situ transformation into silica. The vesicles were characterized by $^1$H NMR spectroscopy, atomic force microscopy, transmission electron microscopy, and dynamic light scattering.



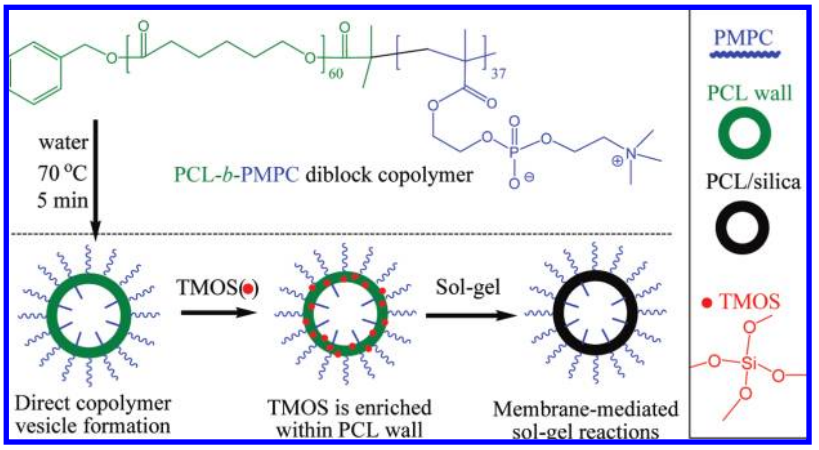
The preparation of block copolymer vesicles by using self-assembly techniques has attracted wide attention recently.[1−7] Such vesicles are composed of hydrophilic coronal chains expressed at both the interior and exterior surface of a hydrophobic membrane. The latter component can solubilize hydrophobic species, whereas the hollow interior is suitable for encapsulation of hydrophilic guest molecules. Thus it is possible to simultaneously deliver both hydrophobic and hydrophilic actives, which could be very useful for biomedical applications. Moreover, block copolymer vesicles are usually much more robust than conventional surfactant-based liposomes, provide a more robust barrier toward diffusion, and offer the intriguing possibility of tunable physicochemical and biological properties.

Poly(ε-caprolactone) [PCL] is a biodegradable polymer and an FDA-approved biomedical material. It has been recently used to prepare PCL-based block copolymer vesicles for drug release applications.[8−10] However, water-miscible organic cosolvents such as THF are typically required to prepare such vesicles. Removal of such cosolvents is essential for potential biomedical applications, but it is rather time-consuming using purification methods such as dialysis. Also, this method is not cost-effective for large-scale preparation. Thus processing techniques such as rehydration (including film swelling and bulk swelling) that avoid cosolvents have attracted increasing attention.[11−16] 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, relatively long time scales (four days) and vigorous agitation are required[10] 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 based on a highly biocompatible block comprising 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) repeat units and a pH-sensitive tertiary amine methacrylate block.[7] Such diblock copolymers have been recently exploited for intracellular delivery, with very high transfection efficiencies and low cytotoxicities being obtained.[17,18] McCormick's group reported that thermo-responsive AB diblock copolymer vesicles could be formed in pure water by simply increasing the temperature above

*To whom correspondence should be addressed. Current address: Department of Chemistry, University of Cambridge, Cambridge, UK. E-mail: jd478@cam.ac.uk.

(1) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967–973.
(2) Antonietti, M.; Förster, S. *Adv. Mater.* **2003**, *15*, 1323–1333.
(3) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244–248.
(4) Du, J. Z.; Chen, Y. M.; Zhang, Y. H.; Han, C. C.; Fischer, K.; Schmidt, M. *J. Am. Chem. Soc.* **2003**, *125*, 14710–14711.
(5) Du, J. Z.; Chen, Y. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 5084–5087.
(6) Du, J. Z.; Armes, S. P. *J. Am. Chem. Soc.* **2005**, *127*, 12800–12801.
(7) Du, J. Z.; Tang, Y. Q.; Lewis, A. L.; Armes, S. P. *J. Am. Chem. Soc.* **2005**, *127*, 17982–17983.
(8) Ghoroghchian, P. P.; Li, G.; Levine, D. H.; Davis, K. P.; Bates, F. S.; Hammer, D. A.; Therien, M. J. *Macromolecules* **2006**, *39*, 1673–1675.
(9) Ahmed, F.; Discher, D. E. *J. Controlled Release* **2004**, *96*, 37–53.
(10) Wittemann, A.; Azzam, T.; Eisenberg, A. *Langmuir* **2007**, *23*, 2224–2230.
(11) Kita-Tokarczyk, K.; Grumelard, J.; Haefele, T.; Meier, W. *Polymer* **2005**, *46*, 3540–3563.
(12) Napoli, A.; Valentini, M.; Tirelli, N.; Muller, M.; Hubbell, J. A. *Nat. Mater.* **2004**, *3*, 183–189.
(13) Discher, B. M.; Won, Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143–1146.
(14) Lee, J. C.-M.; Bermudez, H.; Discher, B. M.; Sheehan, M. A.; Won, Y.-Y; Bates, F. S.; Discher, D. E. *Biotechnol. Bioeng.* **2001**, *73*, 135–145.
(15) Rodríguez-Hernández, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026–2027.
(16) Li, Y. T.; Lotiz, B.; McCormick, C. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 5792–5795.
(17) Lomas, H.; Canton, I.; MacNeil, S.; Du, J. Z.; Armes, S. P.; Ryan, A. J.; Lewis, A. L.; Battaglia, G. *Adv. Mater.* **2007**, *19*, 4238–4243.
(18) Lomas, H.; Massignani, M.; Abdullah, K. A.; Canton, I.; Presti, C. L.; MacNeil, S.; Du, J. Z.; Blanazs, A.; Madsen, J.; Armes, S. P.; Lewis, A. L.; Battaglia, G. *Faraday Discuss.* **2008**, *139*, 143–159.

**Scheme 1. Facile Formation of PCL-*b*-PMPC Diblock Copolymer Vesicles in Pure Water and Their Subsequent Silicification with TMOS in the Absence of Any External Catalyst**






the LCST of the poly(*N*-isopropyl acrylamide) B block.[16] Very recently, we prepared vesicles by direct dissolution of a primary amine-based diblock copolymer, poly(ε-caprolactone)-*block*-poly (2-aminoethyl methacrylate hydrochloride), PCL-*b*-PAMA, in water.[19]

Block copolymer vesicles can be employed as the templates for the preparation of organic/inorganic hybrids or (after calcination) purely inorganic structures. For example, 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.[4−6,20,21] Alternatively, tetramethyl orthosilicate (TMOS) has been used as a soluble silica precursor to *coat* either surfactant vesicles[22−24] or Pluronic-based polymer vesicles.[25] 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,[26] by cationic poly-L-lysine in block copolypeptides,[27] and the cationic coronas of block copolymer micelles.[28] 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.[29] 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.

Very recently, we reported a new membrane-mediated sol−gel reaction in the hydrophobic membrane of PCL-*b*-PAMA block copolymer vesicles in water. 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. The water-immiscible TMOS precursor is solubilized within the hydrophobic PCL membrane, which is subsequently silicified in situ, rather than silica deposition being mediated by the cationic PAMA chains.[19]

Herein we report a facile, convenient method to prepare biocompatible vesicles simply by direct dissolution of a PCL-*b*-PMPC diblock copolymer in pure water at 70 °C, see Scheme 1. PCL is both biocompatible and biodegradable. PMPC is highly biocompatible and surface coatings comprising MPC copolymers are clinically proven to reduce both cell adhesion and protein adsorption.[30−35] Moreover, these vesicles can be subsequently stabilized by aqueous sol−gel chemistry without any concomitant precipitation. No external catalyst was required to direct the silica formation. As a result of its relatively low water solubility, the silica precursor (TMOS) is therefore preferentially solubilized within the hydrophobic PCL membrane. The zwitterionic PMPC chains expressed on the exterior surface of the PCL membrane colloidally stabilize the vesicles and minimize intervesicular cross-linking during in situ silicification. The mechanism requires no external catalyst and is essentially the same as our recently reported *vesicle membrane-mediated sol−gel reaction*.[19]

## Results and Discussion

The diblock copolymer used in this study is $PCL_{60}$-*b*-$PMPC_{37}$, where the subscripts denote the number-average degrees of polymerization of each block. The synthetic route to this

(19) Du, J. Z.; Armes, S. P. *Langmuir* **2008**, *24*, 13710–13716.
(20) Du, J. Z.; Chen, Y. M. *Macromolecules* **2004**, *37*, 5710–5716.
(21) Chen, Y. M.; Du, J. Z.; Xiong, M.; Guo, H. X.; Jinnai, H.; Kaneko, T. *Macromolecules* **2007**, *40*, 4389–4392.
(22) Hubert, D. H. W.; Jung, M.; Frederik, P. M.; Bomans, P. H. H.; Meuldijk, J.; German, A. L. *Adv. Mater.* **2000**, *12*, 1286–1290.
(23) Hubert, D. H. W.; Jung, M.; German, A. L. *Adv. Mater.* **2000**, *12*, 1291–1294.
(24) Hentze, H.-P.; Raghaven, S. R.; McKelvey, C. A.; Kaler, E. W. *Langmuir* **2003**, *19*, 1069–1074.
(25) Wang, H. N.; Wang, Y. H.; Zhou, X. F.; Zhou, L.; Tang, J. W.; Lei, J.; Yu, C. Z. *Adv. Funct. Mater.* **2007**, *17*, 613–617.
(26) Mizutani, T.; Nagase, H.; Fujiwara, N.; Ogoshi, H. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2017–2022.
(27) Cha, J. N.; Stucky, G. D.; Morse, D. E.; Deming, T. J. *Nature* **2000**, *403*, 289–292.
(28) Yuan, J.-J.; Mykhaylyk, O. O.; Ryan, A. J.; Armes, S. P. *J. Am. Chem. Soc.* **2007**, *129*, 1717–1723.
(29) Zhao, D. Y.; Feng, J. L.; Huo, Q. S.; Melosh, N.; Fredrickson, G. H.; Chmelka, B. R.; Stucky, G. D. *Science* **1998**, *279*, 548–552.
(30) Ishihara, K.; Aragaki, R.; Ueda, T.; Watanabe, A.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1990**, *24*, 1069–1077.
(31) Kojima, M.; Ishihara, K.; Watanabe, A.; Nakabayashi, N. *Biomaterials* **1999**, *12*, 121–124.
(32) Ishihara, K.; Ziats, N. P.; Tierney, B. P.; Nakabayashi, N.; Anderson, J. M. *J. Biomed. Mater. Res.* **1991**, *25*, 1397–1407.
(33) Ishihara, K.; Oshida, H.; Endo, Y.; Watanabe, A.; Ueda, T.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1993**, *27*, 1309–1314.
(34) Ishihara, K.; Tsuji, T.; Kurosaki, T.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1994**, *28*, 225–232.
(35) Ishihara, K.; Nomura, H.; Mihara, T.; Kurita, K.; Iwasaki, Y.; Nakabayashi, N. *J. Biomed. Mater.* **1998**, *39*, 323–330.

copolymer is shown in Scheme 2. 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 toluene. Once this polymerization had ceased, excess 2-bromoisobutyryl bromide (relative to PCL-OH) was added to the reaction solution and the resulting esterification produced the desired ATRP macroinitiator ($PCL_{60}$-Br after purification, as judged by $^1$H NMR spectroscopy). The synthesis of the target block copolymer was achieved by ATRP of MPC monomer in a 5:4 THF/methanol mixture at 20 °C for 29 h. The $^1$H NMR spectra shown in Figure S1 (see the Supporting Information) and Figure 1 confirmed the successful synthesis of the $PCL_{60}$-Br macroinitiator and the final $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer, respectively. The degree of esterification of the $PCL_{60}$-Br macroinitiator was estimated to be 99% by comparing the integrated areas of signals b and j (Figure S1, Supporting Information). The mean block composition of $PCL_{60}$-*b*-$PAMA_{37}$ was determined by comparing the integrated areas of signals c and s (see Figure 1A and Table 1). Inspecting Figure S1 in the Supporting Information and Figure 1A, peak j at 1.95 ppm [Br-C($CH_3$)$_2$-COO] in the $PCL_{60}$-Br macroinitiator spectrum is shifted to peak h at 1.72 ppm [$CH_2$-C($CH_3$)$_2$-COO] in the spectrum obtained for $PCL_{60}$-*b*-$PMPC_{37}$; this observation indicated that there was no significant $PCL_{60}$-Br impurity in the diblock copolymer. Thus the $^1$H NMR and GPC data ($M_n$ = 21 800; $M_w/M_n$ = 1.29; 3:1 chloroform/methanol eluent, RI detector) confirmed the successful synthesis and purification of a novel $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer.

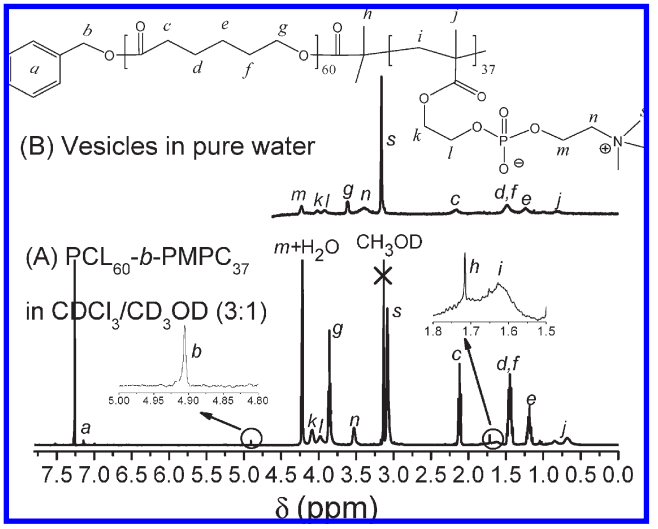



**Figure 1.** $^1$H NMR spectra recorded at 20 °C for (A) the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer in a 3:1 $CDCl_3/CD_3OD$ mixture (which is a good solvent for both blocks); (B) the vesicles formed after direct dissolution of this block copolymer in $D_2O$ (note the attenuation of the PCL signals in this case).

Vesicles were formed spontaneously on direct dissolution of the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer in pure water at 70 °C. The PCL chains form the hydrophobic membrane and the hydrophilic PMPC chains are expressed at both the interior and exterior walls, with the latter chains providing colloidal stability by a steric stabilization mechanism. AFM studies conducted on the dried vesicles provided direct evidence for vesicle formation and yielded an estimated number-average vesicle diameter of 202 ± 59 nm, see Figure 2. The height of the central domain of each vesicle is lower than that at the edge, confirming the expected hollow structure. Areas 1 and 2 have a width of 181 and 227 nm, respectively. However, their heights are only 2.9 and 3.2 nm, respectively. This is perhaps due to the collapse of these relatively soft, deformable

**Scheme 2. Three-Step Synthesis Route to the $PCL_{60}$-*b*-$PAMA_{37}$ Diblock Copolymer**

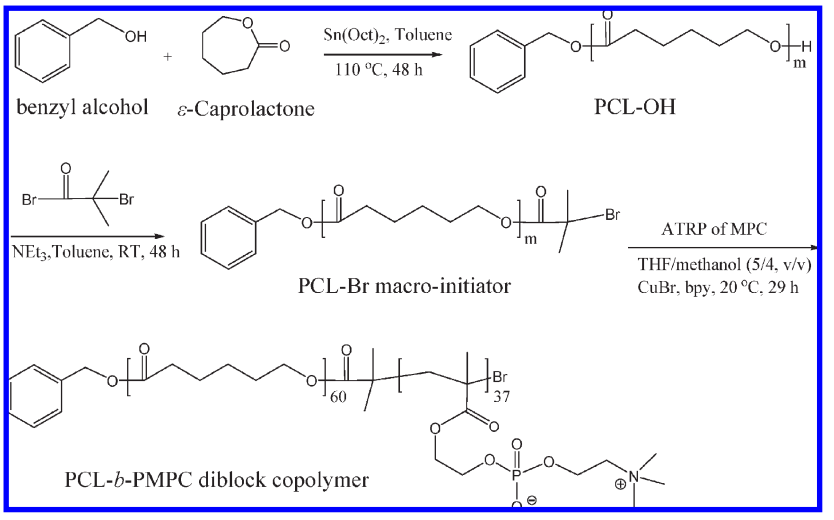



**Table 1. Summary of Relative Peak Areas in $^1$H NMR Spectra of $PCL_{60}$-*b*-$PMPC_{37}$ Diblock Copolymer and Vesicles**[a]

| $^1$H NMR spectra | area of peaks *s*(PMPC) | *c*(PCL) | PMPC/PCL (M) | PCL mol % by $^1$H NMR |
|---|---|---|---|---|
| in $CDCl_3$/MeOD (Figure 1A) | 100 | 35.6 | 37:60 | 100 |
| in pure water (Figure 1B) | 100 | 21.4 | 37:36 | 60 |
| silicified for 41 min (Figure 7A) | 100 | 14.5 | 37:24 | 40 |
| silicified for 134 min (Figure 7B) | 100 | 13.4 | 37:22 | 37 |

[a] The apparent relative PMPC/PCL molar ratio was calculated by comparing the integrated areas of peak s (assigned to PMPC, 37 × 9H) and peak c (assigned to PCL, 60 × 2H in $CDCl_3$/MeOD) by using peak s as the internal standard. The apparent PCL mol % was calculated as follows: for example, in pure water, PCL mol % = 36/60 × 100% = 60%; after 41 min of silicification, PCL mol % = 24/60 × 100% = 40%.

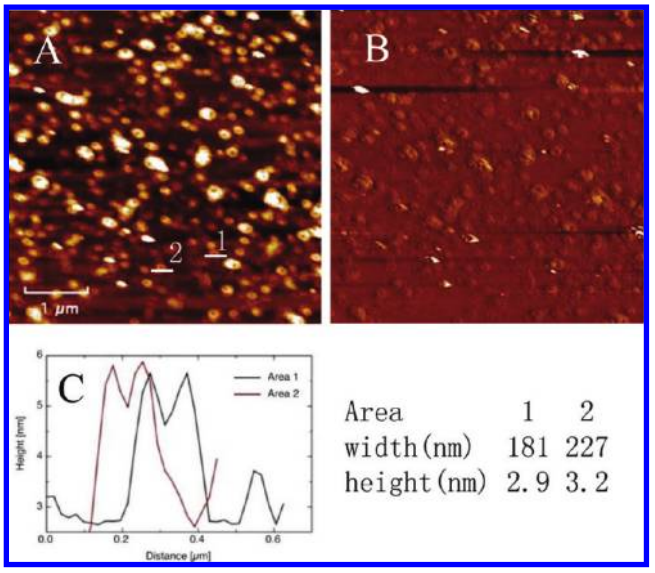



**Figure 2.** AFM data obtained for vesicles prepared by direct dissolution of the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer in water: (A) height image; (B) phase image; and (C) height profile across areas 1 and 2. The vesicle solutions were simply applied as aqueous droplets onto the silicon substrate and allowed to dry at ambient temperature. Note that the brighter vesicles have higher heights than areas 1 and 2.

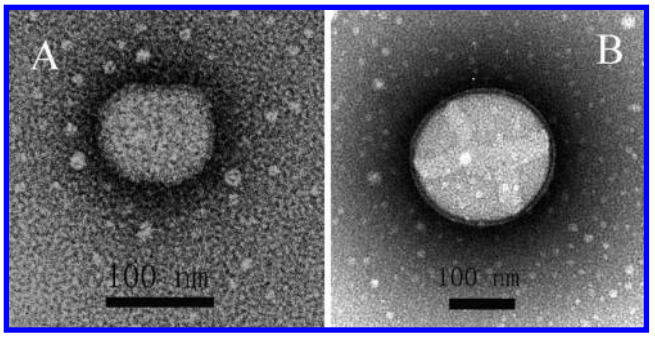



**Figure 3.** TEM images obtained for stained vesicles prepared by direct dissolution of the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer in water: (A) only the PCL vesicle membrane was stained; (B) both the PCL membrane and the surrounding PMPC coronas were stained. The staining reagent used was ammonium heptamolybdate tetrahydrate solution (1.0% aqueous solution) and the staining time was 100 s. Both vesicles were obtained from the same TEM grid.

vesicles on the silicon substrate. Alternatively, there may be a flattening effect during the AFM measurement.

Staining with aqueous ammonium heptamolybdate tetrahydrate solution allowed relatively good quality TEM images to be obtained. Figure 3 shows two typical TEM images of stained vesicles obtained by this approach. A relatively less compact PCL membrane of ~20 nm thickness (see Figure 3A) and PCL membrane plus very loose surrounding PMPC corona (Figure 3B) were visualized. An alternative staining reagent, phosphotungstic acid (PTA), was also employed to increase the contrast. The three-dimensional vesicle morphology is apparent in Figure 4. However, it is possible that the strongly acidic PTA solution may disrupt the vesicle structure. Staining at neutral or higher pH was not attempted because PTA is prone to aerial oxidation at higher pH. DLS studies of the dilute aqueous vesicle solutions (see Figure 5) revealed that the hydrodynamic diameter ($D_h$) for the same vesicles ranged from 40 to 500 nm, with an intensity-average diameter of 131 nm. The vesicle dimensions obtained from DLS analysis are in reasonably good agreement with the AFM studies (after allowing for possible flattening and polydispersity effects) and are also comparable with the TEM images (Figure 3). The above results confirm successful vesicle preparation by the direct dissolution method.

Analysis of the $^1$H NMR spectrum recorded for block copolymer vesicles in $D_2O$ indicates that the PCL signals are only partially attenuated: their apparent degree of solvation is approximately 60% (see Figure 1, plus Table 1 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 $^1$H NMR.[4,6] The apparent degree of solvation of PCL membrane in our previously reported $PCL_{60}$-*b*-$PAMA_{12}$ diblock copolymer vesicles is 34%,[19] which is significantly lower than that determined for the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer vesicles. This is because the latter has a larger hydrophilic volume in the vesicle coronas, which favors membrane solvation. The strongly hydrophilic nature of the PMPC chain may also play an important role in this context.

Despite the partially hydrated nature of the PCL chains, the vesicle membranes formed by the $PCL_{60}$-*b*-$PMPC_{37}$ copolymer can solubilize the poorly water-soluble TMOS rather efficiently. For example, up to 100 $\mu$L 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 $\mu$L of TMOS was sufficient to form visible oil droplets when added to the same volume of pure water. In a further control experiment, $PCL_{60}$ homopolymer was 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. $^1$H NMR spectra recorded for TMOS added to pure $D_2O$ and a vesicle solution in $D_2O$ are shown in Figures 6 and 7, respectively. TMOS (0.67%) was fully hydrolyzed to afford $Si(OH)_4$ after 80 min in pure $D_2O$ and hence became water-miscible on this time scale. A kinetic plot for the rate of TMOS hydrolysis is shown in Figure S2 in the Supporting Information, and indicates a pseudo-first-order process (since the water is present in large excess). It is noteworthy that the TMOS concentration within the vesicle solution is 0.31%, which is only 50% of that in pure $D_2O$. Thus, a shorter reaction time was expected for TMOS hydrolysis in the vesicle solution. Indeed, some TMOS (and also partially hydrolyzed TMOS) was still detected by $^1$H NMR more than 2 h after its addition to the vesicle solution due to its solubilization (and hence protection against hydrolysis) within the hydrophobic PCL membrane. Similar encapsulation has also been reported for hydrophobic dyes within PEO-*b*-PCL vesicle membranes.[8]

In addition to its solubilization, TMOS is subject to concomitant sol−gel reactions within the PCL membrane, especially in the presence of acid or base catalyst. In the absence of any external catalyst, the sol−gel reactions can occur on time scales of several days or even weeks.[20] Thus the solubilized, hydrolyzed TMOS within the PCL membrane is rather slowly converted into silica. Moreover, zwitterionic PMPC contains quaternary ammonium groups, which may interact with anionic silica precursors and hence catalyze sol−gel reactions in the aqueous solution. In related work, we found that a neutral PEO-*b*-PCL diblock copolymer did not catalyze silicification of self-assembled micelles and vesicles.[19] This suggests that the quaternary ammonium groups in the PMPC chains may act as a mild catalyst for silica formation, particularly at the membrane/solution interface.

At a 10:1 TMOS/MPC molar ratio (which corresponds to a 3.15:1 mass ratio of TMOS to diblock copolymer), TEM studies suggest that the sol−gel reaction occurs exclusively within the

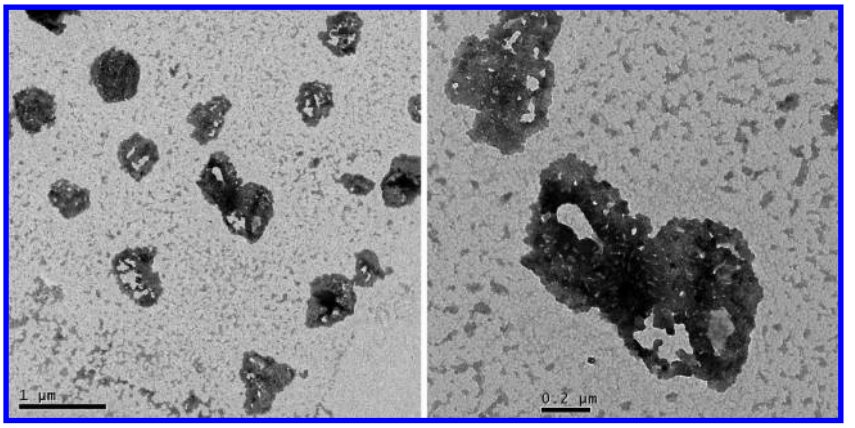



**Figure 4.** TEM images obtained for stained vesicles prepared by direct dissolution of the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer in water. The right-hand image is a close-up of the left-hand image. The staining reagent used was a 1.0% aqueous solution of phosphotungstic acid (PTA) and the staining time was 100 s. The vesicle morphology is clearly apparent, although some vesicle membranes were destroyed by the acidic nature of the PTA solution (pH 1.8).



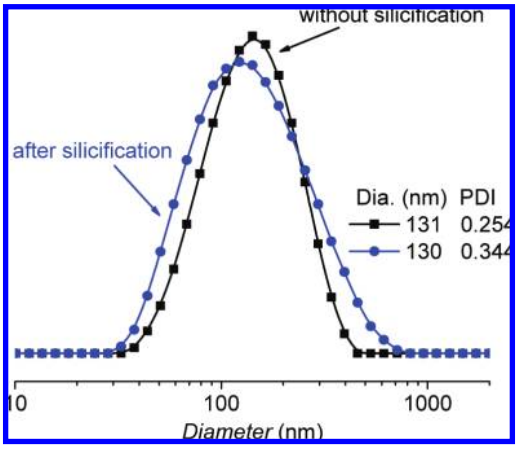



**Figure 5.** Particle size distributions obtained for $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer vesicles prepared by direct dissolution of the copolymer (0.10%) in water at 70 °C, followed by DLS studies at 25 °C.

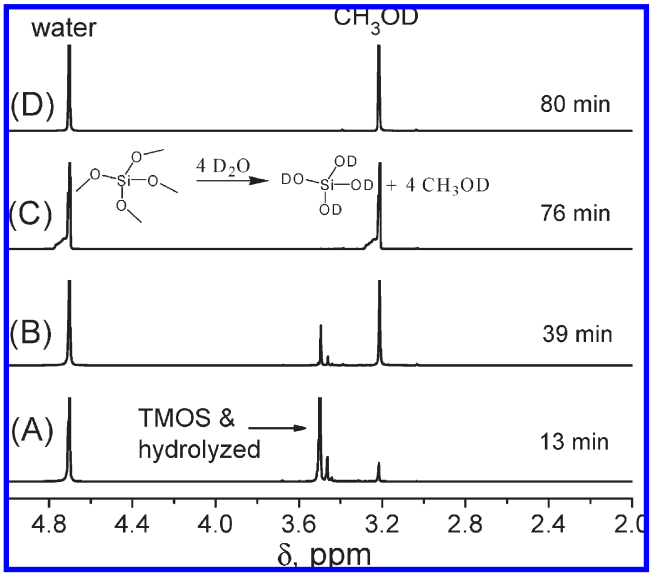



**Figure 6.** $^1$H NMR spectra recorded for TMOS added to pure water as a function of time. TMOS (5.0 $\mu$L) was added to $D_2O$ (0.7442 g) at neutral pH (0.67% TMOS concentration).

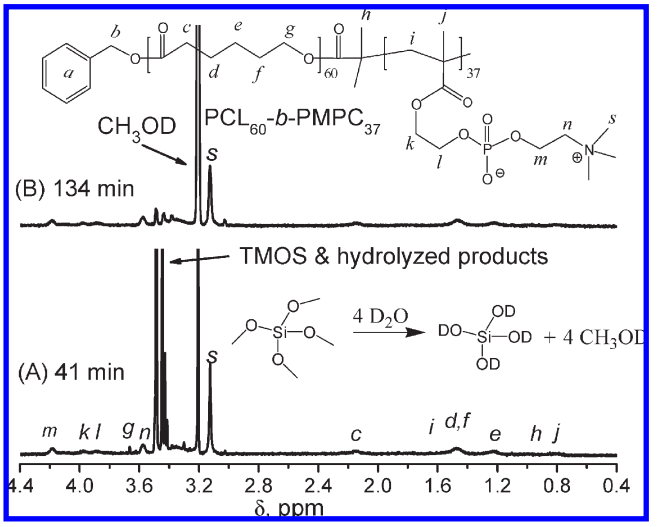



**Figure 7.** $^1$H NMR spectra recorded at 20 °C for the $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer vesicles (0.10%) after silicification with use of a TMOS/MPC molar ratio of 10:1 for (A) 41 min and (B) 134 min. TMOS (6.1 $\mu$L) was added to vesicle solution (2.00 g) in $D_2O$ at neutral pH to silicify the vesicles (0.31% TMOS concentration).

PCL membrane walls, see Figure 8. $^1$H NMR studies confirmed that the PMPC signals remained visible after silicification and the PCL signals were further attenuated from 60% in pure water to 40% after silicification for 41 min (see Figure 7 and Table 1). When the reaction time was increased to 134 min, the PCL signals were attenuated to 37%. 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 PMPC chains will not be adversely affected. 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 (Figure 5). These observations are also consistent with silicification being confined within the PCL membrane, since this should minimize vesicle−vesicle interactions.

According to the $^1$H NMR spectra, the neutral PMPC chains remain well-solvated and mobile, which suggests that they do not act as the locus for silica deposition. The strong attenuation of the PCL signal observed after silicification suggests that its chain

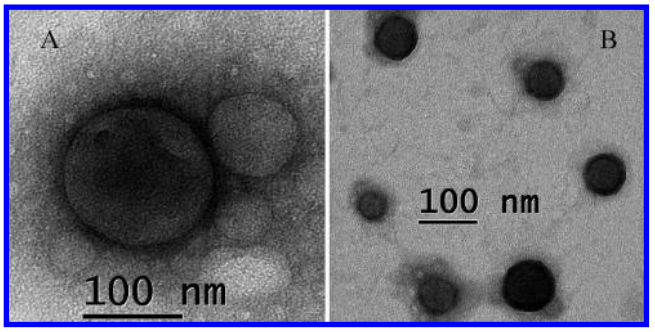



**Figure 8.** TEM images obtained for vesicles after membrane-mediated silicification. The TMOS/MPC molar ratio was 10:1 and the reaction time for silicification was (A) 40 h and (B) 4 days. TEM samples were prepared by allowing 5 $\mu$L of aqueous vesicle solution to dry onto TEM grids. The estimated membrane wall thickness is 12.0 ± 1.0 nm (A) and 13.8 ± 1.0 nm (B).

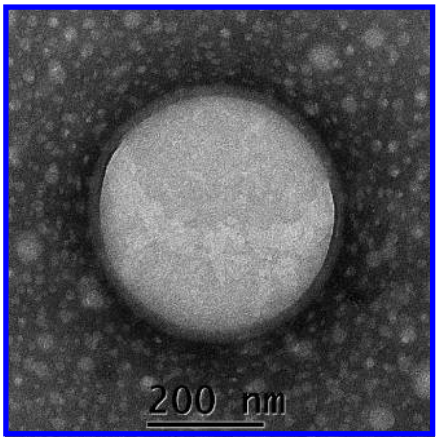



**Figure 9.** TEM image obtained for vesicle after PCL membrane-mediated silicification. The TMOS/MPC molar ratio was 10:1 and the reaction time for silicification was reduced to 6.5 h in the presence of 0.05% triethylamine.

mobility within the membrane is significantly reduced. This sol−gel chemistry also produces methanol, as shown in Figure 7. No TMOS signals were observed after 5 h, indicating its complete hydrolysis. Under neutral conditions, the rate of polycondensation should be much slower than that of hydrolysis (up to several weeks).[20] The TEM contrast for the vesicles would be very poor if little or no silicification had occurred.[20] In fact, good TEM contrast for the silicified vesicles was achieved within 40 h, as shown in Figure 8. This may be because the cationic quaternary nitrogen atoms in the zwitterionic PMPC coronas can catalyze the sol−gel chemistry. To test this hypothesis, we added 0.05% triethylamine catalyst to an aqueous vesicle solution just after adding TMOS. The rate of silicification was significantly accelerated, with "high contrast" vesicles being obtained within 6.5 h, see Figure 9. This offers an alternative route for the acceleration of the sol−gel reaction in the PCL membrane. By comparing the silicification times required for good TEM contrast (e.g., 6.5 h for triethylamine catalyst, 40 h for the PMPC-*b*-PCL copolymer, and several weeks for neutral block copolymer vesicles[20] in neutral water in the absence of any catalyst), the PMPC chains appear to act as a mild catalyst for silicification.

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 on the surface of the vesicle, but membrane-silicified vesicles.

Other conditions for vesicle self-assembly were also explored. For example, $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer (61 wt % PMPC) also formed vesicles spontaneously over a range of copolymer concentrations (0.05−1.0%) in pure water, with no discernible changes in the vesicle dimensions being detected over time. $PCL_{60}$-*b*-$PMPC_7$ diblock copolymer (23 wt % PMPC) was also synthesized to examine the effect of varying the hydrophilic block length on vesicle self-assembly. In this case, vigorous stirring and longer times (several hours) were needed to obtain a 0.1 wt % aqueous solution of vesicles with an intensity-average diameter of 286 nm at 70 °C (see Figure S3 in the Supporting Information). Thus, a certain minimum hydrophilic block length appears to be required for efficient vesicle self-assembly and the final vesicle dimensions depend on the diblock copolymer composition.

The solvent switch method was also evaluated for vesicle preparation. A $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer (8.0 mg) was first dissolved in a mixture of THF (2.0 mL) and methanol (2.0 mL). Then water (4.0 mL) was added gradually to form a blue solution. However, some precipitation was observed after 10 min. After 24 h, all the copolymer had precipitated from the solution. If only 0.2 mL of water was added to the same amount of THF/methanol solution, followed by dialysis against water within 20 min, then an aqueous vesicle solution was formed with minimal precipitation after 24 h. In contrast, it is emphasized that no precipitation was observed and vesicles were formed spontaneously when the same copolymer was directly dissolved in hot water. Thus, direct dissolution appears to be a much better processing route than the solvent switch method, at least for this particular type of diblock copolymer.

In summary, a new class of copolymer vesicles has been obtained by the spontaneous self-assembly of a new biocompatible/partially biodegradable zwitterionic block copolymer in purely aqueous solution without using cosolvents or mechanical stirring. Moreover, the hydrophobic PCL vesicle membrane can be readily silicified by using TMOS to form colloidally stable organic/inorganic hybrid vesicles, which may afford controllable permeability of the membrane by varying the degree of silicification. Unusually, the hydrophobic PCL membrane acts as a scaffold by solubilizing the poorly water-soluble TMOS precursor, while the neutral PMPC chains seem to be a mild catalyst for silicification at the vesicle/aqueous solution interface. It is noteworthy that no external catalyst was required for silicification. This system is subtly different from our previous report of a *vesicle membrane-mediated sol−gel reaction* where the cationic PAMA coronal chains exerted a catalytic effect at the exterior surface of the membrane.[19]

## Experimental Section

**Materials.** 2-(Methacryloyloxy)ethyl phosphorylcholine (MPC; >99%) was kindly donated by Biocompatibles, UK. ε-Caprolactone (Aldrich) was dried azeotropically by using anhydrous toluene to remove traces of water. Stannous 2-ethylhexanoate [$Sn(Oct)_2$; approximately 95%], tetrahydrofuran (THF), triethylamine, 2-bromoisobutyryl bromide, copper(I) bromide (CuBr; 99.999%), 2,2′-bipyridine (bpy), 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.

**Characterization: THF GPC.** Molecular weight distributions of the PCL homopolymers were assessed at 30 °C by using a Polymer Laboratories PL-GPC50 Integrated GPC system equipped with a Polymer Laboratories pump, a PLgel 5 $\mu$m MIXED-C column (300×7.5 mm), a WellChrom K-2301 refractive index detector, a viscometry detector, and a PD 2020 light

scattering detector. The calibration was carried out by using six poly(methyl methacrylate) [PMMA] standards with $M_p$ values ranging from 1 310 to 211 400. The eluent was THF containing 2.0% (v/v) TEA and 0.05% (w/v) BHT and the flow rate was 1.0 mL/min. The data were processed with Cirrus GPC offline GPC/SEC software (version 2.0).

**Chloroform/Methanol GPC.** Analysis of the PCL-*b*-PMPC diblock copolymers was conducted with a Hewlett-Packard HP1090 liquid chromatograph as the pumping unit and two Polymer Laboratories PL Gel 5 $\mu$m Mixed-C (7.5 × 300 mm) columns in series with a guard column at 40 °C connected to a Gilson Model 131 refractive index detector. The eluent was a 3:1 v/v % chloroform/methanol mixture containing 2 mM LiBr at a flow rate of 1.0 mL/min. A series of near-monodisperse PMMA samples were used as calibration standards. Toluene (2 $\mu$L) was added to samples as a flow rate marker. Data analyses were conducted with CirrusTM GPC Software supplied by Polymer Laboratories.

*$^1$H NMR spectra* were recorded with a Bruker AV 400 MHz spectrometer at ambient temperature, using $CDCl_3$, $CD_3OD$/$CDCl_3$, or $D_2O$ as solvents.

*TEM* images were obtained with a Philips CM100 electron microscope operating at 100 kV equipped with a LaB6 gun and a Gatan 1K x 1K digital camera. To prepare TEM samples, 5 $\mu$L of a diluted aqueous vesicle solution was placed on a carbon-coated copper grid, and the water droplet was removed by a pipette, which was connected to a low vacuum line and allowed to evaporate under ambient conditions. Either a 1% aqueous ammonium heptamolybdate tetrahydrate solution or 1% phosphotungstic acid (PTA) was used as a positive stain for the vesicles.

**Synthesis of the $PCL_{60}$-Br Macroinitiator.** This was readily achieved by using a one-pot method. A three-necked flask charged with a magnetic flea, $\varepsilon$-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 with a $N_2$ sparge for 30 min. Benzyl alcohol (1.193 g, 0.0110 mol) was then added via syringe. $Sn(Oct)_2$ (18.8 $\mu$L, $5.52 \times 10^{-5}$ mol) was added by micropipet under a positive nitrogen pressure. The monomer conversion reached 92% after 48 h at 110 °C, as judged by $^1$H NMR. Aliquots of ~1.0 mL were withdrawn periodically for GPC and $^1$H 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, then 2-bromoisobutyryl 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 48 h, excess 2-bromoisobutyryl bromide was reacted with methanol, and the insoluble salt was removed by filtration. The filtrate was concentrated to ~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 (~300 mL). This purification protocol was repeated at least five times to obtain a pure white polymer (85% yield). The $^1$H NMR spectrum of this macroinitiator is shown in Figure S1 in 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 $PCL_{60}$-*b*-$PMPC_{37}$ Diblock Copolymer.** In a typical ATRP protocol, a flask with a magnetic flea and a rubber septum was charged with $PCL_{60}$-Br macroinitiator (1.000 g, 0.1430 mmol) and THF (10 mL). After complete dissolution, a solution of MPC monomer (1.561 g, 5.290 mmol) and bpy ligand (44.60 mg, 0.2860 mmol) in methanol (8 mL) was added. This solution was deoxygenated with a $N_2$ sparge for 30 min before adding Cu(I)Br (0.0250 g, 0.1430 mmol). The [MPC]:[PCL-Br]:[CuBr]:[bpy] relative molar ratios were 37:1:1:2. The MPC polymerization was conducted under a nitrogen atmosphere at 20 °C. After 29 h, the reaction solution was diluted with water and purified by dialysis against water. A fine white powder (1.60 g) was obtained after freeze-drying. A $^1$H NMR spectrum of the purified block copolymer was recorded with use of a 3:1 $CDCl_3$/$CD_3OD$ solvent mixture.

**Preparation of Vesicles.** The following protocol was adopted. $PCL_{60}$-*b*-$PMPC_{37}$ diblock copolymer (typically 100.0 mg, but varied to obtain different copolymer concentrations) was dissolved in water (100 mL). Full copolymer dissolution to form a near-transparent bluish aqueous vesicle solution was achieved after heating to 70 °C for 5 min. For the $PCL_{60}$-*b*-$PMPC_7$ diblock copolymer, vigorous stirring and longer time (several hours) were required to obtain a cloudy solution (suggesting only partial dissolution).

**Solubility of $PCL_{60}$-Br Macroinitiator in TMOS.** A 10 mL glass vial was charged with $PCL_{60}$-Br macroinitiator (4.0 mg) and TMOS (400 mg). The macroinitiator was only partly soluble at 21 °C, but dissolved immediately at 30 °C.

**Solubility of TMOS in Aqueous $PCL_{60}$-*b*-$PMPC_{37}$ Vesicle Solution.** The solubility of TMOS in the aqueous vesicle solution was evaluated according to the following protocol: Ten 10 $\mu$L aliquots of TMOS were added sequentially to a $PCL_{60}$-*b*-$PAMA_{12}$ aqueous vesicle solution (0.10 wt %; 1.0 mL), with full solubilization being observed within 10 s in each case. In contrast, just 5 $\mu$L of TMOS formed visible oil droplets when added to the same volume of pure water (these droplets disappeared after 10 min due to the in situ hydrolysis of the TMOS).

**Acknowledgment.** TSB (ex DTI) is thanked for a postdoctoral fellowship for J.D. through project 264 of the MNT initiative. Biocompatibles (UK) is thanked for supplying the MPC monomer. S.P.A. is the recipient of a five-year Royal Society/Wolfson Research Merit Award. Dr. Andrew Parnell is thanked for AFM studies.

**Supporting Information Available:** Additional $^1$H NMR spectra, kinetics data and DLS results. This material is available free of charge via the Internet at http://pubs.acs.org.