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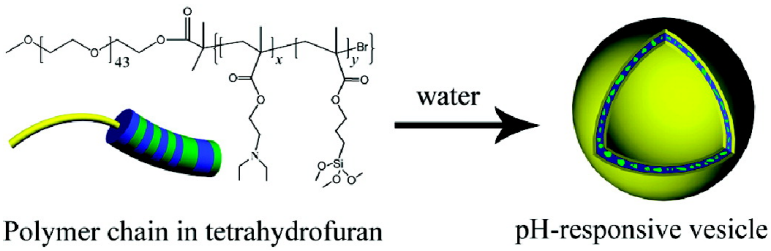

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pH-Responsive Vesicles Based on a Hydrolytically Self-Cross-Linkable Copolymer

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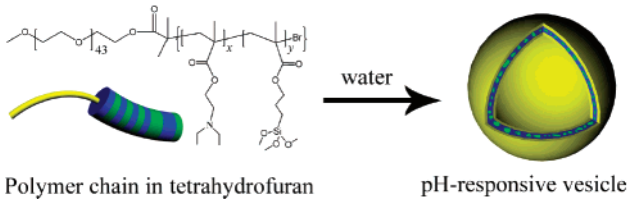
Amphiphilic block copolymers can form a range of supramacromolecular assemblies with spherical, cylindrical, and vesicular morphologies.[1−5] Water-dispersible polymeric vesicles are more durable than conventional liposomes and hence are promising nanosized vehicles for the protection and delivery of water-soluble drugs and proteins. The field of block polymer vesicles has been recently reviewed by Antonietti and Förster[3] and also Hamley.[5] Recently, Du and Chen reported a range of cross-linked vesicles based on poly(ethylene oxide)-*block*-poly[3-(trimethoxysilyl)propyl methacrylate] (PEO-*b*-PTMSPMA) copolymers in either methanol/water or DMF/water mixtures.[6] Lecommandoux et al. reported polypeptide-based block copolymer vesicles whose size[7] or core-corona structure[7b] responded to changes in the solution pH. In related work, Deming and co-workers described a new type of stimulus-responsive polypeptide vesicle by conformation-specific assembly.[8] Herein we report a new type of shape-persistent polymeric vesicle with pH-tunable membrane permeability. These vesicles are formed by the self-assembly of a pH-responsive, hydrolytically self-cross-linkable copolymer, poly(ethylene oxide)-*block*-poly[2-(diethylamino)ethyl methacrylate-*stat*-3-(trimethoxysilyl)propyl methacrylate], [PEO-*b*-P(DEA-*stat*-TMSPMA)], in THF/water mixtures; see Scheme 1. This block copolymer was synthesized by statistical copolymerization of 2-(diethylamino)ethyl methacrylate (DEA) and 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) in methanol at room temperature using a poly(ethylene oxide)-based macro-initiator and a standard Atom Transfer Radical Polymerization (ATRP) protocol.[9] This route gave much lower polydispersities ($M_w/M_n = 1.10-1.20$) than those reported for the statistical copolymerization of methyl methacrylate with TMSPMA in anisole at 70 °C using a similar PEO_{45} macro-initiator ($M_w/M_n = 1.58-1.78$).[6d] Typical gel permeation chromatography and ^{1}H NMR data for this PEO_{43}-*block*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) copolymer are provided in the Supporting Information (Figures S1 and S2A).

DEA homopolymer (PDEA) is pH-responsive; it dissolves in water at low pH as a weak cationic polyelectrolyte, but it becomes insoluble above pH 7 due to deprotonation of its tertiary amine groups.[10] We found that replacing up to 50 mol % of the DEA residues with TMSPMA comonomer did not affect this pH-responsive behavior significantly. On addition of D_2O to a PEO_{43}-*block*-P[DEA_{40}-*stat*-$TMSPMA_{40}$] copolymer solution in d_8-THF (see Figure S2B) to give a final 2:1 D_2O/d_8-THF solution, the peaks assigned to the P(DEA-*stat*-TMSPMA) blocks disappeared, while the PEO peak remained unchanged (see Figure S2C). A CH_3OD signal was observed due to in situ hydrolysis of the $-Si(OCH_3)_3$ groups to $-Si(OD)_3$, which subsequently react further to produce siloxane cross-links. The intensity of this CH_3OD signal relative to the PEO signal increases with time (see Figure S2D). A kinetic plot indicated that the rate of hydrolysis was fastest within the first 30 h (Figure S3). The basic DEA residues in the copolymer catalyzed the rates of hydrolysis and vesicle cross-linking. In the absence of any external catalyst, stable cross-linked vesicles were obtained within a few hours via self-catalysis, whereas at least 1 month was required for the (noncatalytic) cross-linking of a PEO_{45}-*b*-$PTMSPMA_{59}$ precursor under the same conditions.[6b] Addition of less than 0.1 wt % triethylamine further enhanced the rate of hydrolysis of the TMSPMA groups, especially within the first 10 h.

Scheme 1. Formation of pH-Responsive Block Copolymer Vesicles[a]



[a] Yellow, hydrophilic PEO; green, pH-responsive DEA residues; blue, hydrolytically self-cross-linkable TMSPMA residues.

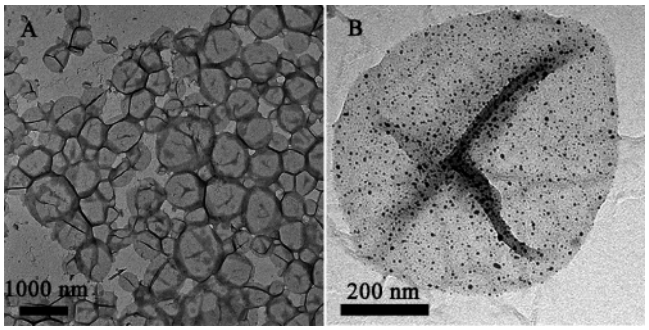


Figure 1. TEM images of (A) vesicles prepared using the PEO_{43}-*block*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) copolymer in 1:2 v/v THF/water at an initial copolymer concentration of 40.0 g/L, and (B) the same vesicles decorated with gold nanoparticles located solely within the vesicle walls.

Although the addition of triethylamine accelerated the rate of cross-linking, transmission electron microscopy (TEM) studies indicated no significant influence on the final vesicle morphology (compare Figure 1A with Figures S4 and S5). The mean vesicle diameter estimated by TEM from Figure 1A is 630 ± 250 nm. However, the mean vesicle diameter and polydispersity are influenced by several parameters, such as the initial copolymer concentration (C_{ini}), the water content (C_w), the block copolymer composition, the type of common solvent employed, etc. For example, the mean vesicle diameter obtained by TEM for $C_{ini} = 10.0$ mg/mL ($C_w = 52.9$ wt %) is 260 ± 80 nm (see Figure S6), which is more uniform than that shown in Figure 1A.

Figure 1B shows a TEM image of vesicles decorated with gold nanoparticles. First, aqueous $HAuCl_4$ solution was added to a dilute aqueous solution of vesicles (as shown in Figure 1A), which leads to protonation of the basic DEA residues and the concomitant incorporation of $AuCl_4^-$ as a counterion. The in situ reduction of

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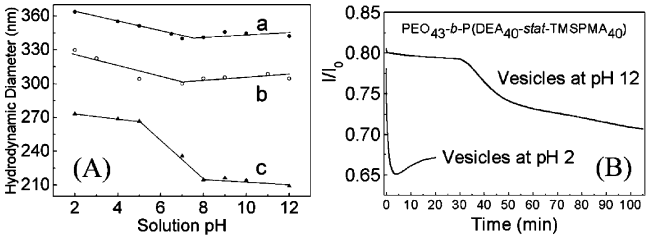

Figure 2. (A) Variation of hydrodynamic vesicle diameter with solution pH: PEO_{43}-*b*-P(DEA_{40}-*stat*-$TMSPMA_{40}$) vesicles prepared (a) with and (b) without the triethylamine catalyst; (c) PEO_{43}-*b*-P(DEA_{60}-*stat*-$TMSPMA_{10}$) vesicles prepared using triethylamine. (B) Change in the relative fluorescence intensity of rhodamine B dye with time after rapid mixing with an aqueous vesicle solution. These data were obtained using stopped-flow apparatus to ensure rapid mixing of the dye with the vesicles.

this Au(III)-based anion by $NaBH_4$ produces zero-valent gold nanoparticles of 3.8 ± 1.5 nm (see Figures S7 and S8) that are confined within the vesicle walls. Although this procedure was primarily undertaken to "stain" the vesicles and also to confirm the presence of the amine-functionalized DEA residues within the vesicle walls, it is noteworthy that colloidal gold catalysts are of increasing interest for various synthetic transformations[11] and that vesicle-supported precious metal-based catalysts may offer advantages in terms of catalyst recovery.[12]

Using the triethylamine catalyst leads to larger vesicles, as judged by dynamic light scattering (DLS); see curves *a* and *b* in Figure 2A. Addition of triethylamine after vesicle formation increased the solution pH from 7 to around 10. This leads to deprotonation of the DEA residues, which may cause some degree of vesicle reorganization prior to cross-linking. Smaller vesicles were formed when a copolymer with a higher DEA content was employed under the same conditions (compare curve *c* with curve *a*). The intensity-average hydrodynamic diameter, D_H, of these vesicles varies with solution pH; see Figure 2A. D_H increased monotonically as the solution pH was lowered below pH 7, which corresponds to the approximate pK_a of DEA homopolymer. Presumably, the cross-linked walls of the vesicles swell due to protonation of the DEA residues. This suggests that the permeability of the vesicle walls should be pH-sensitive. ^{1}H NMR studies (see Figure S2; spectra D and E) also supported this hypothesis. D_H increased slightly above pH 7. This is probably due to weak vesicle aggregation since their isoelectric points are around pH 8.4 (see Figure S9). However, macroscopic precipitation was not observed over the pH range investigated. This is, in part, due to the steric stabilization conferred by the PEO chains and perhaps also due to the very low Hamaker constant expected for vesicles, which largely comprise water. In addition, Figure 2A indicates that the sensitivity of the vesicles to changes in pH increases as expected for higher DEA contents (the maximum diameter increases by 7, 10, and 31%, i.e., the maximum volume increases by 22, 33, and 123% for curves *a*, *b*, and *c*, respectively). Moreover, vesicle cross-linking is clearly effective; otherwise, complete dissociation would occur at low pH due to protonation of the DEA-based blocks. The differing vesicle swelling ratios indicate variable permeability of the vesicle walls; that is, higher degrees of cross-linking should lead to lower wall permeabilities. A similar effect has been reported for poly(acrylic acid)-based hollow spheres.[13] To test this hypothesis, we rapidly mixed empty vesicles with an aqueous solution of rhodamine B dye using stopped-flow apparatus (see Figure 2B) at pH 2 and 12. Much lower fluorescence efficiencies were obtained at pH 2 (due to self-quenching of the rhodamine B dye inside the vesicles) compared to that at pH 12. This is due to the much slower permeation of the dye into the vesicles in alkaline solution, which reduces the probability of self-quenching.[14] Thus, the permeability of the vesicle wall is highly pH-dependent, as expected. We believe that the fluorescence intensity decreases initially at pH 2 due to efficient self-quenching of the dye within the vesicle walls. After a few minutes, the dye also penetrates the aqueous interior of the vesicles, leading to less efficient self-quenching. Hence, there is a local minimum in the fluorescence versus time plot.

The number-average vesicle diameter estimated from the TEM image in Figure 1A is 630 ± 250 nm. This value is significantly greater than the D_H of 340 nm obtained from DLS for the same vesicles in aqueous solution at pH 7 and 20 °C. Similar results were obtained from vesicles under other conditions. For example, vesicles prepared at C_{ini} = 120.0 g/L and C_w = 69.2 wt % had a TEM diameter of 1070 ± 620 nm, whereas the corresponding D_H was 600 nm. These discrepancies indicate significant flattening of vesicles adsorbed onto the TEM grid; indeed, there is some evidence for such deformation in Figure 1B. It is very difficult to determine the mean wall thickness of larger vesicles (see Figure 1A) due to this buckling effect. However, a mean wall thickness of 24 ± 1 nm can be estimated by TEM for smaller vesicles (<100 nm) since these are less prone to buckling on drying. According to earlier studies,[6a,b] the vesicle wall thickness should be essentially independent of the vesicle diameter. This wall thickness of 24 nm is comparable to the contour length of 20.1 nm calculated for the hydrophobic P(DEA_{40}-*stat*-$TMSPMA_{40}$) block, which suggests that these chains are interdigitated within the vesicle walls.

In summary, new block copolymer vesicles were prepared from self-assembly of a hydrolytically self-cross-linkable block copolymer in THF/water mixtures. DLS and fluorescence studies indicate that the permeability of the vesicle walls is pH-sensitive. These vesicles can also be decorated with gold nanoparticles; this provides further structural insights and may offer some possibilities for vesicle-supported precious metal catalysts.

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Supporting Information Available: Full experimental section, including synthesis details and GPC, NMR, TEM, DLS, and fluorescence measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Cameron, N. S.; Corbierre, M. K.; Eisenberg, A. *Can. J. Chem.* **1999**, *77*, 1311−1326. (b) Luo, L.; Eisenberg, A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1001−1004.
(2) 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.
(3) Antonietti, M.; Förster, S. *Adv. Mater.* **2003**, *15*, 1323−1333.
(4) Jenekhe, S. A.; Chen, X. L. *Science* **1999**, *283*, 372−375.
(5) Hamley, I. W. *Soft Matter* **2005**, *1*, 36−43.
(6) (a) Du, J. Z.; Chen, Y. M.; Zhang, Y. H.; Han, C. C.; Fischer, K.; Schmidt, M. *J. Am. Chem. Soc.* **2003**, *125*, 14710−14711. (b) Du, J. Z.; Chen, Y. M. *Macromolecules* **2004**, *37*, 5710−5716. (c) Du, J. Z.; Chen, Y. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 5084−5087. (d) Du, J. Z.; Chen, Y. M. *Macromolecules* **2004**, *37*, 6322−6328.
(7) (a) Chécot, F.; Lecommandoux, S.; Gnanou, Y.; Klok, H.-A. *Angew. Chem., Int. Ed.* **2002**, *43*, 1339−1343. (b) Rodríguez-Hernández, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026−2027.
(8) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. *Nat. Mater.* **2004**, *3*, 244−248.
(9) Ma, Y.; Tang, Y.; Billingham, N. C.; Armes, S. P.; Lewis, A. L.; Lloyd, A. W.; Salvage, J. P. *Macromolecules* **2003**, *36*, 3475−3484.
(10) Liu, S. Y.; Armes, S. P. *Angew. Chem., Int. Ed.* **2002**, *41*, 1413−1416.
(11) Tsunoyama, H.; Sakurai, H.; Ichikuni, N.; Negishi, Y.; Tsukuda, T. *Langmuir* **2004**, *20*, 11293−11296.
(12) Ley, S. V.; Ramarao, C.; Gordon, R. S.; Holmes, A. B.; Morrison, A. J.; McConvey, I. F.; Shirley, I. M.; Smith, S. C.; Smith, M. D. *Chem. Commun.* **2002**, 1134−1135.
(13) Sauer, M.; Meier, W. *Chem. Commun.* **2001**, 55−56.
(14) Park, M.; Deng, S.; Advincula, R. C. *Langmuir* **2005**, *21*, 5272−5277.

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