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Jianzhong Du, Yiqing Tang, Andrew L. Lewis, and Steven P. Armes

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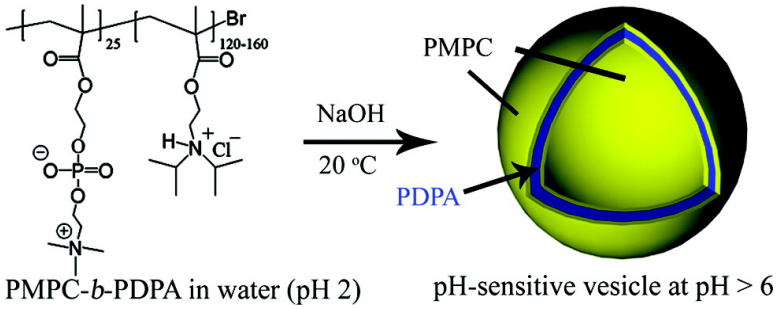

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pH-Sensitive Vesicles Based on a Biocompatible Zwitterionic Diblock Copolymer

Jianzhong Du,[†] Yiqing Tang,[‡] Andrew L. Lewis,[‡] and Steven P. Armes*,[†]

Department of Chemistry, The University of Sheffield, Brook Hill, Sheffield S3 7HF, United Kingdom, and Biocompatibles UK Ltd., Chapman House, Farnham Business Park, Weydon Lane, Farnham, Surrey GU9 8QL, United Kingdom

Received September 22, 2005; E-mail: s.p.armes@shef.ac.uk

Since their discovery several decades ago,[1] liposomes have been widely studied for drug delivery and controlled release applications.[2] However, the bilayer membranes in liposomes are relatively thin and readily deformable; this leads to facile vesicle fusion and inadequate barrier properties. Recently, micellar self-assembly techniques have been extended to prepare block copolymer vesicles.[3−8] Compared to conventional surfactant-based liposomes, polymer-based vesicles have much thicker membrane walls (offering a greater barrier to diffusion), are more durable/robust, and offer the intriguing possibility of tunable physicochemical and biological properties by simply varying the block copolymer structure. Common organic solvents, such as DMF, THF, or 1,4-dioxane, are usually required to prepare vesicles.[9] Removal of such cosolvents after vesicle formation is time-consuming and can be problematic. Thus vesicle preparation techniques that avoid such solvents, such as film rehydration (film swelling), bulk swelling, and electroformation, have attracted increasing attention.[10] For example, Tirelli et al. prepared oxidation-responsive vesicles in pure water by film rehydration followed by extrusion.[11] This method was also exploited by Discher et al. to prepare a range of vesicles using poly(ethylene oxide)-based diblock copolymers.[12,13] Recently, Lecommandoux et al. prepared vesicles by the simple self-assembly of a peptide-based diblock copolymer in pure water.[14] Herein we report a new class of diblock copolymer vesicles based on a highly biocompatible monomer, 2-(methacryloyloxy)ethyl phosphorylcholine (MPC), and a second monomer, 2-(diisopropylamino)ethyl methacrylate (DPA), which confers pH sensitivity to the membrane wall; see Scheme 1 and Figure 1. These PMPC-*b*-PDPA diblock precursors can be easily synthesized from commercially available monomers in high yields using Atom Transfer Radical Polymerization in methanol at room temperature without requiring protecting group chemistry.[15] Similar PMPC-*b*-PDPA diblocks have been previously reported for the preparation of biocompatible pH-responsive micelles.[15] We now demonstrate vesicle formation simply by preparing the same diblocks, albeit with greater asymmetries. These PMPC-*b*-PDPA vesicles can be prepared directly in purely aqueous solution without any cosolvents and are colloidally stable at physiological pH (see below). Moreover, vesicle dissociation occurs completely below pH 6, which suggests the possibility of intracellular delivery of water-soluble drugs and proteins. Since these new vesicles contain the biomimetic phosphorylcholine motif, they can be considered to be very close polymeric analogues of conventional liposomes. Thus they are expected to have a number of biomedical applications as nanosized delivery vehicles. Typical gel permeation chromatography (GPC) and ^{1}H NMR results for selected PMPC-*b*-PDPA copolymers are shown in Table S1 and Figure S1 (see Supporting Information).

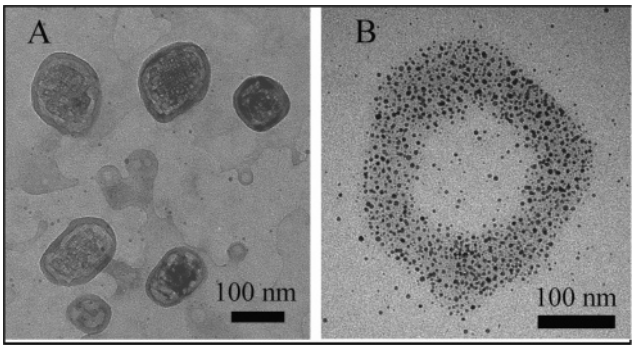


Figure 1. TEM images of (A) vesicles prepared from $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer at pH 7; (B) vesicles in (A) decorated with gold nanoparticles located within the vesicle walls after in situ reduction of $HAuCl_4$ (pH 6.4).

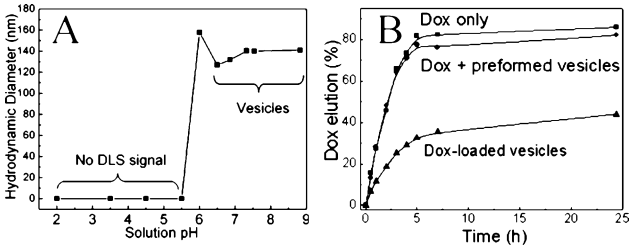
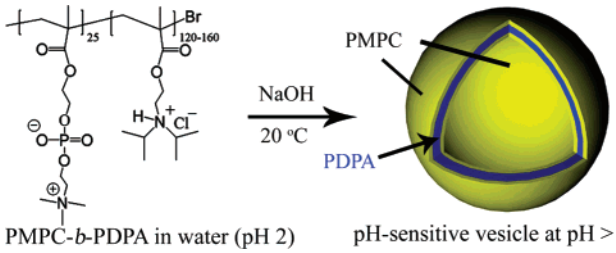


Figure 2. (A) Variation of hydrodynamic diameter of self-assembled colloidal aggregates formed by a $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer in aqueous solution versus solution pH (the initial copolymer concentration at pH 2 was 1.0 g/L). (B) Doxorubicin (Dox) elution profile from $PMPC_{25}$-*b*-$PDPA_{120}$ vesicle solution (pH 7.5 saline buffer at 20 °C).

Scheme 1. Formation of PMPC-*b*-PDPA Block Copolymer Vesicles



PDPA homopolymer dissolves in water below pH 6 as a weak cationic polyelectrolyte, but becomes insoluble above approximately pH 6 due to deprotonation of its tertiary amine groups (its pK_a is around 6.3[16]). As shown in Figure 2A, no signal can be detected by Dynamic Light Scattering (DLS) for the $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer in water (pH < 5.5), which indicates its molecular dissolution under these conditions. At approximately pH 6, a hydrodynamic diameter (D_H) of 160 nm is indicated, as the PDPA block becomes hydrophobic in situ. This D_H value decreases to 140 nm at higher solution pH. Transmission electron microscopy

[†] The University of Sheffield.
[‡] Biocompatibles UK Ltd.

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(TEM) studies revealed that, above pH 7, these copolymeraggregates exist solely as vesicles; see Figures 1A and 2A. Angular dependence measurements by DLS for the vesicles shown in Figure 1A confirmed that the apparent diffusion coefficient was independent of the scattering vector, which suggests spherical morphologies of low polydispersity (see Figure S2).[17] The number-average particle diameter of the vesicles in Figure 1A is approximately 115 ± 34 nm by TEM, and the mean wall thickness is estimated to be 17 ± 1 nm. The intensive-average diameter reported by DLS for the same vesicles is 140 nm (at pH 7). TEM studies also revealed that an ill-defined micellar structure was formed at around pH 6 (see Figure S3A), which is consistent with the DLS observations (Figure 2A). Figure S3 also confirmed that these vesicles remained colloidally stable up to pH 10. The vesicles dissociate below pH 6 (see Figure 2A) due to protonation of the PDPA block; no colloidal aggregates were observed by TEM under these conditions.

The vesicle wall thickness of 17 nm estimated from Figure 1A is somewhat lower than the contour length of 30 nm calculated for the hydrophobic $PDPA_{120}$ block. The wall thickness also depended on the mean degree of polymerization of the PDPA block, as expected. For example, the vesicle wall increased to 37 ± 4 nm (see Figure S4A and Figure S5) for a $PMPC_{25}$-*b*-$PDPA_{160}$ copolymer and decreased to 11 ± 1 nm for a $PMPC_{25}$-*b*-$PDPA_{70}$ copolymer. After selective staining for 2 min using an ethanolic solution of uranyl acetate, the PMPC chains in the vesicle exterior became visible (Figure S4B). Also, vesicles with a wall thickness of 22 nm comprising 180 repeat units of a hydrophobic block (contour length is 45 nm) were recently reported.[18] The above observations suggest that the hydrophobic PDPA chains are interdigitated within the vesicle walls. A power law of $t = kN^b$ describes the relationship between the vesicle wall thickness (t) and the hydrophobic block unit number (N).[19] The exponent b and constant k are calculated to be 1.168 and 0.067, respectively, by plotting ln(t) versus ln(N); see Figure S6. As shown in Table S1, the copolymer composition affects the morphology of the self-assembled aggregates significantly. $PMPC_{25}$-*b*-$PDPA_n$ copolymers formed vesicles under the investigated conditions when n ranged from 120 to 160 (Figure S4 and Figure S5). Reducing n to 70 for the same PMPC block length led to the coexistence of micelles and vesicles (Figure S7), while shorter PDPA blocks produced solely micelles.[15] However, copolymer precipitation, rather than vesicle formation, was observed above pH 6 for $PMPC_{25}$-*b*-$PDPA_{263}$ and $PMPC_{10}$-*b*-$PDPA_{91}$ copolymers. Thus stable vesicles cannot be formed if the PMPC block is too short or if the PDPA block is too long. The initial concentration (C_{ini}) of $PMPC_{25}$-*b*-$PDPA_{120}$ at pH 2 also has a significant effect on the vesicle formation. The aggregates that are formed at a C_{ini} of 1.0, 5.0, and 10.0 g/L are shown in Figure S9. The solution turbidity increases for higher C_{ini}; TEM studies revealed that both vesicles and larger, ill-defined aggregates coexisted at a C_{ini} of 5.0 g/L (Figure S9), while only complex non-vesicular aggregates were formed at 10.0 g/L (Figure S10).

Figure 1B shows a TEM image of vesicles decorated with gold nanoparticles. A reaction scheme for this functionalization is shown in Figure S11. First, dilute aqueous $HAuCl_4$ solution was added to an aqueous vesicle solution (see Figure 1A) to *partially* protonate the PDPA chains and hence incorporate $AuCl_4^-$ as a counterion at pH 6.4−7.0. The in situ reduction of this anionic Au(III) species by $NaBH_4$ produced zero-valent gold nanoparticles of 4.4 ± 1.6 nm (Figure S12). This procedure was undertaken to selectively stain the vesicles so as to confirm the presence of the PDPA chains with in the vesicle walls. The stained vesicle is significantly larger than the precursor vesicles (compare Figure 1A with 1B) because staining involves partial protonation of the PDPA chains; this confirms the pH sensitivity of these vesicles.

Doxorubicin (Dox) is a water-soluble anti-cancer drug in its hydrochloride salt form. Dox encapsulation/release profiles obtained for the vesicles shown in Figure 1A are reported in Figure 2B. Data (squares) obtained for a control experiment utilizing an aqueous solution of 0.556 g/L Dox in the absence of any vesicles indicated rapid drug elution, as expected. Similar data (circles) were obtained after mixing two aqueous solutions comprising Dox and 1.0 g/L *preformed* vesicles for 24 h, followed by dialysis against a pH 7.5 saline buffer for 15 h. Using this protocol, the initial Dox concentration immediately after dialysis was 0.081 g/L, and the maximum possible doxorubicin loading within the preformed vesicles is around 16%. However, the release profile suggests that little or no loading occurred. In contrast, an aqueous solution of Dox-loaded vesicles prepared by mixing 18.25 g/L Dox with 1.0 g/L $PMPC_{25}$-*b*-$PDPA_{120}$ copolymer at pH 2, followed by adjustment to pH 7.4, produced a much slower drug elution profile (triangles). For these latter drug-loaded vesicles, the Dox concentration was 0.136 g/L after subsequent dialysis against pH 7.5 saline buffer for 15 h. The loading efficiency is estimated to be approximately 27%, and the release profile indicates significantly retarded release of the drug at this pH due to its entrapment within the vesicles.

In summary, a new class of pH-sensitive polymer vesicles has been prepared by self-assembly of a biocompatible diblock copolymer in purely aqueous solution simply by controlling the solution pH. These vesicles are authentic polymeric analogues of conventional surfactant-based liposomes and are expected to have biomedical applications as nanosized delivery vehicles.

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Supporting Information Available: Synthesis details and summaries of the various characterization studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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