The pH-induced vesicle-to-micelle morphology transition of a THP-protected polymer

The pH induced vesicle to micelle morphology transition of a THP-protected polymer

Kay E. B. Doncom[a], Helen Willcock[a] and Rachel K. O'Reilly[a*]

[a] University of Warwick, Department of Chemistry, Gibbet Hill, Coventry, CV4 7AL

Correspondence to: Rachel O'Reilly (E-mail: *Rachel.OReilly@warwick.ac.uk*)

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ABSTRACT

A diblock copolymer consisting of tetrahydropyranyl acrylate (THPA) as a pH-deprotectable block, and a permanently hydrophobic block, methyl acrylate (MA), was synthesized by RAFT polymerization using a quaternary amine functionalized, hydrophilic, RAFT chain transfer agent. The polymer self-assembled in water to form vesicles with D_h = 130 nm, as determined by DLS and cryogenic transmission electron microscopy. Acid catalyzed deprotection of the THPA units to yield acrylic acid resulted in a vesicle to micelle morphology transition, as evidenced by the decrease in hydrodynamic diameter to D_h = 19 nm and the observation of micelles by dry state transmission electron microscopy.

KEYWORDS: pH-responsive, morphology transition, vesicle, micelle, RAFT, diblock copolymer

INTRODUCTION

Amphiphilic block copolymers, consisting of at least one block that is hydrophilic and at least one block that is hydrophobic, undergo self-assembly in aqueous media in order to minimize the unfavourable interactions between the hydrophobic block and the surrounding water.[1] The morphology adopted upon self-assembly is dependent upon stretching of the hydrophobic chains in the core, the interfacial tension between the hydrophobic core and the surrounding aqueous environment and the repulsion between the hydrophilic chains in the corona.[2] These three factors can be related to the amphiphilic balance of the polymers, i.e. the ratio of hydrophilic to hydrophobic fractions.

Stimuli-responsive polymers are ones which undergo a change in hydrophilicity (*i.e.* they become either more hydrophilic or more hydrophobic) in response to an external stimulus.[3] Incorporating these responsive polymers into block copolymers can lead to control over the morphology adopted by the resultant self-assembled system. The application of the stimulus causes a change in the hydrophilicity of the responsive block and therefore affects the overall amphiphilic balance of the polymer chain and if drastic enough can induce a change in the morphology adopted in solution.

The amphiphilic balance can be affected by changing the physical environment of the polymer, for example by the addition of salts and additives which promote the solubility of one block over the other.[4,5] Another way to alter the amphiphilic balance is to cause a chemical change within the polymer itself, either reversibly or irreversibly.

One stimulus that has been explored within the literature is a change in pH of the solution.[6-8] The application of pH as a stimulus can cause a reversible change within the polymer, *i.e.* the protonation of amine units to render them

hydrophilic,[9-11] or can cause an irreversible chemical change such as the removal of a protecting group. Protected acid monomers have previously been utilized in the preparation of otherwise synthetically challenging amphiphilic block copolymers.[12-17]

Tetrahydropyranyl acrylate (THPA) is a protected acid monomer that has been shown to be polymerizable by RAFT polymerization techniques and can be deprotected either thermally or through the use of acetic acid.[12-14] As the THPA deprotection is acid catalyzed, the reaction can be considered to be self-catalytic and has been shown to be very efficient under mild conditions. In several examples by Petzetakis *et al.* THPA was used during the synthesis of polylactide-polyTHPA acid block copolymers and then deprotected to form polylactide-polyacrylic acid block copolymers, which self-assemble into cylinders in aqueous solution *via* crystallization-driven self-assembly.[12-14] Klaikherd *et al*. have also synthesized an amphiphilic block copolymer consisting of poly(N-isopropylacrylamide), PNIPAM and THP-protected HEMA.[18] Upon self-assembly below the lower critical solution temperature or cloud point of the PNIPAM block the block copolymer self-assembled to form micelles with a PNIPAM corona and THP-protected HEMA core, but upon lowering the pH, the THP protecting group was cleaved, resulting in micelle dissociation to yield purely hydrophilic unimers.

There are several examples of systems which undergo a micelle to vesicle transition in response to the application of a particular stimuli.[19-22] However the reverse transition is perhaps of more interest. The destruction of the central water pool within the vesicle, for example by transition from vesicle to micelle, can allow for the triggered release, and hence delivery, of hydrophilic payloads.[10,23,24] However, the vesicle to micelle transition in response to changes in pH has been relatively unexplored. One example from Eisenberg and co-workers, utilized a triblock copolymer consisting of polyacrylic acid, polystyrene and poly 4-vinyl pyridine which self-assembled in DMF/THF/H_2O mixtures. At pH 1 the polymers formed vesicles but between pH 3 – 11 solid aggregates were formed.[25]

Herein we report the synthesis of a diblock copolymer consisting of a hydrophilic head group, a pH-deprotectable THPA block and a hydrophobic MA block. The self-assembly behaviour of the diblock copolymer was investigated by DLS and TEM analysis. The pH-response of the nanostructures was demonstrated by treatment with acetic acid to deprotect the THPA and the subsequent vesicle to micelle morphology transition confirmed by DLS and TEM analysis.

EXPERIMENTAL

Materials

2,2'-Azobis(2-methylpropionitrile) (AIBN) was recrystallized from methanol and stored in the dark at 4°C. Methyl acrylate (MA) was distilled over CaH_2 and stored at 4°C. THPA was synthesized as described in the literature[26] and stored at -7°C. All other materials were used as received from Sigma-Aldrich. Tetrahydrofuran (THF) and *N, N*-dimethylformamide (DMF) were used as received from Fisher Scientific unless stated otherwise. Dry DMF was used as received from Sigma-Aldrich. Dialysis tubing was supplied by Medicell with a molecular weight cut off of 3.5 kDa.

Characterization

^{1}H NMR spectroscopy and ^{13}C NMR spectroscopy were obtained at 400 and 125 MHz respectively with a Bruker DPX-400 spectrometer in $CDCl_3$ or DMSO unless otherwise stated. Chemical shifts are reported in parts per million (ppm) and referenced to the residual solvent peak ($CHCl_3$ ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.16 ppm, DMSO ^{1}H = 2.50 ppm).

SEC data was obtained using HPLC grade DMF with 2% LiBr with a flow rate of 1 mL min^{-1}, on a set of two PLgel 5µm Mixed-D columns with a guard column. SEC data was analyzed using Cirrus Software based on polymethylmethacrylate (PMMA) standards. Infrared spectroscopy was recorded on a Perkin-Elmer Spectrum 100 FT-IR ATR unit. Mass spectra were recorded on a Bruker Esquire 2000 ESI spectrometer.

Dynamic Light Scattering (DLS) analysis was performed on a Malvern Zetasizer Nano ZS instrument operating at 25°C with a 4 mW He-Ne 633 nm laser module. Measurements were made at an angle of 173° (back scattering) and results were analyzed using Malvern DTS 5.02. All determinations were made in triplicate unless otherwise stated (with 10 measurements recorded for each run).

TEM samples were prepared by placing an oxygen-plasma treated, carbon coated copper grid film side down onto a droplet of the solution to be analyzed. After two minutes the excess liquid was blotted with filter paper and the grid allowed to dry. The sample was then stained using a 1% uranyl acetate solution for 15 seconds (unless otherwise stated), blotted with filter paper to remove excess liquid and the grid allowed to dry. Samples were analyzed with a JEOL TEM-2011 microscope, operating at 200 keV. Samples for cryo-TEM prepared in the following manner; a droplet of solution (10 µL) was placed on a holey carbon coated copper grid and the excess removed by blotting. The sample was then vitrified by plunging into liquid ethane. The grid was transferred to a cryo stage and imaged using a JEOL TEM-2011 operating at 120 keV.

Synthesis of quaternary amine CTA 1

The quaternary amine functionalized CTA was synthesized in a three step procedure. Firstly dodecylsulfanyl ([4-(hydroxymethyl) phenyl] methylsulfanyl) methanethione was synthesized as previously reported.[12] In brief, dodecanethiol (1 g, 4.9 mmol) was added to a stirred suspension of potassium phosphate (1.04 g, 4.9 mmol) in acetone (10 mL). After 10 minutes carbon disulfide (1.12 g, 14.7 mmol) was added and the solution stirred for 2 hours, at which point 4-(chloromethyl) benzyl alcohol (0.77 g, 4.9 mmol) was added. The reaction mixture was then stirred at room temperature for a further 19 hours, filtered and all volatiles removed under reduced pressure. The crude product was purified by dissolving in CH_2Cl_2 (20 mL) and washing with 0.1 M hydrochloric acid (20 mL), water (3 x 20 mL) and saturated brine solution (3 x 20 mL). The organic layer was dried over magnesium sulfate, filtered and washed with CH_2Cl_2 and cold hexane to give a yellow solid (1.7 g, 87%). IR spectroscopy (ν_{max}/cm^{-1}): 3360 (O-H), 2957 (alkane C-H), 2916 (alkane C-H), 2850 (alkane C-H), 1614 (aromatic C=C), 1512 (aromatic C=C), 1485 (aromatic C=C), 1061 (thiocarbonyl S=C). ^{1}H NMR spectroscopy ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3H, $^3J_{H-H}$ = 9 Hz, $(CH_2)_9CH_3$), 1.2-1.45 (m, 18H, $(CH_2)_9CH_3$), 1.7 (m, 2H, $SCSSCH_2CH_2$), 3.37 (t, 2H, $^3J_{H-H}$ = 9.8 Hz, $SCSSCH_2CH_2$), 4.61 (s, 2H, $ArCH_2SCSS$), 4.68 (d, 2H, J_{H-H} = 7.6 Hz, $HOCH_2Ar$), 7.33 (m, 4H, Ar*H*). ^{13}C NMR spectroscopy ($CDCl_3$, 500 MHz): δ = 14.1, 22.7, 28.0, 28.9, 29.1, 29.4, 29.5, 29.6, 29.7, 31.9, 37.1, 41, 65, 127.3, 129.5, 134.6, 140.4, 223.7.

The next step in the procedure involved the bromination of the alcohol functionality.[27] The alcohol functionalized CTA (1.5 g, 3.8 mmol) was dissolved in diethyl ether/DMF (10:1 v/v, 55 mL total volume) under nitrogen. Phosphorous tribromide (0.36 mL, 3.8 mmol) was added drop wise at 0 °C. The reaction was allowed to come to room temperature and stirred for 3 hours. The crude product was washed with sodium hydrogen carbonate solution (3 x 50 mL), water (3 x 50 mL) and saturated brine (3 x 50 mL), dried over magnesium sulfate, filtered and the solvent removed under reduced pressure. The crude product was purified *via* flash column chromatography on silica gel using 9:1 petroleum ether: ethyl acetate as the eluent, giving a yellow solid (1.37 g, 79 %). IR

spectroscopy (ν_{max}/cm^{-1}): 2956 (alkane C-H), 2916 (alkane C-H), 2849 (alkane C-H), 1469 (aromatic C=C), 1060 (thiocarbonyl S=C). ^{1}H NMR spectroscopy ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3H, $^3J_{H-H}$=9 Hz, $(CH_2)_9CH_3$), 1.2-1.45 (m, 18H, $(CH_2)_9CH_3$), 1.7 (m, 2H, $SCSSCH_2CH_2$), 3.37 (t, 2H, $^3J_{H-H}$=9.8 Hz, $SCSSCH_2CH_2$), 4.47 (s, 2H, $ArCH_2SCSS$), 4.60 (d, 2H, $^3J_{H-H}$=7.6 Hz, $HOCH_2Ar$), 7.33 (m, 4H, Ar*H*). ^{13}C NMR spectroscopy ($CDCl_3$, 500 MHz): δ = 14.2, 22.7, 28.0, 28.9, 29.1, 29.4, 29.5, 29.6, 29.7, 31.9, 33.0, 37.1, 40.8, 129.4, 129.7, 135.7, 137.3, 223.5.

The bromine functionalized CTA was then reacted with trimethylamine in order to achieve the quaternary amine functionalized CTA. The bromine functionalized CTA (1 g, 2.2 mmol) was dissolved in petroleum ether (50 mL) under a flow of nitrogen. Trimethyl amine (1 M in THF, 10.8 mL, 10.8 mmol) was added slowly and the reaction stirred at room temperature for 19 hours before the precipitated product was isolated by vacuum filtration, washed with petroleum ether (3 x 30 mL) and dried *in vacuo* to give **CTA 1** as a bright yellow solid (0.86 g, 91%). IR spectroscopy (ν_{max}/cm^{-1}): 3010 (aromatic C-H), 2955 (alkane C-H), 2919 (alkane C-H), 2850 (alkane C-H), 1614 (aromatic C=C), 1512 (aromatic C=C), 1485 (aromatic C=C), 1467 (aromatic C=C), 1060 (thiocarbonyl S=C). ^{1}H NMR spectroscopy ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3H, $^3J_{H-H}$=9 Hz, $(CH_2)_9CH_3$), 1.2-1.45 (m, 18H, $(CH_2)_9CH_3$), 1.7 (m, 2H, $SCSSCH_2CH_2$), 3.39 (m, 11H, , $SCSSCH_2CH_2$ and $(CH_3)_3N$), 4.63 (s, 2H, $ArCH_2SCSS$), 5.05 (d, 2H, $^3J_{H-H}$=7.6 Hz, $HOCH_2Ar$), 7.40 (d, 2H, $^3J_{H-H}$=7.6 Hz, $(CH)_2CCH_2S$), 7.61 (d, 2H, $^3J_{H-H}$ = 8 Hz, $(CH_3)_3NCH_2C(CH)_2$). ^{13}C NMR spectroscopy ($CDCl_3$, 500 MHz): δ = 14.1, 22.7, 28.0, 28.96, 29.1, 29.3, 29.5, 29.6, 29.6, 29.57, 29.6, 31.9, 37.3, 40.3, 52.7, 68.3, 126.8, 130, 133.4, 138.8, 223. LR-ESI-MS found: 440.2 $(M)^+$ $C_{24}H_{42}NS_3$, 381.1 $C_{21}H_{33}S_3$. HR-ESI $C_{24}H_{42}NS_3$ $(M)^+$ 440.2474 (calcd), 440.2478 (found).

Synthesis of THPA homopolymer

CTA 1 (83.1 mg, 0.16 mmol), THPA (2.0 g, 12.8 mmol) and AIBN (2.6 mg, 0.02 mmol) were dissolved in dry DMF (4 mL, 1: 2 w: v compared to monomer) and placed in an oven dried ampoule with a stirrer bar, under the flow of nitrogen. The solution was degassed at least three times by successive freeze-pump-thaw cycles and released to and sealed under nitrogen. The polymerization mixture was then heated at 65°C for 1 hour 10 minutes. The polymerization mixture was rapidly cooled to stop the reaction and the resulting polymer purified by precipitation into diethyl ether (300 mL) once and hexanes (300 mL) twice to afford chain end functionalized homopolymer, **2**, M_n (^{1}H NMR) = 5.0 kDa, M_n (DMF SEC) = 5.3 kDa, $Đ_M$ = 1.11. IR spectroscopy (ν_{max}/cm^{-1}): 2940 (alkane C-H), 2868 (alkane C-H), 1732 (ester C=O), 1443 (aromatic C=C), 1020 (thiocarbonyl S=C). ^{1}H NMR spectroscopy ($CDCl_3$, 400 MHz): δ = 0.85 (t, 3H, $^3J_{H-H}$=6.8 Hz, $(CH_2)_9CH_3$ of CTA end group), 1.21-1.40 (m, 20H,$CH_2(CH_2)_{10}CH_3$ in CTA), 1.41-2.72 (br m, C*H* and CH_2 in polymer backbone and THPA side chain), 3.34 (m, 11H, $(CH_3)_3N^+$ and $SCSSCH_2(CH_2)_{11}CH_3$), 3.61-3.90 (br d, 58H, OCH_2CH_2 in THPA side chain), 4.91 (br m, 2H, $(CH_3)_3NCH_2Ar$), 5.93 (br s, 29H, OC*H*O THPA side chain), 7.27 (br s, 2H, Ar*H*), 7.54 (br s, 2H, Ar*H*).

Synthesis of diblock copolymer

Polymer **2** (0.2 g, 0.04 mmol), MA (0.13 g, 1.45 mmol), and AIBN (1.2 mg, 0.008 mmol) were dissolved in dry DMF (0.5 mL) and placed in an oven dried ampoule with a stirrer bar, under the flow of nitrogen. The ampoule was degassed at least three times and released to and sealed under nitrogen. The polymerization mixture was then heated at 65 °C for 2 hours. The polymerization was stopped by submerging the vessel in liquid nitrogen and the resulting polymer purified by precipitation into hexanes (3 x 300 mL) to afford chain end functionalized diblock copolymer, **3**, M_n (^{1}H NMR) = 7.6 kDa, M_n (DMF SEC) = 10.5 kDa, $Đ_M$ = 1.12. IR spectroscopy (ν_{max}/cm^{-1}): 2940 (alkane C-H), 2868 (alkane C-H), 1732 (ester C=O), 1443 (aromatic C=C), 1020 (thiocarbonyl S=C). ^{1}H NMR spectroscopy ($CDCl_3$, 400 MHz): δ = 0.87 (t, 3H, $^3J_{H-H}$=6.8 Hz, CH_2CH_3 of CTA end group),

1.21-1.30 (m, 20H, $CH_2(CH_2)_{10}CH_3$ of CTA end group), 1.31-2.50 (br m, C*H* and CH_2 in polymer backbone and THPA side chain), 3.33 (m, 11H, $(CH_3)_3N$+ and $SC{=}SSCH_2(CH_2)_{11}CH_3$), 3.61-3.90 (br m, 150H, OCH_2CH_2 in THPA side chain and OCH_3 in MA side chain), 4.89 (br m, 2H, $(CH_3)_3NCH_2Ar$), 5.90-6.04 (br s, 30H, OC*H*O THPA side chain), 7.27 (br m, 2H, Ar*H* in CTA head group), 7.54 (br m, 2H, Ar*H* in CTA head group).

Self-assembly of polymer

Solvent switch

The polymer was dissolved in THF at a concentration of 0.5 $mgmL^{-1}$ and an equal volume of water slowly added at a rate of 0.6 $mLmin^{-1}$, with stirring. The solution was then transferred to a dialysis bag (MWCO 3.5 kDa) and dialyzed against 18.2 $M\Omega cm^{-1}$ water, incorporating at least six water changes.

Thin film formation

The polymer was dissolved in THF in a round bottom flask at a concentration of 0.25 $mgmL^{-1}$. After stirring for one hour, the solvent was slowly removed *in vacuo* with rotation of the flask. This left a thin film of polymer coating the sides of the flask. 18.2 $M\Omega cm^{-1}$ water was then added to a concentration of 0.25 mg mL^{-1} and then solution stirred at 30 °C for three days.

Direct dissolution

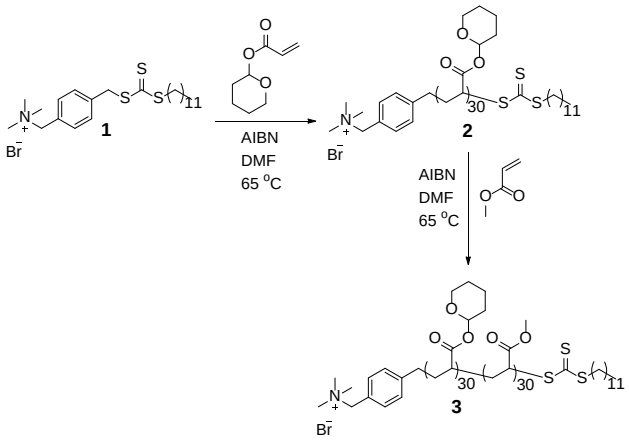
The polymer was stirred in 18.2 $M\Omega\ cm^{-1}$ water at a concentration of 0.25 $mgmL^{-1}$ in an oil bath maintained at 30 °C for three days.

Deprotection of THP groups

A solution of self-assembled polymer at 0.25 $mgmL^{-1}$ was stirred overnight with acetic acid (1 equivalent per THPA unit) with heating to 65 °C. The solution was then cooled to room temperature and the pH of the solution adjusted to 8 in order to analyze the assemblies by DLS and TEM.

RESULTS AND DISCUSSION

Synthesis of the end functionalized diblock copolymer



Scheme 1: The synthetic route to end group functionalized diblock copolymer, **3**.

The morphology adopted in solution by a self-assembling polymer is affected by the amphiphilic balance of the block copolymer and often micelles are formed when the hydrophilic and hydrophobic blocks are roughly equal in length, with vesicle formation occurring when the hydrophobic block is longer than the hydrophilic block.[1] Therefore in order to achieve vesicles, a diblock copolymer bearing a permanently hydrophobic block, a hydrophobic block which is pH-deprotectable and a hydrophilic end group was designed (see **Scheme 1**). In the protected form both blocks are hydrophobic and therefore self-assembly is solely directed by the hydrophilic end group. To ensure the hydrophilic functionality was present at the end of each polymer chain, a previously synthesized bromine functionalized CTA[27] was reacted with trimethyl amine to afford **1**, a positively charged quaternary amine functionalized CTA. This RAFT agent was used to polymerize THPA in order to form the acid deprotectable block bearing a terminal hydrophilic functionality, **2**, M_n (^{1}H NMR) = 5.0 kDa, M_n (DMF SEC) = 4.8 kDa, $Đ_M$ = 1.08. The narrow dispersity observed by SEC analysis,

along with the agreement between theoretical molecular weight and calculated molecular weight, shows that the polymerization proceeded with good control. The UV trace of the SEC at 309 nm is in good agreement with the RI trace showing that the trithiocarbonate group has been retained throughout the polymerization and analysis by ^{1}H NMR spectroscopy shows that there has been no significant deprotection of the polymer during polymerization (see ESI).

The homopolymer was then chain extended with methyl acrylate to form a diblock copolymer with a positively charged tertiary amine end group, **3**, M_n (^{1}H NMR) = 8.2 kDa, M_n (DMF SEC) = 8.5 kDa, $Đ_M$ = 1.07. Again, the narrow dispersity seen by SEC analysis, and predicted molecular weight calculated from end group analysis by ^{1}H NMR spectroscopy, shows that the polymerization proceeds with good control. The efficient chain extension of the homopolymer was demonstrated by SEC analysis (**Figure 1**). Again, analysis by ^{1}H NMR spectroscopy confirmed that no deprotection of the THP block had occurred during the chain extension reaction (see ESI).

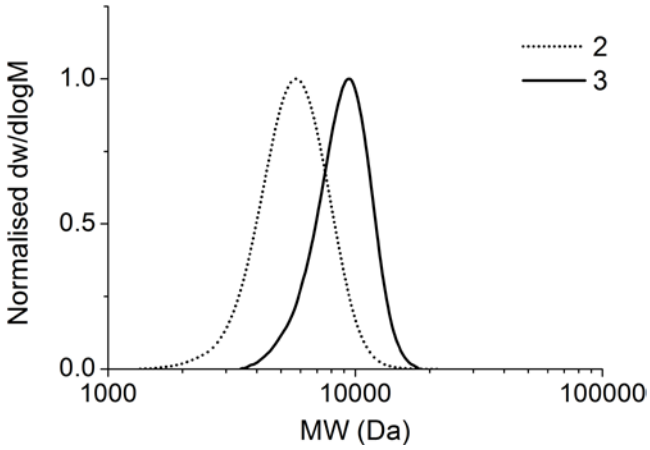


Figure 1: DMF SEC chromatograms showing the shift to higher MW upon chain extension of homopolymer **2** to diblock copolymer **3**

Self-assembly of the THP-protected polymer

Similar block lengths for the THPA block and the MA block were targeted to ensure that in the protected form vesicles would form upon self-assembly of the diblock, due to the entire polymer chain being hydrophobic and therefore self-assembly being driven by the hydrophilic quaternary amine functionalized end group. Upon deprotection of the THP block, micelles should form as the ratio of hydrophilic to hydrophobic fractions of the polymer will be approximately equal.

In order to investigate the self-assembly properties of the protected polymer, different self-assembly techniques were investigated. Solvent switch and thin film formation (described in the experimental section) proved to be unsuitable methods for self-assembly due to precipitation of the polymer during the assembly process. Therefore the polymer was self-assembled by direct dissolution into water at a concentration of 0.25 mg mL^{-1}. After stirring for three days at 30 ^{o}C a transparent polymer solution was obtained. This solution was analyzed by DLS and a population with D_h by number of 130 ± 2 nm with a $Đ$ of 0.11 was observed. As can be seen in **Figure 2**, the sizes obtained from intensity, volume or number data, generated by DLS analysis, correlate well.

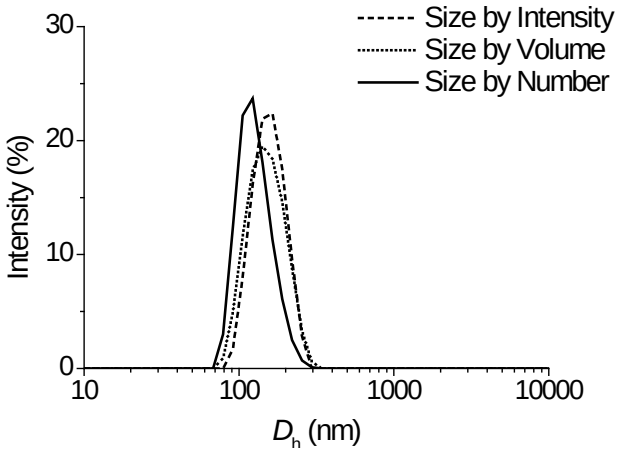


Figure 2: Size by intensity, volume and number traces obtained by DLS analysis for a solution of **3** after self-assembly by direct dissolution at a concentration of 0.25 mgmL^{-1}

The size of the vesicles remained stable for several days after formation (see ESI). This solution was analyzed by TEM to further characterize the morphology of the self-assembled structures. Upon staining with uranyl acetate solution, vesicles with a D_{av} of 136 ± 23 nm were observed (see **Figure 3**). In order to further prove that these structures were vesicles, a sample was analyzed by cryo-TEM. Spherical structures with a clear bilayer were observed, showing that the polymer is assembling to form vesicles (see **Figure 3**, inset). Contrary to the cryo TEM image, the edges of the vesicles observed by dry state TEM did not appear to be completely smooth, with what appears to be smaller nanostructures budding off from the vesicle. We propose this is due to the staining method as uranyl acetate is an acidic stain (pH *ca.* 4.5) and may cause some deprotection of the THPA block. Indeed by leaving the stain on the grid for longer periods of time, more micelles were observed clustered around the vesicles. Therefore it appears that the acidic nature of the stain is causing the vesicles to start to deprotect upon the grid and this leads to the formation of spherical micelles (see **Figure 4**)

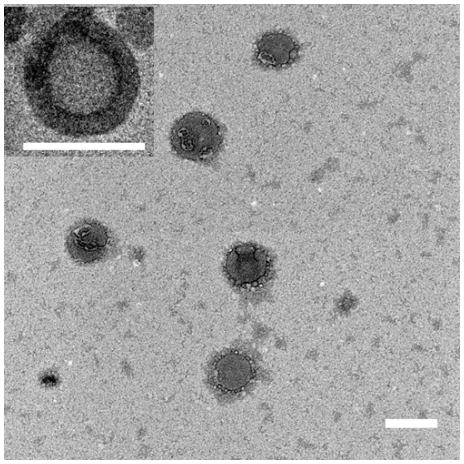


Figure 3: TEM image of a self-assembled solution of **3** at 0.25 mgmL^{-1} stained with uranyl acetate for 15 seconds, scale bar = 200 nm, and inset, a vesicle imaged by cryo-TEM

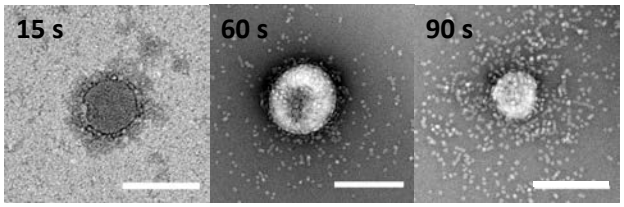
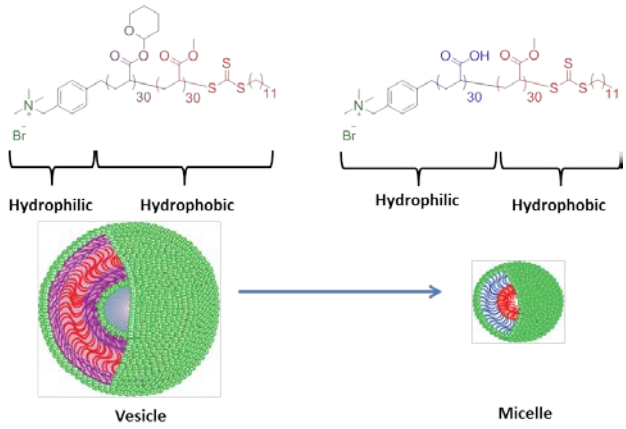


Figure 4: TEM images of a self-assembled solution of **3** stained with uranyl acetate for different lengths of time, scale bar = 200 nm in all cases.

Deprotection of the polymer



Scheme 2: The morphology transition associated with the deprotection of the hydrophobic THPA units to form hydrophilic acrylic acid.

Tetrahydropyran is a protected acid which can be deprotected either thermally or by an acid-catalyzed reaction.[12-14] Upon deprotection the hydrophobic THP side are removed, releasing dihydropyran (DHP) and the polymer formed is hydrophilic polyacrylic acid (see **Scheme 2**). In order to induce a vesicle to micelle morphology transition a vesicle solution of **3** at 0.25 mg mL^{-1} was heated at 65 °C overnight, with glacial acetic acid (1 equiv. per THPA side chain). This resulted in the polymer precipitating, as the polyacrylic acid block is insoluble in acidic

solution. In order to allow the deprotected polymer to self-assemble the solution was basified with NaOH solution until the pH of the solution was approximately 8. This solution was then stirred overnight to allow the self-assembled morphologies to stabilize. Based on the almost equal ratio of hydrophobic to hydrophilic blocks, the expected morphology for the deprotected polymer would be spherical micelles. Analysis by DLS showed a decrease in the hydrodynamic diameter of the self-assembled structures with a population with a D_h of 19 ± 1 nm (see **Figure 5**). Some larger structures are visible in the size by intensity, caused by the micelles aggregating due to the polyelectrolyte effect (see ESI).[28] Analysis of this solution by dry state TEM with staining showed a population of micelles with a D_h of 22 ± 4 nm (see **Figure 6**).

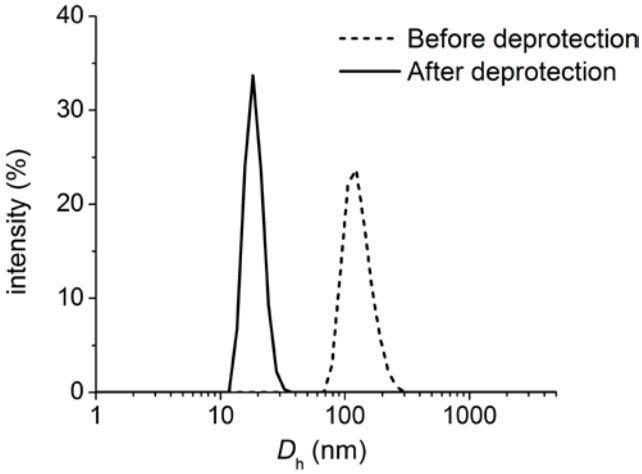


Figure 5: DLS traces of self-assembled diblock copolymer **3** at 0.25 mgmL^{-1} before and after deprotection with acetic acid

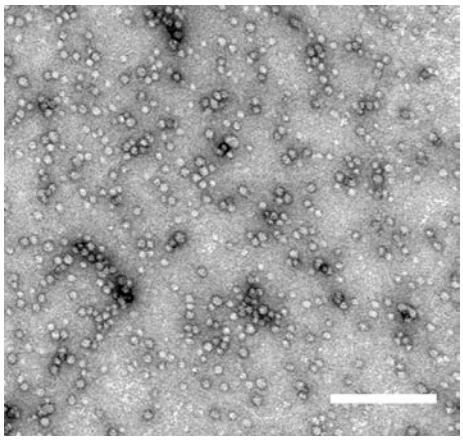


Figure 6: TEM image of micelles formed after deprotection of **3** with acetic acid, stained with uranyl acetate, scale bar = 200 nm.

CONCLUSIONS

We have presented the synthesis of a pH-deprotectable diblock copolymer by RAFT polymerization utilizing a quaternary amine functionalized CTA. The resulting diblock copolymer has a narrow dispersity and self-assembles into vesicles in aqueous media via direct dissolution. The vesicles were characterized by DLS and cryogenic TEM. Addition of acetic acid resulted in the deprotection of the responsive block and a resultant morphology transition to spherical micelles, which were characterized by DLS and TEM. Such a vesicle to micelle morphology transition has potential in the encapsulation and release of a hydrophilic payload from within the central water pool of the vesicles.

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GRAPHICAL ABSTRACT

Kay E. B. Doncom, Helen Willcock and Rachel K. O'Reilly

The pH induced vesicle to micelle morphology transition of a THP-protected polymer

A diblock copolymer containing a pH-deprotectable block and a hydrophilic end group was synthesized by RAFT polymerization. The vesicle to micelle morphology was demonstrated by DLS and TEM.

GRAPHICAL ABSTRACT FIGURE