Supporting Information associated with the paper:

Controlling Vesicle Membrane Permeability with Catalytic Particles

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Contents

Amphiphillic Di-block Copolymer Characterisation

Poly(*n*-butyl methacrylate)-*block*-poly(2-(dimethylamino) ethyl methacrylate), pBMA-*b*-pDMAEMA, (M_n = 20000 g mol^{-1}, PDI = 1.13) was obtained by ATRP as described in our previous work[1].

The molar mass distribution of the diblock copolymer was determined using gel permeation chromatography (GPC) on the Agilent Varian 390-LC (refractive index detection, mixed E columns and chloroform mobile phase). Plot (a) in figure 1 shows the reported molecular weights. The first block, poly(*n*-butyl methacrylate) has a molecular weight of 13500 $gmol^{-1}$ (degree of polymerisation (DP) of 94) and a molar-mass dispersity of 1.10. The diblock copolymer has a molecular weight of 20000 $gmol^{-1}$ and a molar-mass dispersity of 1.13, therefore giving the second block a weight of 6500 $gmol^{-1}$. The block ratio is compared to that reported by H^1 NMR spectroscopy. Using the ratio of the integrals of the O-CH_2- protons for the DMAEMA and BMA blocks, the DP of the DMAEMA block can be calculated based on that reported for the BMA block, see figure 2. A DMAEMA: BMA ratio of 1:2.53 gives a DP of 37 for the DMAEMA block (5800 $gmol^{-1}$).

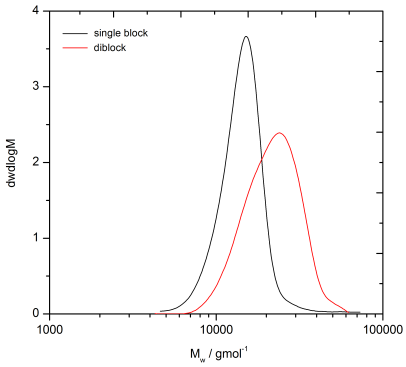

Fig S1 Molar mass distribution plot of the BMA block (black) and the di-block copolymer (red).

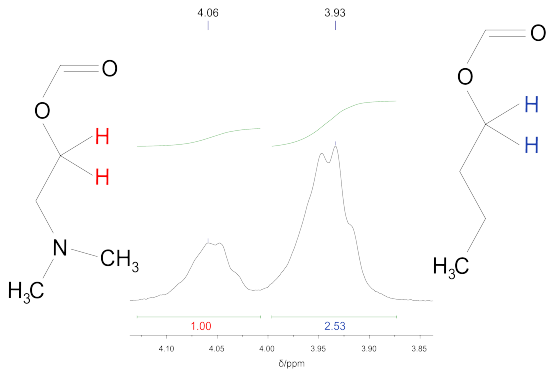

Fig S2 The two proton environments used to assess block ratio alongside their H^1 NMR peaks (See full spectra in appendix).

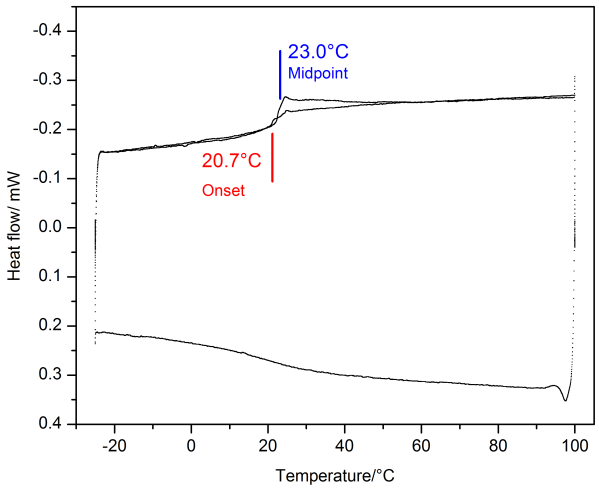

Fig S3 The DSC curve of pBMA-b-pDMAEMA showing the onset and midpoint glass transition temperature.

CHN analysis (CE 440 Elemental Analyser with thermal conductivity detector - Warwick Analytical Services Ltd[2]) confirms this block ratio; GPC/NMR reported a C, H and N weight percentage of 65, 10 and 3%, respectively, whilst CHN analysis reported 66, 10 and 3%. Differential scanning calorimetry (DSC 1 STAR^e system) reported a glass transition temperature of 20.67 – 24.60 °C (onset-mid), in agreement with that reported in literature (figure 3).[3] Two heating cycles and one cooling cycle were carried out (-25 to 100°C) at a rate of 2 K min^{-1} with 25 minute isotherms. The nitrogen flow rate was 40 mL min^{-1}.

Microfluidic Device Construction and Vesicle Formation

(a) Materials

Standard wall borosilicate glass capillaries (GC100-10, OD 1.0 mm, ID 0.58 mm and GC200-7.5, OD 2.0 mm, ID 1.16 mm) were purchased from Harvard Apparatus. Clear Tygon tubing (0.8 mm ID) was purchased from Cole-Parmer. Evo-Stik Two Part epoxy resin was applied to seal the capillaries where necessary. Solutions were introduced to the microfluidic device through the tubing attached to syringes driven by positive displacement syringe pumps (Harvard Apparatus, PHD 2000 series). Weller Dispensing Needles KDS3012P (GA 30, ID 0.15 mm) and KDS1812P (GA 18, ID 0.97 mm) were used to introduce the inner phase and outer/intermediate phases, respectively).

(b) Preparation of Vesicles by Microfluidics

A glass capillary with an inner and outer diameter of 1.16 mm and 2.0 mm, respectively, forms the outer casing of the device and contains the flow of the outer phase. For these studies, a 5 wt.% PVA aqueous solution is used to provide additional stabilisation during vesicle formation. Within this, two capillaries with inner and outer diameters of 0.58 mm and 1.0 mm, respectively, were tapered with a laser puller to allow flow focusing of the middle organic phase (injection to 110 µm and collection to 120 µm). These tapered capillaries were aligned to create a flow-focused junction where double droplets are formed. A 5 mg mL^{-1} solution of p(BMA)$_{94}$-*b*-p(DMAEMA)$_{37}$ in chloroform is used as a volatile intermediate phase. Upon evaporation of the chloroform, the amphiphilic copolymer self-assembles into a lamellar type structure forming vesicles. In the case of particle embedded vesicles, an additional 6 mg mL^{-1} of manganese oxide particles is added to the intermediate phase and the solution ultrasonicated to disperse the particles. The inner phase, a 0.2 mol dm^{-3} sodium fluoride, 0.2 mol dm^{-3} sodium sulphate or 0.003 mol dm^{-3} congo red solution, is introduced at the junction via a 0.15 mm ID dispensing needle. Optimum flow rates for vesicle synthesis were 0.15 mL min^{-1} for the outer phase, 0.005 mL min^{-1} for the middle phase and 0.005 mL min^{-1} for the inner phase. Once formed by the device, vesicles were collected in outer phase solution. The average inner and outer diameter of double emulsion droplets was 311

and 328 µm, respectively. 20 minutes of sample collection corresponds to a total of *ca.* 6000 vesicles. The total surface area of this sample is 1.93 x 10^{-3} m^2 and the theoretical vesicle wall thickness is 242 nm. Optical images of the produced objects were obtained by a Leica DM 2500M optical microscope and accompanying Nikon D5100 camera. Electron microscopy images were obtained on a Zeiss Supra 55VP electron microscope with an attached cryo-SEM unit.

Cryo-SEM images show a wall thickness of 100-200 nm with manganese dioxide particles (*ca.* 1-2 µm) embedded in approximately a third of the vesicle surface. Distribution of manganese dioxide particles is not uniform across all vesicles due to a non-homogenous distribution of particles in the initial intermediate phase solution and the higher density of particles with respect to the solvent phase (causing movement during chloroform evaporation).

Based on a theoretical unilamellar wall thickness of 22.4 nm (a bilayer formed from fully extended chains overlapping in their hydrophobic region), it is clear that a unilamellar structure is not formed. The vesicle wall may be multilamellar or less ordered.

Release Measurements

(a) Triggered release of sodium sulfate from vesicles: visual release studies

A sample of sodium sulfate encapsulated vesicles was added to a reservoir containing 1 mL of 0.1 mol dm^{-3} barium chloride solution. The sample was placed under the Leica DM 2500M optical microscope and vesicle rupture was observed following the addition of 0.1 mL of 30 wt.% hydrogen peroxide solution; upon catalytic reaction of the embedded manganese oxide particles with peroxide in the continuous phase oxygen bubbles start to form rapidly. These oxygen bubbles disrupted the membrane sufficiently enough to cause vesicle collapse, as demonstrated in the supporting video. Upon vesicle rupture, a white crystal precipitate of barium sulfate is formed following the reaction of sodium sulfate and barium chloride.

(b) Triggered release of sodium fluoride from vesicles: quantitative release studies

Samples of vesicles loaded with sodium fluoride solution (at a fluoride ion concentration of 3800 ppm) were prepared following the procedure outlined in section (i). A sample of vesicles collected after 20 minutes of microfluidic device output (a total inner volume of 0.1 mL) was collected into 3 mL of outer phase solution. During collection and the first hour of each experiment, vesicles were agitated in solution to prevent destabilization caused by prolonged contact with each other during the chloroform evaporation stage. This was achieved by circulating the outer phase solution at the location of the vesicles. Agitation was continued throughout the experiment. To prevent an imbalance of osmotic pressure across the vesicle wall, sodium chloride is added to the outer phase at a chloride ion concentration of 3800 ppm. Changes in the fluoride ISE response in the outer phase solution were measured over a period of 900 minutes; following a calibration of the ISE with fluoride ion standards, the fluoride ion concentration in the outer phase is calculated.

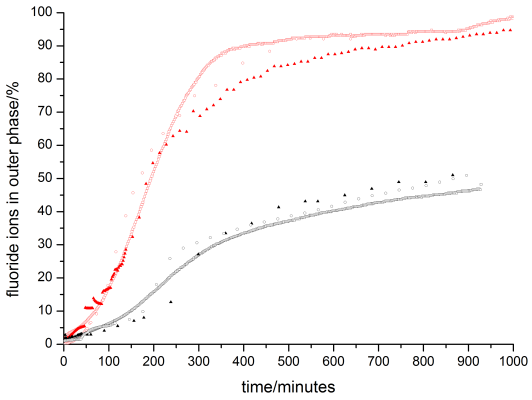

Fig S4 Fluoride ion release profiles of vesicles with (black) and without (red) embedded manganese oxide particles

After this time, the sample is sonicated and the vesicles

ruptured, allowing for a 100% release value to be calculated. This procedure was followed for vesicles with and without manganese oxide particles in their lamellar walls in the absence of a hydrogen peroxide trigger, as show in figure 4.

Vesicles with manganese oxide particles in their walls were then treated with hydrogen peroxide triggers. 3.0 mL of 30 wt.% hydrogen peroxide was added to a sample of vesicles at 50 minutes resulting in rapid vesicle rupture and 100% release after 64 minutes. Temporary increases in fluoride ion release were triggered by 0.2 mL additions of 3 wt.% hydrogen peroxide at 150, 250 and 340 minutes.

Vesicles were monitored for changes in size after triggered release. 3 hours after the addition of a 0.3 wt% hydrogen peroxide trigger, the vesicle wall thickness appeared to have increased by *ca.* 4µm. After two more identical triggers and 24 hours, vesicles shape and size varied (see figure 6 and 7).

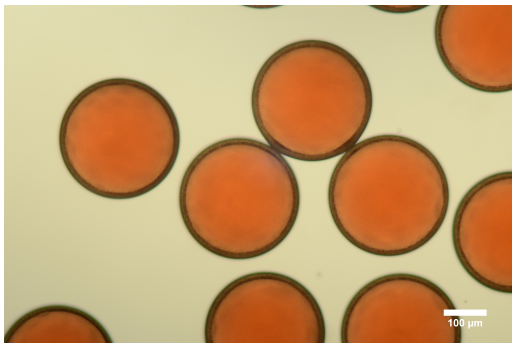

Fig S5 Congo red loaded vesicles embedded with manganese oxide particles before three 0.2 mL 3 wt% hydrogen peroxide triggers.

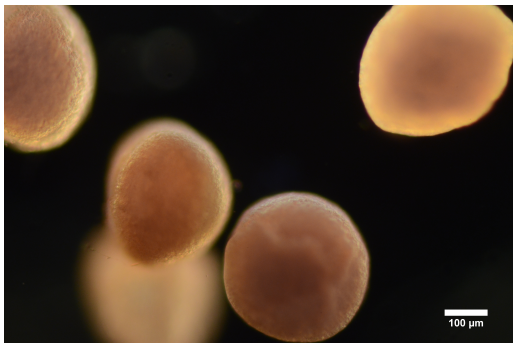

Fig S6 Vesicles of figure 6, 24 hours after.

To confirm the role of the manganese oxide catalytic reaction, blank water triggers were added to vesicles in an equivalent fashion (see figure 7); a comparatively small increase in fluoride ion release is observed in this case (with respect to the peroxide trigger).

(c) Triggered release of congo red dye from vesicles: quantitative release studies

Samples of particle-embedded vesicles loaded congo red solution were prepared following the procedure outlined in section (i). A sample of vesicles collected after 20 minutes of microfluidic device output (a total inner volume of 0.1 mL) was collected into 3 mL of PVA outer phase solution. Vesicles were agitated as in part (iii). Over a period of *ca.* 1300 minutes, 1 mL aliquots of the outer phase were removed from the sample and their UV-Vis spectra measured over the wavelength range of 200-850 nm. After each measurement, the aliquots were returned to the sample. For each sampling point, the concentration of congo red dye was calculated.

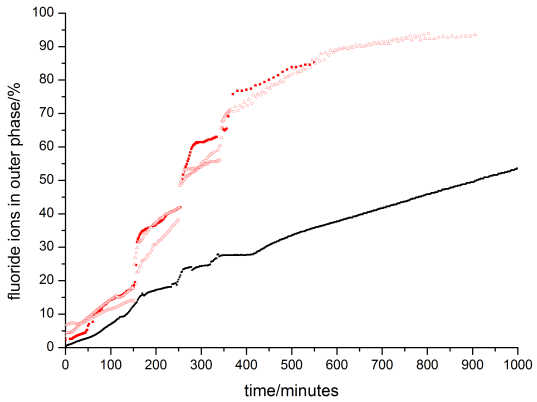

Fig S7 Fluoride ion release of particle-embedded vesicles with hydrogen peroxide (red) and water (black) triggers at 150, 250 and 340 minutes.

Given the known molar extinction coefficient of 2500 L mol^{-1} cm^{-1} and path length of 1 cm, the Beer-Lambert law was used to plot a calibration curve by taking the absorbance value of the wavelength maximum, 505 nm, of

congo red. From this plot and the absorbance values of each sampling point, congo red concentration is calculated. Percentage of dye release is calculated by comparison of concentration at a given time and after total release (based on initial vesicle loading).

(d) Quantification of manganese by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) detection

Samples of vesicles monitored in the release studies have a total manganese concentration of 69358 ppb after the addition of three 0.2 mL 3 wt.% hydrogen peroxide triggers. This value is taken as the expected outer phase manganese concentration if 100% of particles became dissociated with the membrane on application of the trigger (assuming particles only fell into the outer phase, not the inner phase).

After the application of the triggers to the sample, elemental manganese content in the outer phase was determined by ICP-OES. This technique has a lower limit of detection of 25 ppb; a required solid content of < 0.1 % for analysis made a sample dilution necessary, increasing the limit of detection to 1250 ppb. Results indicated that the concentration of manganese in the outer phase was indistinguishable from that of the manganese-free blanks. The loss of manganese (IV) oxide to the outer phase from the vesicles was therefore ≤ 1.8 %, with respect to the manganese (IV) oxide particles embedded in the vesicle walls.

Confocal Microscopy Images

Congo Red loaded polymer vesicles were observed with a confocal laser scanning microscope (Leica TCS SP5, Leica Microsystems). Figure 7 of the main article (512 x 512 pixels, 8 bit pixel depth, 4.0x digital zoom, 32 line average) was acquired on a Leica DMI6000 microscope using a HC PL FLUOTAR 10.0x 0.30 dry objective lens, at a scan speed of 8000 Hz. Congo red was visualised using an argon laser (λ = 504 nm, 30 % intensity) with a spectral detection window of 560 – 671 nm. Data was collected and analysed using the Leica LAS-AF confocal acquisition software.

Appendix

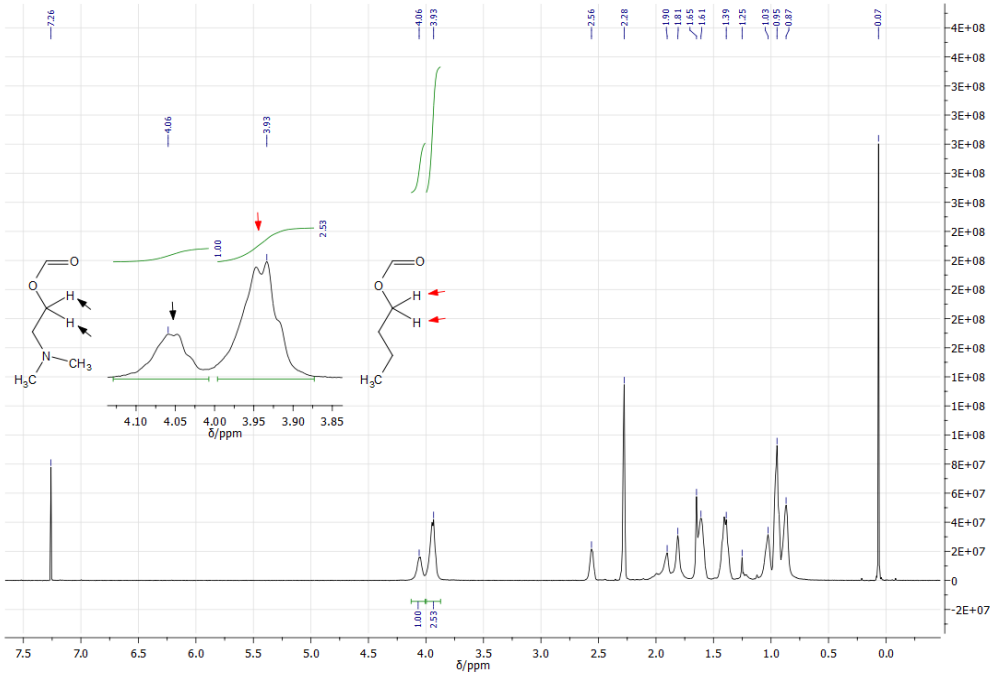

Fig S8 The full H[1] NMR spectrum of Poly(*n*-butyl methacrylate)-*block*-poly(2-(dimethylamino) ethyl methacrylate).

^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ = 0.07 (s, 2H); 0.80-1.1 (m, 9.2 H); 1.39 (m, 3H); 1.6 (m, 3H); 1.8 – 2.0 (m, 3H); 2.3 (s, 3H); 2.6 (m, 1H); 3.9 (m, 2.5 H); 4.0 (m, 1H).

References

[1] R. Chen, D. J. G Pearce, S. Fortuna, D. L. Cheung, S. A. F. Bon, *J. Am. Chem. Soc.* 2011, **133**, 2151–2153

[2] Warwick Analytical Services; University of Warwick Science Park, CV4 7EZ, UK. A sub group of Exeter Analytical.

[3] *Polymer Handbook*, 3rd Edition, Ed. J. Brandrup, E. H. Immergut, Wiley Interscience, Chichester, West Sussex, 1989, ch. VI, p. 218 and 258