Supplementary Material (ESI) for Chemical Communications

Hollow polymer particles that are pH-responsive and redox sensitive: two simple steps to triggered particle swelling, gelation and disassembly

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Experimental Details

***MATERIALS*:**

Methylmethacrylate (MMA), methacrylic acid (MAA) and *t*-butylmethacrylate (*t*-BMA) were purchased from Aldrich and were used as received. Azoisobutyronitrile (AIBN) was purchased from VWR and was used as received. *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, Aldrich > 97%), *N*-Hydroxysuccinimide (NHS, Aldrich, 98%), cystamine dihydrochloride (Aldrich, 98%) and pyrene methylamine hydrochloride (Aldrich) were also used as received. Poly(vinyl alcohol) (PVA) was purchased from Aldrich and was 98% hydrolysed (M_n = 14 - 22,000 g/mol). All solvents were reagent grade.

Preparation of poly(MMA-co-t-BMA)

A typical synthesis is as follows. 0.25 g of AIBN was dissolved in 85 ml of tetrahydrofuran (THF) in a three neck flask and nitrogen purged under reflux conditions at 55° C for 30 min. A mixture of MMA (5.48 g), *t*-BMA (4.52 g) and AIBN (0.025 g) dissolved in THF (17 ml) was added to the solution under nitrogen at a uniform rate over a period of 90 min. After the feed the reaction was allowed to proceed overnight. After cooling, the solution was precipitated and extensively rinsed using aqueous methanol (25% water) before drying at 80 ^{o}C overnight. The ^{1}H NMR data and assignments for poly(MMA-*co*-*t*-BMA) are shown in Fig. S1. The mol.% of MMA was calculated from the integrated areas of the *t*-BMA (d) and methoxy (c) protons. The copolymer contained 60 mol.% MMA and 40 mol.% *t*-BMA as deduced by ^{1}H NMR spectroscopy data.

Preparation of poly(MMA-co-MAA)

1g of poly(MMA-*co*-*t*-BMA) was dissolved in 15 ml of 1,4 dioxane. Then 1 g of concentrated HCl solution (37 %) was carefully added while stirring. The solution was placed in a single-necked flask and heated at 80° C under reflux overnight. After cooling, the solution was precipitated by addition to excess water and thoroughly rinsed with water before drying at 80 ^{o}C. The ^{1}H NMR spectrum for poly(MMA-*co*-MAA) appears in Fig. S2. Potentiometric titration data of the particles (Fig. S3(a)) showed that the copolymer contained 39 mol.% MAA.

Preparation of hollow pH-responsive poly(MMA-co-MAA) particles

The method used to prepare the hollow particles is related to solvent evaporation, which is itself, a versatile and well accepted method for preparing drug-loaded particles[1]. The differences are that a co-solvent that is water soluble is added an the copolymer is amphiphilic. 1 g of poly(MMA-*co*-MAA) was dissolved in 22 ml of a mixed CH_2Cl_2 (84 vol.%) / methanol (16 vol.%) solvent. This was then added into 60 ml of water containing 1 wt.% PVA sheared at 10,000 rpm (whilst cooled to 0 ^{o}C), using a Silverson LR4 high speed mixer. The emulsification continued for 30 seconds after

addition of the polymer solution. The emulsion was then allowed to stir gently overnight to enable removal of the CH_2Cl_2. The product was purified by repeated centrifugation and redispersion in water in order to remove excess PVA. It was then filtered through a 50 μm filter.

Preparation of poly(MMA-co-t-BMA) particles

The particles were made using a similar method to that above; however, methanol was not used.

Preparation of reversibly crosslinked poly(MMA-co-MAA/Cyst) microspheres

To 100 g of 1.5 wt% poly(MMA-*co*-MAA) dispersion maintained at a pH of 6.8 (using 0.1M phosphate buffer) 1.4g of EDC and 0.9 g NHS were added. The dispersion was stirred for 15 mins before adding 0.15 g of air-oxidised cystamine dihydrochloride (Aldrich, 98%) at room temperature. The reaction was allowed to proceed for about 18 h and the product was purified by repeated centrifugation and redispersion in water. Elemental analysis showed that the purified polymer contained 1.2 wt.% S which corresponds to 3.8 mol.% cystamine. This is in good agreement with results from Ellman's test, which gave a cystamine content of 4.0 mol.%

Preparation of fluorescently-labelled poly(MMA-co-MAA)/cyst particles

The method used to label the particles with pyrene methylamine followed that given by Holappa et al.[2] The method used was the same as that described above for cystamine. 1 mol.% of pyrene methylamine hydrochloride was added alongside the cystamine.

***PHYSICAL MEASUREMENTS*:**

GPC. GPC was conducted using a Shodex R101 Refractive index detector and was run using THF at room temperature. The columns contained Phenomenex Phenogel 5um beads with 500, 10^4 and 10^6 angstrom pore sizes. The flow rate was 1ml/min.

NMR ^{1}H NMR was obtained in *d*-acetone using a Bruker 300 instrument.

Potentiometric titration was conducted using a Mettler Toledo DL15 Titrator. Titration was performed on 40 ml of a 1 wt.% uncrosslinked dispersion using 0.1M NaOH. The titrations were performed over a period of 24 h.

Elemental analysis was obtained at the School of Chemistry, University of Manchester using a Thermo Scientific Flash 2000 organic element analyser. The thiol content in the copolymer was also characterized by using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid) using a standard curve obtained with cystamine.

Optical microscopy was conducted with an Olympus BX41 microscope and white transmitted light.

Fluorescence miscroscopy was conducted using a Nikon Eclipse 50i microscope. The sample was illuminated using a mercury lamp filtered using a DAPI (blue) filter which allows transmission of light at 358 nm.

SEM was performed using a Philips XL30 FEG SEM instrument. Dispersions were deposited on SEM stubs by evaporation at room temperature. Gels were freeze-fractured using liquid nitrogen.

Rheology measurements were performed at 25 ^{o}C using a TA AR-G2 rheometer using a 250 μm gap with a 20 mm diameter steel plate at 1% strain. The dispersions and gels investigated were allowed to stand for 30 min prior to measurement.

***Measurement of Swelling Ratio*:**

A drop of 0.1 wt% dispersions in water was placed between a microscope slide and cover slip. A drop of 0.1 M buffer solution (of specific pH) was added at the edge of the slip and allowed to diffuse between the slide and slip. Particles were tracked using a digital video camera as the buffer solution diffused through the dispersion and the images analysed to determine average diameters and volume swelling ratios. The error bars in Fig. 2(d) represent the standard deviation of the mean

value. At least 21 particles were analysed to determine a swelling ratio for each pH used.

SUPPLEMENTARY FIGURES

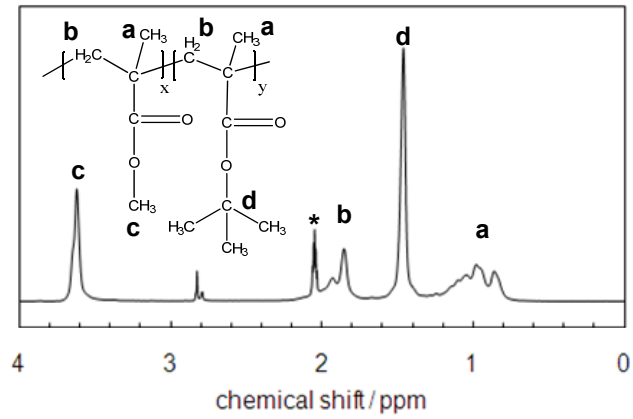

Figure S1. NMR spectrum for poly(MMA-*co*-*t*-BMA). The asterisk represents the acetone peak.

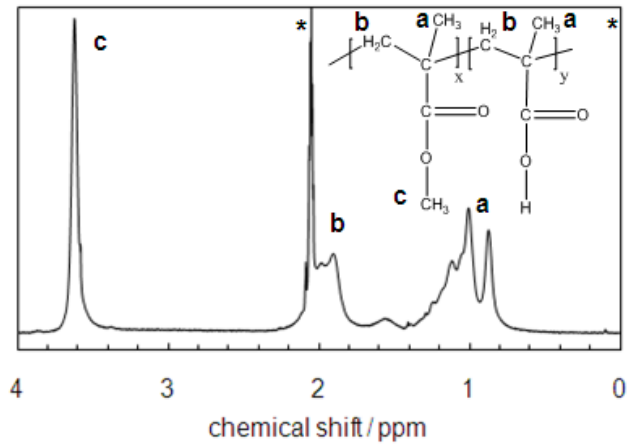

Figure S2. NMR spectrum for poly(MMA-*co*-MAA). The asterisk represents the acetone peak.

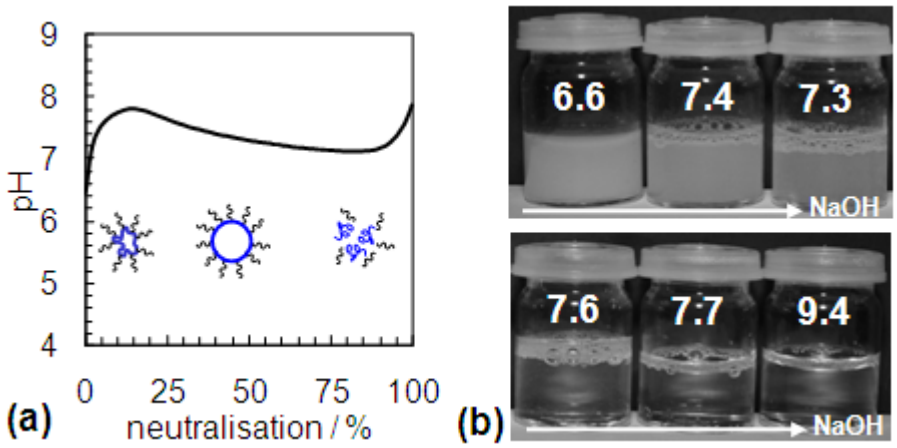

Figure S3. (a) Variation of pH and with neutralisation for poly(MMA-*co*-MAA) particles, (b) Poly(MMA-*co*-MAA) dispersions as a function of pH (shown on tubes). The samples contained 0.1 wt% copolymer and the direction of increasing NaOH addition is shown by the arrows.

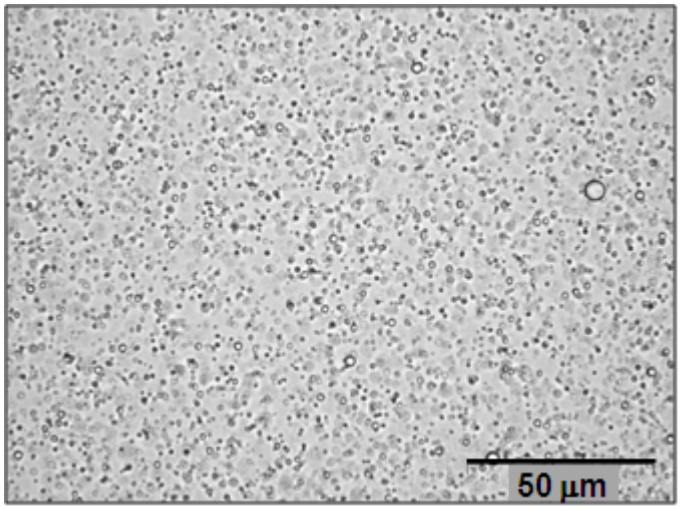

Figure S4. Optical micrograph of poly(MMA-*co*-*t*-BMA) particles.

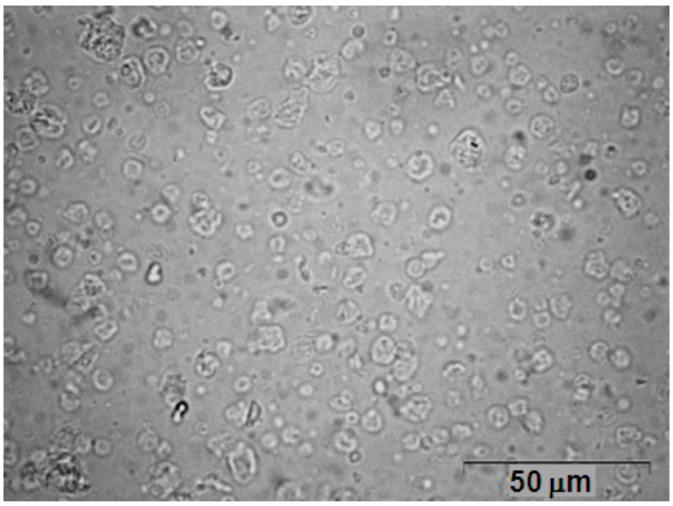

Figure S5. Optical micrograph of swollen poly(MMA-*co*-MAA/Cyst) particles at a pH of 9.2.

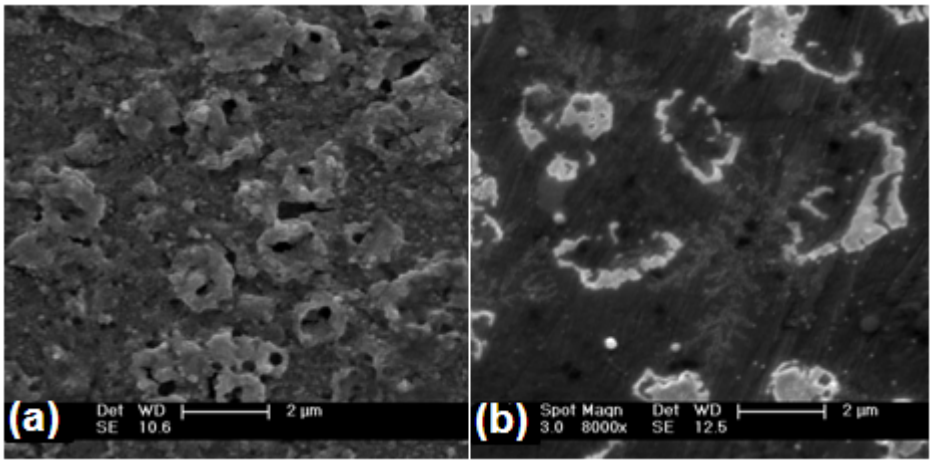

Figure S6. Scanning electron micrographs of reversibly crosslinked poly(MMA-*co*-MAA/Cyst) dispersions deposited on SEM stubs from a pH of 9.5. For (b) 0.05 M glutathione was added to a 0.1 wt% dispersion for 10 min prior to a deposition.

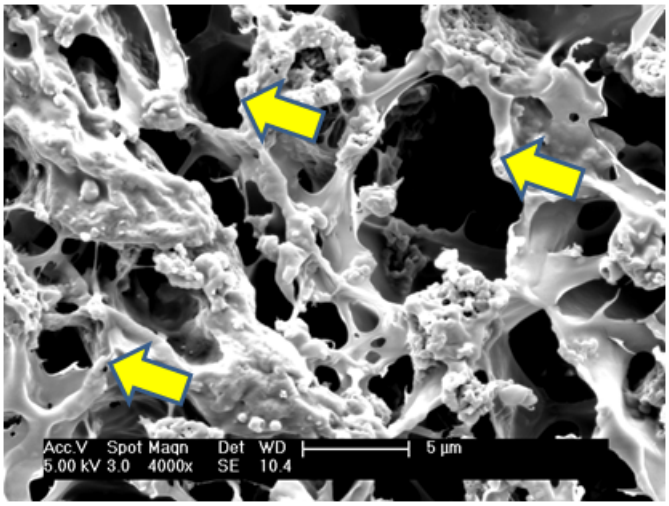

Figure S7. Scanning electron micrograph of freeze-dried poly(MMA-*co*-MAA/Cyst) gel. The pH was 8.5, and the total concentration prior to freeze-drying was about 8 wt%. The arrows show pores.

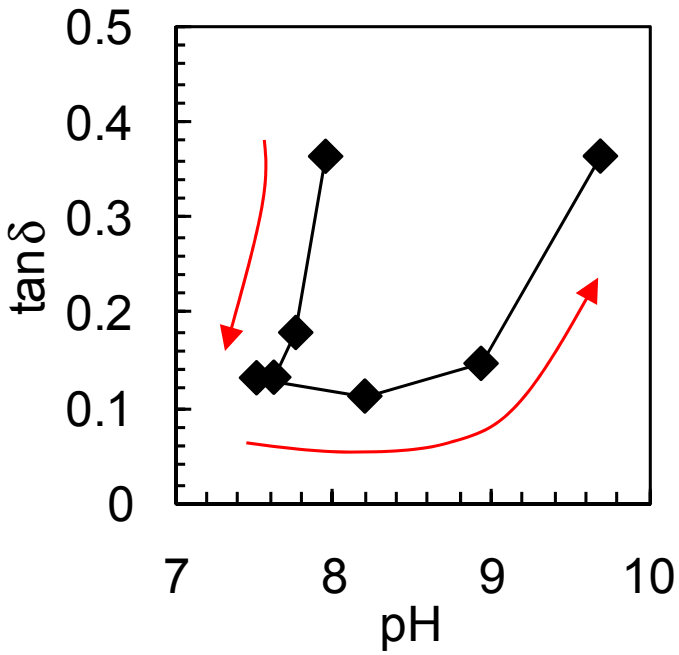

Figure S8 Variation of G' with pH for a 5 wt.% poly(MMA-*co*-MAA/Cyst) dispersion. The data were measured at 1 Hz and 1% strain. The arrows indicate the changes with pH as NaOH was added.

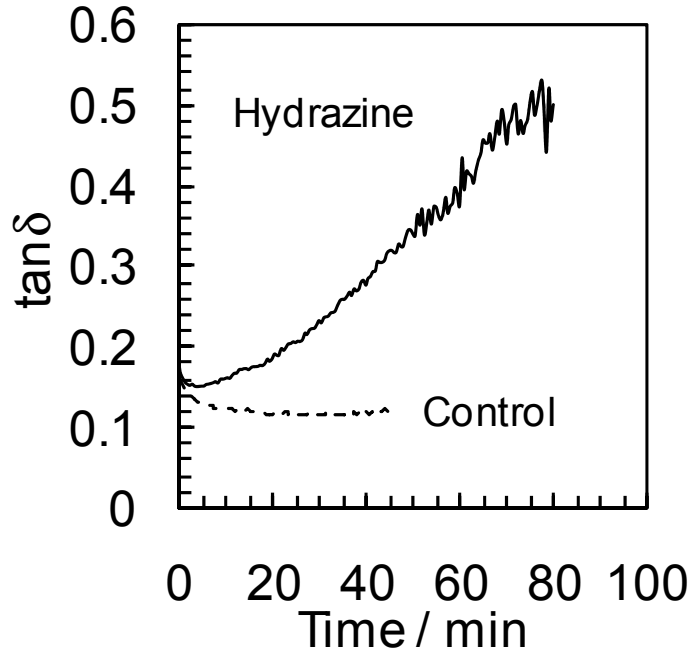

Figure S9. Variation of tanδ (= *G''*/*G'*) with time for poly(MMA-*co*-MAA/Cyst) 5 wt% dispersion at pH = 7.5 after addition of 0.05 M hydrazine with time. Data for a control system are also shown for comparison.

References

1. S. Freiberg and X. X. Zhu, *Int. J. Pharmaceut.*, 2004, **282**, 1.
2. S. Holappa, L. Kantonen, F. M. Winnik and H. Tenhu, *Macromolecules*, 2004, **37**, 7008.