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PAPER

Tuning the properties of pH-responsive and redox sensitive hollow particles and gels using copolymer composition

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Biodegradable pH-responsive hollow polymer particles offer excellent potential for preparing high performance biomaterials. Unfortunately, the established methods for pH-responsive hollow particle preparation are laborious and difficult to scale up. Recently, we reported that pH-responsive hollow particles could be prepared using solvent evaporation [Bird *et al.*, *Chem. Commun.*, 2011, **47**, 1443]. Here, we greatly expand and extend that work by investigating four new pH-responsive hollow particle systems based on poly(MMA-*co*-MAA) (methyl methacrylate and methacrylic acid) and poly(EA-*co*-MAA) (EA is ethyl acrylate). The hollow polymer particles were crosslinked with cystamine after preparation to give redox sensitive, biodegradable, hollow particles. For one of the systems a remarkable particle-in-hollow particle morphology was observed. The pH-triggered swelling of the hollow particles was studied and pH-triggered release of a model solute from these new hollow particles was demonstrated. The dispersions formed physical gels in the physiological pH range. The hollow particle physical gels had elastic modulus values as high as 4000 Pa at low total polymer concentrations. The swelling properties of the particles and the mechanical properties of the gels were tuneable using copolymer composition. The particles and gels could be disassembled with glutathione. The properties of these new gel-forming dispersions imply they have good potential for future application as injectable gels for regenerative medicine.

Introduction

Hollow polymer particles are of major research interest because of their large range of potential applications. These include biomaterial applications such as drug delivery,¹ gene delivery,² artificial cells^{3,4} and regenerative medicine.⁵ They also have application in surface coatings,⁶ catalysis,⁷ light weight fillers, encapsulation of cosmetics and vessels for confined reactions.^{3,8} The introduction of environmental response enhances application opportunities, especially in the context of biomaterials.⁹ The seminal studies involving hollow particle preparation were performed by Caruso *et al.*^{10,11} using colloidal templating combined with layer-by-layer assembly. Typically, colloidal templating involves using silica particles as a sacrificial template to construct the polymer coating.^{10–12} Dissolution of silica requires HF,^{11,13} which is a hazardous material. Recently, we demonstrated that biodegradable pH-responsive polymer particles could be prepared using solvent evaporation.¹⁴ The particles were both pH responsive and redox sensitive. The dispersions formed gels at moderate pH values and they could be disassembled using

reducing agents. Our new method for preparing pH-responsive hollow particles (described below) is simple, uses pre-formed copolymers, and does not require the use of a pre-prepared colloidal template, layer-by-layer assembly or HF. Here, we expand on our earlier study and extend it substantially by using new copolymers to demonstrate the generality of our approach. We also test our hypothesis that the pH at which these new hollow particles swell and also the elastic modulus of the hollow particle gels are tuneable through the composition of the pH-responsive copolymers used for their construction.

The most commonly used method for preparing hollow polymer particles requires layer-by-layer polymer shell assembly and a colloidal template.^{10,15,16} Other approaches to preparing hollow polymer particles involve *in situ* polymerisation,^{17,18} solid stabilised emulsions,¹⁹ or coacervation.²⁰ Recently, calcium carbonate has been used as a colloidal template.²¹ A simple scalable method for hollow particle preparation that has potential to be applied easily for biomaterial applications is lacking. Recent *in situ* polymerisation techniques have involved reactive surfactants,²² photocrosslinking²³ and a RAFT-based templating approach.²⁴ Approaches based on evaporation of solvents to form hollow capsules are much simpler. Li *et al.* constructed hollow particles using amphiphilic azo homopolymers.²⁵ Their method involved dissolving the copolymer in THF and then slowly adding water to form the hollow particles over a period of 60 h. The concentrations

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prepared appeared to be low and their technique would seem challenging to scale up. Lensen *et al.*²⁶ prepared biodegradable hollow particles using a specially synthesised polylactide polymer terminated by pentadecafluoro-1-octanol. They dissolved their polymer in a decane/ CH_2Cl_2 solution which was then emulsified. Evaporation of the CH_2Cl_2 forced the polymer to form a hollow polymer shell at the decane/water interface. The decane was removed by lyophilisation. In contrast to those methods^{25,26} our approach (a) uses a pH-responsive copolymer, (b) a cosolvent (methanol) that is soluble in the aqueous phase and (c) does not require lyophilisation. Our method is also well suited to deliver high particle concentration dispersions and scale up.

Our research group has a strong interest in establishing pH-responsive polymer colloid dispersions that form physical²⁷ or covalently-linked^{28,29} gels under physiological pH conditions. Injectable gels that are load supporting provide the opportunity to repair the mechanical properties of damaged intervertebral discs³⁰ (IVDs) and cartilage. To provide load support inside the body, pH-responsive particles with high RCOOH concentrations are essential because they can provide high swelling pressures at physiological ionic strengths.³¹ We have explored injectable physically gelled microgel dispersions for this purpose.³¹ An injectable cell/gel mixture requires built in microporosity for extracellular matrix growth.³² Because of this we investigate here pH-responsive hollow particles in order to establish new injectable load supporting gels for future regenerative medicine applications. The present study focuses on physical properties and does not consider biocompatibility.

Our approach for preparing biodegradable pH-responsive hollow particles is depicted in Scheme 1. The pH-responsive copolymers used to prepare the hollow polymer particles here

are poly(MMA-*co*-MAA) (methyl methacrylate and methacrylic acid) and poly(EA-*co*-MAA) (ethyl acrylate). These copolymers are abbreviated as M-*x*MA and E-*x*MA, respectively, (Scheme 1(a)) and *x* represents the mole percentage of MAA. MMA is used in bone cement and MAA is a hydrolysis product of MMA. It follows that the M-*x*MA systems should have lower potential regulatory barriers to application. E-*x*MA is used here to establish the generality of our approach.

The first step of preparing our biodegradable pH-responsive hollow particles (Step 1, Scheme 1(b)) involves addition of a solution of copolymer in a CH_2Cl_2 /methanol co-solvent blend to water containing poly(vinyl pyrrolidone) (PVP) under high shear. The rapid diffusion of methanol to the water phase caused precipitation of the copolymer at the CH_2Cl_2 /water droplet interface. This process and CH_2Cl_2 evaporation results in hollow particles. The second step (Scheme 1(b)) involved reaction of cystamine (C) with some of the MAA groups on the copolymer shell to incorporate reversible crosslinking *via* disulfide groups. The cystamine is essential for enhancing particle stability by preventing dissolution of the particles when the pH approaches the pK_a of the particles. This method gave biodegradable, pH-responsive hollow poly(MMA-*co*-MAA)/cystamine (M-*x*MA-*y*C) or poly(EA-*co*-MAA)/cystamine (E-*x*MA-*y*C) particles. The value for *y* is the mole percentage of cystamine incorporated.

In the present study we expand and greatly extend the earlier communication¹⁴ by investigating four new pH-responsive hollow particle dispersions. These include hollow E-*x*MA-*y*C particles which are new. The new E-*x*MA-*y*C particle gels are also shown to have high modulus values at low concentrations. We demonstrate pH-triggered release of a model solute from

Scheme 1 (a) Synthesis of the pH-responsive copolymers and (b) schematic representation of the two-step method used to prepare reversibly cross-linked hollow pH-responsive particles.

M-40MA-4C particles for the first time. The study also establishes that the pH-triggered particle swelling and gelation behaviours of these new particles and dispersions are tuneable through copolymer composition. A novel, particle-in-hollow particle morphology is also reported in this study.

Experimental

Materials

MMA (99%), EA (99%) and *t*-BMA (98%) were purchased from Aldrich and used as received. Azobisisobutyronitrile (AIBN, purity greater than 97%) was purchased from VWR and was used as received. *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, purity greater than 97%), *N*-Hydroxysuccinimide (NHS, 98%), cystamine dihydrochloride (98%) and pyrene methylamine hydrochloride (95%) were all purchased from Aldrich and used as received. Poly(vinyl alcohol) (PVA) was purchased from Aldrich and was 98% hydrolysed ($M_n = 14\,000$ – $22\,000\text{ g mol}^{-1}$). PVP was also purchased from Aldrich (average molecular weight = $40\,000\text{ g mol}^{-1}$). All solvents were reagent grade and water was Milli-Q quality.

Preparation of poly(MMA-*co*-*t*-BMA) and poly(EA-*co*-*t*-BMA)

An example synthesis is given in the following for poly(MMA_{0.60}-*co*-MAA_{0.40}). AIBN (0.25 g, 1.5 mmol) was dissolved in 85 ml of THF in a three-neck flask under nitrogen and refluxed at 55 °C for 30 min. A mixture of MMA (5.48 g, 54.7 mmol), *t*-BMA (4.52 g, 31.8 mmol) and AIBN (0.025 g, 0.15 mmol) dissolved in THF (17 ml) was added to the solution at a uniform rate over 90 min. After the feed the reaction was allowed to proceed overnight. After cooling, the solution was precipitated and rinsed using aqueous methanol (25% water) before drying at 80 °C overnight. The ¹H NMR spectra and assignments for poly(MMA-*co*-*t*-BMA), abbreviated as M-*x*BMA, are shown in Fig. S1(a). For these abbreviations *x* represents the mole percentage of *t*-BMA. An equivalent process was used to prepare the poly(EA-*co*-*t*-BMA) copolymers. The ¹H NMR spectrum and assignments for poly(EA_{0.52}-*co*-*t*-BMA_{0.48}) (abbreviated as E-48BMA) are shown in Fig. S1(c).

Preparation of poly(MMA-*co*-MAA) and poly(EA-*co*-MAA)

Poly(MMA-*co*-*t*-BMA) (1 g) 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 °C. ¹H NMR spectra for poly(MMA-*co*-MAA) appear in Fig. S1(b). These copolymers are abbreviated as M-*x*MA where *x* represents the mole percentage of MAA. An equivalent process was used to prepare the poly(EA-*co*-MAA) copolymers (abbreviated as E-*x*MA). Those ¹H NMR spectra and assignments appear in Fig. S1(b) and (c).

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

A solvent evaporation method was used to prepare the hollow particles. Solvent evaporation is a versatile and well accepted method for preparing drug-loaded particles.³³ E-*x*MA (1 g) was dissolved in 22 ml of a mixed CH₂Cl₂ (84 vol.%) / methanol (16 vol.%) solvent. This solution was then added into 60 ml of water containing 4 wt.% PVP and sheared at 10 000 rpm using a Silverson LR4 high speed mixer whilst being cooled at 0 °C. The emulsification continued for 30 s after addition of the polymer solution. The emulsion was then allowed to stir gently overnight to remove CH₂Cl₂. The product was purified by repeated centrifugation and redispersion in water to remove excess PVP. It was then filtered through a 50 μm filter. The poly(EA-*co*-MAA) particles were prepared using an equivalent procedure. The M-40MA particles were prepared using PVA in place of PVP according to the earlier report.¹⁴ We found that PVP gave an improved yield of the hollow particles and because of this PVP was used for the four new hollow particle systems studied here. The use of PVP did not significantly affect hollow particle properties.

Preparation of reversibly crosslinked hollow poly(MMA-*co*-MAA)/cystamine and poly(EA-*co*-MAA)/cystamine particles

To 100 g of 1.5 wt% M-40MA dispersion (6.4 mmol of MAA) maintained at a pH of about 6.4 (using 0.1 M phosphate buffer), EDC (1.4 g, 7.3 mmol) and NHS (0.9 g, 7.8 mmol) were added. The dispersion was stirred for 15 min at room temperature before adding air-oxidised cystamine dihydrochloride (0.15 g, 0.67 mmol). The reaction was allowed to proceed for about 18 h and the product was purified by repeated centrifugation and redispersion in water. The cystamine contents were determined by microanalysis (School of Chemistry, The University of Manchester). The poly(MMA-*co*-MAA)/cystamine particles are abbreviated as M-*x*MA-*y*C. The value for *y* is the mole percentage of cystamine incorporated. The preparation of fluorescently labelled M-*x*MA-*y*C particles was described in detail earlier¹⁴ and involved the use of pyrene methylamine. The method used to prepare the other M-*x*MA-*y*C particles and poly(EA-*co*-MAA)/cystamine particles (abbreviated as E-*x*MA-*y*C) was similar to that above. For preparation of the E-*x*MA-*y*C particles the pH used was about 5.8. The overall yield of M-*x*MA-*y*C and E-*x*MA-*y*C particles was more than 80% based on the mass of respective M-*x*MA or E-*x*MA copolymer used. These methods gave very good reproducibility and the particle sizes of consecutive batches could not be distinguished.

Physical measurements

GPC was conducted using a Shodex R101 Refractive index detector and THF at room temperature. The columns contained Phenomenex Phenogel 5 μm beads with 500, 10⁴ and 10⁶ Å pore sizes. The number-average molar mass (M_n) and polydispersity (PD) are quoted in this work. ¹H NMR spectra were obtained in acetone-*d*₆ using a Bruker 300 instrument. Potentiometric titrations were conducted using a Mettler Toledo DL15 Titrator and addition of 1.0 M NaOH. All pK_a values quoted here are apparent values.³⁴ Optical microscopy was conducted with an

Olympus BX41 microscope. Fluorescence microscopy 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. Thin sections of gel were rapidly frozen in liquid nitrogen ($-196\text{ }^{\circ}\text{C}$) and then freeze-dried. Rheology measurements were performed at $25\text{ }^{\circ}\text{C}$ using a TA AR-G2 rheometer and a $250\text{ }\mu\text{m}$ gap with a 20 mm diameter steel plate. For measurements of the swelling ratio a drop of dispersion ($0.1\text{ wt\%}$ in water) was placed between a microscope slide and cover slip. A drop of 0.1 M buffer solution 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 constant diameters and volume swelling ratios (Q). At least 21 particles were analysed to determine Q for each pH used. The value for Q , was calculated using

$$Q = \left(\frac{d}{d_c}\right)^3 \quad (1)$$

where d and d_c are the diameters of the hollow particles at a given pH and in the non-swollen (collapsed) state, respectively. In cases where the particles were not spherical the diameter used was the mean diameter of the equivalent sphere. The pH values for d_c used were those where the particles did not exhibit swelling and are shown in Fig. 3(e) at $Q = 1.0$.

Release measurements

These measurements were performed using M-40MA dispersions (60 mL , $0.05\text{ wt\%}$). Rhodamine B ($0.01\text{ wt\%}$) pH = 7.25 phosphate buffer were added and the dispersion stirred for 1 h before 2 mL of HCl (4 M) was added. The dispersion was then purified by repeated centrifugation with pH = 4 buffer. The final dispersion was concentrated to $0.1\text{ wt\%}$. Aliquots of the dispersion were taken and diluted in water and buffer to give samples containing $0.01\text{ wt\%}$ particles at a range of pH values. The samples were allowed to rest for 4 h before the particles were removed *via* centrifugation and supernatant analysed using UV-visible spectroscopy (PerkinElmer Lambda 25 UV/visible Spectrometer). A calibration curve enabled quantification of Rhodamine B release.

Results and discussion

Copolymer characterisation

The vast majority of the results presented in this study are for new systems. For comparison, some results are also presented from the earlier study¹⁴ for systems based on M-40MA. M- x MA and E- x MA were prepared by acid hydrolysis of the respective precursor M- x BMA or E- x BMA copolymers (See Scheme 1(a)). The compositions of the latter were determined by ^1H NMR spectroscopy (see Fig. S1). The M_n values for these copolymers (Table 1) were in the region of about 8000 to $30\,000\text{ g mol}^{-1}$. The PD values are close to the value of 2.0 expected for conventional free-radical copolymerisation. The ^1H NMR spectra for M- x MA and E- x MA (Fig. S1) show the disappearance of the t -BMA

peaks, due to hydrolysis, confirming the conversion of the t -BMA groups to MAA to form M- x MA and E- x MA. The x values used (*i.e.*, mol.% of MAA) for those copolymers (Table 1) were obtained from analysis of the ^1H NMR spectra for the parent M- x BMA and E- x BMA copolymers (Fig. S1).

Non-crosslinked hollow particle characterisation

During the process of solvent evaporation (Step 1, Scheme 1(b)) a copolymer shell formed around the droplets shortly after emulsification (see Fig. S2(a)). The rapid diffusion of methanol from the droplets decreases the solvent quality for the copolymer and forced copolymer precipitation at the $\text{CH}_2\text{Cl}_2/\text{water}$ interface. The non-crosslinked hollow M-40MA particles were collapsed (and crumpled) when the CH_2Cl_2 had evaporated (Fig. S2(b)). The generality of our method was tested using E- x MA copolymers. Spherical, hollow, particles were produced using E-32MA (Fig. S2(c)). We reported earlier¹⁴ that the amphiphilic nature of the copolymer was important for hollow particle formation. Hollow particles were not formed when M-40BMA was used (Fig. S2(d)) because the hydrophobic copolymer was confined to the droplet interior during solvent evaporation. SEM images of M-21MA particles showed the particles to be hollow and spherical (see Fig. S2(e)).

Values for x (mol.% MAA) within M- x MA and E- x MA were also estimated by pH-titration using dispersed (non-crosslinked) hollow particles (See Table 1). Representative titrations are shown in Fig. 1. (A full data set are shown in Fig. S3.) The generally good agreement for the mol% MAA between the two methods (^1H NMR and titration) confirms good conversion of the pre-cursor M- x BMA or E- x BMA copolymers to their respective M- x MA or E- x MA copolymers. The pH *vs.* neutralisation plots for all of the M- x MA dispersions in pure water showed a pH maximum. A representative example is shown in Fig. 1(a). This was most pronounced for the systems with largest x values. However, the maxima were less pronounced in the presence of 0.1 M electrolyte and also when x decreased (see Fig. S3). For all the M- x MA dispersions titrated in the presence of 0.1 M KCl the pH remained reasonably constant in the neutralisation region of about 20 to 60% . This buffering behaviour did *not* occur for the E- x MA dispersions (see Fig. 1(b)). Neutralisation of M- x MA results in exposure of buried MAA groups by a pH-triggered structural transition. On the other hand E- x MA behaves more like a conventional polyelectrolyte, with increasing neutralisation occurring more uniformly. We propose that the much higher glass transition temperature (T_g) of the poly(MMA) segments (*cf.* poly(EA)) oppose pH-triggered swelling. The T_g values for poly(MMA) and poly(EA) are 99 and $-23\text{ }^{\circ}\text{C}$, respectively.³⁵ Eventually, the ionic repulsive interactions become sufficiently strong to force the outer shell to swell, enabling an influx of OH^- groups to the inner part of the M- x MA hollow particle shells. This exposes new RCOOH groups and this opposes further pH increase. The inset of Fig. 1(a) shows that the dispersions dissolved once the pK_a was reached (which was 7.3 for M-40MA in water).

The pK_a for the M- x MA particles was about 1.0 pH unit lower when the titration was conducted in the presence of 0.10 M KCl compared to pure water (Fig S3(b)). This type of behaviour is known for polyacid gels.³⁴ The pK_a used here was that measured

Table 1 Summary of copolymer compositions and properties^a

Code (M-xMA or E-xMA)	Composition	M_n^b /(g mol ⁻¹)	PD	Mol.% MAA ^c	Mol.% MAA ^d	pK_a^e
M-21MA	Poly(MMA _{0.79} -co-MAA _{0.21})	23,450	2.0	21.2	21.4	7.9
M-27MA	Poly(MMA _{0.73} -co-MAA _{0.27})	17,600	1.8	27.0	26.4	7.6
M-34MA	Poly(MMA _{0.66} -co-MAA _{0.34})	16,900	1.7	34.2	32.5	7.1
M-40MA	Poly(MMA _{0.60} -co-MAA _{0.40})	27,300	1.9	40.0	41.3	6.5
M-48MA	Poly(MMA _{0.52} -co-MAA _{0.48})	21,400	1.8	47.5	50.6	6.3
E-32MA	Poly(EA _{0.68} -co-MAA _{0.32})	7,680	1.8	31.7	33.4	5.9
E-48MA	Poly(EA _{0.52} -co-MAA _{0.48})	20,500	1.8	48.1	46.3	5.6

^a M-40MA is from the earlier study.¹⁴ ^b GPC data are for M-xBMA and E-xBMA. ^c This value corresponds to x used throughout this paper and was based on ¹H NMR analysis of M-xBMA or E-xBMA. ^d Based on titration of M-xMA or E-xMA particles in the presence of 0.1 M KCl. ^e Values measured for the respective M-xMA or E-xMA particles in the presence of 0.1 M KCl.

in 0.10 M KCl because it is closer to the high ionic strength environment *in vivo*. It is noted that the pK_a decreased (with or without added electrolyte) as x increased for the M-xMA and E-xMA dispersions (Fig. S3(b)). This general trend has been reported for MAA-containing microgels.³⁶ The particles with the lowest x values have the highest MMA or EA contents and the greatest extent of inter-segment and intra-shell hydrophobic attraction. We propose that this more effectively opposes pH-triggered swelling and alters the proton dissociation equilibrium in favour of the RCOOH groups, which increases the pK_a .

Reversibly crosslinked pH-responsive hollow particles

pH-responsive reversibly crosslinked polymer particles were prepared by reacting cystamine with the M-xMA or E-yMA particles at room temperature using EDC/NHS activation (Step 2, Scheme 1(b)). The mol.% of cystamine incorporated into the hollow particles was about 3.5 to 3.9 mol.% for the M-xMA-yC copolymers and 2.7 to 2.8 mol.% for the E-xMA-yC copolymers (See Table 2). This implies that up to 37% of the available RCOOH groups were able to react with cystamine under the conditions employed here. The number-average particle sizes for the as-made (non-swollen) reversibly crosslinked hollow particles (Table 2) were determined by optical microscopy. The particles had high coefficients of variation (CV) because they were prepared using an emulsification-based method. Centrifugation could be used to reduce the polydispersity if required.

The crosslinked hollow particles were investigated in the as made (collapsed) state using optical microscopy and SEM (See Fig. 2.). The optical image for the M-21MA-4C particles (Fig. 2 (a)) is remarkable because some of the hollow particles contained particles (indicated with arrows) – See inset of Fig. 2(a). This is the first example of pH-responsive particle-in-hollow particle dispersions to the best of our knowledge. They are analogous to double emulsions.³⁷ Because an emulsion method was used to prepare these particles (Step 1, Scheme 1(b)) we propose that a transient water-in-oil-in-water emulsion phase was involved in the formation of these particles within hollow particles. They were obtained using the copolymer with the lowest MA content (M-21MA) and hence overall polarity. This could be an important factor that enables transient water-in-oil-in-water emulsion formation and requires further investigation.

Scanning electron micrographs are shown for deposited particles in Fig. 2(d)–(f). The M-40MA-4C particles are mostly collapsed and have a doughnut type morphology. This type of morphology is often observed for collapsing micrometre-sized droplets or hollow particles and was discussed in detail by Bahadur *et al.*³⁸ and Kryzanek *et al.*¹² SEM images were obtained for a E-32MA-3C particle (Fig. 2(e)) shows that it completely collapsed during the drying process used for SEM. This shows that these particles have a thin, flexible, shell. Fig. 2(f) shows a number of collapsed E-48MA-3C particles. Not all particles appear uniform and a coarse morphology is evident. This could be due to crumpling, as shown for E-32MA-3C in

Fig. 1 Titration data for non-crosslinked hollow particle dispersions. The data in (a) show the effect of added electrolyte on the titrations for M-40MA dispersions. The inset shows digital photographs of the dispersions in pure water at selected pH values (shown). The data shown in (b) enable comparison for M-48MA and E-48MA and were obtained in the presence of 0.1 M KCl.

Table 2 Reversibly crosslinked hollow particle compositions, swelling and gel properties

Copolymer code	Mol. Cyst ^a /%	$d/\mu\text{m}$ (CV ^b)	pH _{gel} ^c	Q_{pHgel} ^d	G'_{pHgel} ^e /Pa	γ^*_{pHgel} ^f /%
M-21MA-4C	3.9	2.5 (48%)	—	—	—	—
M-40MA-4C	3.8	2.4 (50%)	7.5	4.0 ^g	385	—
M-48MA-4C	3.5	2.0 (36%)	7.4	4.5	780	4.0
E-32MA-3C	2.7	2.2 (52%)	7.0	4.0	2,700	2.0
E-48MA-3C	2.8	3.1 (75%)	6.9	4.9 ^g	3,980	5.0

^a Mol.% cystamine incorporated. ^b Coefficient of variation. ^c pH at the gelation point – See text. ^d Value for Q at pH_{gel}. ^e Storage modulus at pH_{gel}.

^f Onset yield strain at pH_{gel}. ^g Interpolated values from Fig. 3(e).

Fig. 2(e). This is supported by the inset for Fig. 2(f) which shows a higher magnification image of a collapsed hollow particle.

We reported pH-triggered swelling images for hollow M-40MA particles in the earlier communication.¹⁴ Here we show, for the first time, pH-triggered swelling for hollow particles based on a poly(EA) copolymer (E-32MA-3C). See Fig. 3(a). These images show that pH-triggered swelling for these hollow particles is rapid. The shell thickness is estimated to be about 1 to 1.5 μm from the images shown for this system in Fig. 3(a). A characteristic swelling time (τ_{sw}) can be estimated using the following equation^{39,40} and a gel diffusion coefficient (D_g) of the order of $10^{-7} \text{ cm}^2 \text{ s}^{-1}$.⁴¹ We use the thickness of the shell of the hollow particles (L_{sh}) as an effective gel length.

$$\tau_{\text{sw}} = \frac{L_{\text{sh}}^2}{\pi^2 D_g} \quad (2)$$

It follows that for hollow particles with a 1.0 μm shell, a swelling time of the order of about 10 ms should result. This simple calculation supports the suggestion that it is the diffusion of the buffer that is rate limiting for the swelling of these pH-responsive hollow particles.

Optical images for selected swollen particles are shown in Fig. 3(b) and (d). Fig. 3(c) shows hollow swollen particles with pyrene methylamine covalently linked to the shells. Generally, it can be seen from Fig. 2(a)–(c) and 3(a)–(d) that for both of the M- x MA-4C and E- x MA-3C systems the tendency for the particles to retain their spherical shape increased with decreasing x . We speculate that at low pH (below the respective pK_a values) inter-segment hydrogen bonding between RCOOH groups favoured a collapsed (buckled) hollow particle morphology which was locked in place to a large extent by cystamine crosslinking.

pH-dependent swelling data are presented in Fig. 3(e) for all of the crosslinked hollow particle dispersions studied. The pH values at approximately the inflection points for E-32MA-3C and M40-MA-4C swelling transitions are 7.1 and 7.5, respectively. These values can be compared to the pK_a s for E-32MA and M-40MA, measured in 0.1 M KCl, of 5.9 and 6.5, respectively (Table 1). The reason for the differences is a reduced MAA content (x) in the crosslinked particles due to reaction with cystamine (Table 2) and the presence of crosslinking, which will oppose swelling. The data sets for E-48MA-3C, M-48MA-4C

Fig. 2 Micrographs for as made crosslinked hollow particles. (a) to (c) show optical images M-21MA-4C, M-40MA-4C and E-48MA-3C particles, respectively. The arrows in (a) show hollow particles containing particles. The inset shows an expanded view of one of these particles. (d) to (f) show SEM images for M-40MA-4C, a collapsed E-32MA-3C particle and E-48MA-3C particles, respectively. All the particles were obtained at pH values of about 6.

Fig. 3 Swelling of reversibly crosslinked pH-responsive hollow particles. (a) Optical images of a E-32MA-3C particle as it swells from the collapsed state to a swollen state in response to buffer at pH = 8.0. The time (seconds) is shown. (b) Optical micrograph and (c) fluorescence image of M-40MA-4C particles at a pH of about 9.2–9.5. The particles for (c) were labelled using pyrene methylamine and the arrows identify hollow particles. (d) Optical micrograph of E-48MA-3C particles at pH = 8.5. Some swollen hollow particles are identified with arrows (e) Shows swelling ratio vs. pH data for various M- x MA- y C and E- x MA- y C hollow particles with representative error bars.

and M-21MA-4C are more limited and inflection points cannot be clearly discerned. However, it can be seen that for each series Q increases with x , as expected. The increased swelling of the E- x MA- y C series compared to the M- x MA- y C series could be due to the lower cystamine incorporation for the former. The lower T_g value for poly(EA) may also play a role as we have found that EA-based microgels swell more than MMA-based ones.²⁸

pH-triggered release from reversibly crosslinked hollow particles

We used Rhodamine B as a model active to demonstrate pH-triggered release using our M-40M-4C particles (See Fig. 4). To demonstrate release we added a drop of concentrated NaOH solution to one side of a Rhodamine B-loaded dispersion (initially at pH = 4.0) located on a sample stage of the fluorescence microscope and recorded the progress of the NaOH solution front with time. Fig. 4(a) and (b) show the progress of the NaOH front, which released Rhodamine B and gave a red background (visible on online edition). The particles remained intact after swelling. Fig. 4(c) shows a fluorescence image for a non-swollen hollow particle containing absorbed Rhodamine B. This image shows that prior to release these particles contained the dye throughout the shell and also inside the hollow particle. An irregular distribution of intensity is evident from the shell (Fig. 4(c)) indicating that the Rhodamine B in the shells was

not uniformly distributed. This might be caused by phase separation of the dye within the polymer shell. The fact that the dye is also present inside the hollow particles shows that the shells must have had a low average pore size compared to the size of Rhodamine B.

We quantified release of the dye from the Rhodamine B-loaded hollow particles using UV-visible spectroscopy (see Fig. 4(d)). pH-triggered release is clearly evident. It can also be seen that there was some leakage at low pH. The maximum rate of pH triggered release for these particles is in the vicinity of 7.0 (Fig. 4(d)) and this coincides with the onset of pH-triggered hollow particle swelling (Fig. 3(e)). All of the dye was released when the pH reached 7.6. This demonstrates good potential for future pH-triggered release from these types of pH-responsive hollow particles *in vivo*.

pH-triggered inter-connected hollow particle gel morphology and mechanical property tuning

Addition of NaOH solution to concentrated hollow particle dispersions triggered particle swelling and physical gel formation. This can be seen from Fig. 5(a) and (b). By analogy to pH-responsive microgels,²⁸ physical gels form when the effective volume fraction of the dispersed hollow particles ($\phi_{hp(eff)}$) reaches a critical value, $\phi_{hp(eff)}^*$. When $\phi_{hp(eff)} = \phi_{hp(eff)}^*$ there

Fig. 4 pH-triggered release of Rhodamine B from pH-responsive hollow M-40-4C particles. (a) and (b) show diffusion of a NaOH solution front through a dispersion initially at a pH of about 4.0. The arrows show the direction of diffusion. (c) Shows a higher magnification image of a hollow particle containing Rhodamine B at a pH of about 4.0. (d) Shows a release vs. pH profile for the hollow particle dispersion containing absorbed Rhodamine B. The release fraction is ratio of the mass of Rhodamine B released to the hollow particle mass. The error bars are shown.

is no longer significant space between the neighbouring particles and stress is distributed across hollow particle contacts which gives rise to a load supporting network. The value for

$\phi_{hp(eff)}$ will be an increasing function of Q (which can be controlled by the pH, Fig. 3(e)) and also hollow particle concentration.

For all of the M-*x*MA-4C dispersions the pH decreased at first after addition of NaOH due to the buffering effect discussed earlier. Physical gels could be formed at pH = 7 for particle concentrations as low as 2.5 wt.% for E32-MA-3C and 1.4 wt.% for E48-MA-3C. SEM images of freeze dried M-*x*MA-4C and E-*x*MA-3C gels (Fig. 5(c) – (f)) show morphologies consisting of inter-connected pores with sizes in the range of about 1–10 μ m. This range of pore sizes (polydisperse) is comparable to that of the parent swollen hollow particles (Fig. 3(a)–(d)). Fig. 5(e) shows a side-on-view with evidence of collapsed hollow particles forming the gel periphery. The highly porous micrometre-scale morphology, which appears to be inter-connected, is potentially useful for future tissue scaffold applications. We term these gels (which consist of a continuous crosslinked network) inter-connected hollow particle gels because this describes both their morphology and the unique components from which they are made.

The inter-connected porosity of the gels (Fig. 5(c)–(f)) would seem at first glance to be compatible with disulfide exchange since this is known to be pH-dependent with increasing reaction rates at pH values close to the pK_a of the thiol groups.⁴² The pK_a of cystamine is 8.35.⁴³ The images shown in Fig. 5(e) and (f) were obtained from particle gels formed at a pH of 7.1. This is more than 1.2 pH units lower than the pK_a of cystamine. A referee pointed out that under these circumstances a little less than 10% of the thiol groups will be deprotonated and that this is more than sufficient for disulfide exchange to occur. We do not have any data that unambiguously rules out disulfide exchange from contributing to the pH-triggered gelation or the morphologies apparent in Fig. 5. However, we have observed similar pH-triggered gelation to that observed in this work and similar gel

Fig. 5 pH-triggered gelation of concentrated hollow particle dispersions. (a) and (b) shows digital photographs of inverted tubes of M-40MA-4C and E-48MA-3C dispersions at different pH values (shown). (c) and (d) show scanning electron micrographs for freeze-dried M-40MA-4C (pH = 8.5) and M-48MA-4C (pH = 7.4) gels, respectively. SEM images for E-32MA-3C (pH = 7.1) and E-48MA-3C (pH = 7.1) are shown in (e) and (f), respectively. All of the gels were prepared using 5 wt.% dispersions.

morphologies to those shown in Fig. 5 using M-40MA particles functionalised with aminoethyl methacrylate and crosslinked by free-radical chemistry.⁴⁴ The crosslinking mechanism in that case involved -C-C- bonds and *not* thiols. It follows that disulfide exchange is not the dominant cause of the morphologies apparent in Fig. 5.

Physical gelation occurs when neighbouring particles expand to an extent where there is no longer space sufficient available for particles to move past each other. Portions of neighbouring particle shells are effectively in contact. We propose that the pH-triggered physical gelation of the hollow particles will cause uneven stresses being placed on the particle shells due to differences in the neighbouring particle environment. In other words, the forces on a given part of an expanding shell will be affected by the other shell portions (from neighbouring swelling particles) that come into direct contact during gel formation. There will also be regions of a shell which are not contacted by neighbouring shells. This should give locally uneven forces and deformations of the shell walls. At a molecular level the ability of local network domains to expand (and even fragment) will vary depending on their location within the shell with respect to other impinging shells. This imbalance in macroscopic and molecular scale forces will be accentuated by the high particle polydispersity of the present systems. We propose that the inter-connected morphologies result from pH-triggered fragmentation of segments of hollow particle shells due to locally high stress environments.

Dynamic rheology data were obtained for 5 wt.% M-*x*MA-4C or E-*x*MA-3C dispersions at different pH values to probe the mechanical properties of these pH-responsive inter-connected hollow particle gels. (See Fig. 6.) Fig. 6(a) to (c) shows G' (storage modulus) and $\tan\delta$ ($= G''/G'$, G'' is the loss

modulus) values measured at 1 Hz from frequency sweep experiments. (The frequency sweep data are shown in Fig. S4.) These data show the unusual rheological behaviour for the M-*x*MA-4C gels (Fig. 6(a) and (b)). For these dispersions addition of NaOH caused an increase in G' (and a decrease in $\tan\delta$) and a decrease in pH. The latter was due to swelling of the particles and exposure of buried RCOOH groups. When the pH exceeded 7.5 further addition of NaOH caused a decrease in G' and an increase in $\tan\delta$. At high pH values (9.7) the elasticity of the gel becomes weaker. In contrast, the E-*x*MA-3C inter-connected hollow particle gels did not show pH hysteresis. This is due to the lower T_g for these particles and less buffering during neutralisation.

The data from Fig. 6 (b) and (c) enable the estimation of critical pH values for gelation (pH_{gel}). The pH_{gel} values is taken as the smallest pH value corresponding to a minimum for $\tan\delta$. The pH_{gel} values for these dispersions are shown in Table 2. The values of pH_{gel} for E-32MA-3C and M-40MA-4C very close to (or the same as) the pH values at the swelling inflection points (Fig. 3(e)). This shows that gelation is caused by the pH-triggered swelling of the hollow particles.

The G' values measured at pH_{gel} , i.e., G'_{pHgel} (Table 2) decrease in the order: E-48MA-3C > E-32MA-3C > M-48MA-4C > M-40MA-4C. This is the same order as x decreases for each series. However, the values for Q at pH_{gel} (Q_{pHgel} , Table 2) do not follow this order. Therefore, factors other than Q govern the elastic modulus for these gels. A possible explanation is that the more flexible E-*x*MA-3C particles provide a higher interfacial contact area between neighbouring particles in the gel, giving a higher number of load distributing crosslinks per unit volume.

Frequency sweep data are shown for the inter-connected hollow particle gels in Fig. S4. It is noteworthy that the frequency sweep that E-40MA-4C is exceptional in that it has a negligible frequency dependence of $\tan\delta$ and also the lowest $\tan\delta$ values of all the gels. This inter-connected hollow particle gel forms an ideal network according to the Winter-Chambon criteria.^{45,46} Strain sweep measurements were also conducted (see Fig. S5.). The values for the onset yield strain (γ^*) were determined for the gels. This corresponds to the point where G' falls to 95% of its value at 1% strain.⁴⁷ These values are plotted in Fig. 6(d). These gels are moderately brittle, which is not surprising given they are physical gels. The gels also show evidence of a maximum in γ^* . For M-48MA-4C and E-48MA-3C this is followed by a decrease and then a further increase to a value higher than any of the original values. It is suggested that NaOH triggered breaking of the disulfide bonds at high pH (e.g., greater than 9.1) increases ductility. This would involve disulfide exchange.

Triggered disassembly of pH-responsive inter-connected hollow particle gels

Biodegradability is very important for potential applications of injectable gel-forming dispersions. This was tested earlier¹⁴ using hydrazine, which is a strong reductant. Hydrazine caused a rapid decrease in G' and the gel flowed within 20 min. Here, we investigated glutathione because it is a natural reductant present within cells. Glutathione also caused disassembly of the network, albeit at a slower rate than hydrazine. This can be seen from the

Fig. 6 Variation of G' and $\tan\delta$ with pH for hollow particle gels. (a) Shows the variation of G' with pH. (b) and (c) show the variation of $\tan\delta$ with pH for the dispersions from (a). (d) Shows the variation of the onset yield strain with pH. The data were measured using a particle concentration of 5 wt%. The data for (a) to (c) were obtained from frequency sweep experiments. The solid arrows in (a)–(c) show the approximate pH values at which the particle Q values are at their respective inflection points (See text). The circular dotted arrows show the direction of NaOH solution addition. The lines are guides to the eye. The error bars for each figure apply to all of the data.

Fig. 7 Triggering gel disassembly using glutathione. (a) SEM image of M-40MA-4C gel after addition of 5 mM glutathione. The SEM image was obtained 10 min after glutathione addition. (b) Shows tubes for E-32MA-3C and the effect of added glutathione (5 mM) after one week. The gel had begun to flow noticeably after 48 h at *ca.* 20 °C. The pH was approximately 7.1.

Fig. 7(a) (compare to Fig. 5(c)). The matrix has the appearance of “melting”. This is visual evidence of glutathione-triggered disassembly of the gel. We also tested E-32MA-3C gel to determine if glutathione-triggered disassembly could also occur. A gel-to-fluid was observed for a 5 wt.% dispersion after about 2 days when 5 mM of glutathione had been added (Fig. 7(b)). This shows the generality of our approach to copolymers other than those based on poly(MMA).

Conclusions

A range of new pH-responsive hollow particles and particle gels have been investigated in this work. Our new hollow particle preparation method was successfully used to prepare E-*x*MAA-3C particles and appears to be generally applicable. The E-*x*MAA-3C systems exhibited a decreased pH for their pH-triggered swelling and inter-connected hollow particle gel formation. Our method should apply to any amphiphilic random copolymer containing high MAA contents. The M-21MA-4C particles showed a remarkable particle-in-hollow particle morphology. More generally, the pH at which the hollow particles showed their maximum swelling increase corresponded with gel formation. This pH range (6.9 to 7.5) resides within the window of physiological pH values. The hollow particles show rapid pH-triggered swelling. pH-triggered release of a model solute (Rhodamine B) was demonstrated from the M-40M-4C particles. This occurred at a pH that should be well-suited for potential use *in vivo*. Like the M-*x*MAA-4C dispersions the E-*x*MAA-3C gels could be transformed to a fluid by addition of a reducing agent. Here, we used glutathione. The physically gelled E-*x*MAA-3C dispersions had much higher elastic modulus values compared to the M-*x*MAA-4C system. The E-48MA-3C gel was noteworthy in this regard. At pH = 7 and 5 wt.% it had a modulus of about 4000 Pa and $\tan\delta$ values that were nearly frequency independent. Our hollow particles were prepared using solvent evaporation using pre-formed polymers and did not involve the use of small-molecule surfactants. The pK_a and also pH at which swelling occurs (and physical gels form) can be controlled by the MAA content used to prepare the copolymer. The elasticity of the physical gels is also tuneable through copolymer composition. Both Q and G'

increase with x . Because of these factors, the new (injectable) gels studied here would seem to have good potential for future biomaterials applications. However, biocompatibility studies are required and these are planned for future work.

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