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Tailoring pH-responsive acrylic acid microgels with hydrophobic crosslinks for drug release

B. Lu,[a] M. D. Tarn,[a] N. Pamme*[a] and T. K. Georgiou*[b]

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Amphiphilic microgels based on the hydrophilic acrylic acid (AA) and hydrophobic crosslinks of different compositions were synthesised using a lab-on-a-chip device. The microgels were formed by polymerising hydrophobic droplets. The droplets were generated *via* a microfluidic platform and contained a protected form of AA, a hydrophobic crosslinker (ethylene glycol dimethacrylate, EGDMA) and a free radical initiator in an organic solvent. Following photopolymerisation and subsequent hydrolysis, AA based microgels of amphiphilic nature were produced and it was demonstrated that they can successfully deliver both hydrophilic as well as hydrophobic moieties. The model drug delivery and the swelling ability of the microgels were influenced by the pH of the aqueous solution as well as the crosslinking density and hydrophobic content of the microgels.

Introduction

Microgels are gel particles with sizes ranging from 0.1 to 100 μm, and consisting of three-dimensionally crosslinked polymer networks. Such microgels have many exciting applications, including bioapplications in drug delivery,[1–10] tissue engineering,[2] and biosensing,[11] as well as applications in areas of the oil industry,[12] organic dye removal,[13,14] coatings[15] and food industry.[16] Depending on the chemistry of the polymer chains, microgels can be dispersed in organic and/or aqueous solvents. For bioapplications like drug delivery it is important that the microgels are dispersed in water, *i.e.* they should contain hydrophilic components that will stabilise and disperse the microgels in water. However, it would be more advantageous if the microgels were amphiphilic, *i.e.* if they contained both hydrophobic and hydrophilic groups, as they would be able to encapsulate and release both hydrophilic and hydrophobic drugs.

Amphiphilic microgels, however, are challenging to fabricate because most of the production methods, such as conventional emulsion templating, involve the formation of droplets in an immiscible continuous phase. The droplets contain pre-gels or polymerisable reagents that are later reacted to form the microgel. Thus, when fabricating hydrophilic microgels, the hydrophilic reagents are dispersed in the droplets while the continuous phase (CP) is an organic solvent in which the reagents have reduced or no solubility. The opposite applies when forming hydrophobic microgels; hydrophobic reagents are dispersed in the droplets while the CP is usually an immiscible aqueous solution. In order to fabricate amphiphilic microgels, one hydrophilic reagent and one hydrophobic would have to be stabilised and mixed together in a droplet without diffusing into the CP. However, if the CP is aqueous then the hydrophilic reagent will diffuse from the droplets to the CP and *vice versa*. If the CP is based on oil or an organic solvent, the hydrophobic reagents will diffuse from the droplets to the CP. Thus, amphiphilic microgels are usually prepared using post-modifications[1,4,7,17–21] and/or multi-step procedures.[6,22] Commonly, hydrophilic microgels are fabricated and then modified to produce amphiphilic microgels by covalently or electrostatically binding amphiphilic or hydrophobic moieties.[1,4,7,18–21]

In this study we have used a microfluidic device to form droplets that were subsequently polymerised and hydrolysed to fabricate amphiphilic microgels that were based on acrylic acid (AA, ionisable and hydrophilic) and hydrophobic crosslinks based on the hydrophobic crosslinker ethylene glycol dimethacrylate (EGDMA). The application of a microfluidic platform offers the advantages of generating droplets, and consequently microgels, of narrower size distribution, while the size of the droplets can be tailored by varying the flow rates.[16,23–26] Thus, the microgels are tailorable in terms of their size but also in terms of their composition since the crosslinking density, and so amphiphilicity, can also be varied by adjusting the molar ratio of the components. Finally, when using a microfluidic device, immediate reactions following droplet generation can be performed, thereby enabling the polymerisation of some relatively unstable and easily hydrolysed monomers. For example, (meth)acrylic monomers when in contact with water tend to hydrolyze to (meth)acrylic acid,[27] but (meth)acrylic acid will not be miscible in an organic solvent that contains another hydrophobic (macro)monomer or (macro)crosslinker in order to produce amphiphilic microgels.

[a] Department of Chemistry, University of Hull, HU6 7RX, Hull, UK. E-mail: n.pamme@hull.ac.uk
[b] Department of Materials, Imperial College London, SW7 2AZ, London, UK. E-mail: t.georgiou@imperial.ac.uk

Therefore, in order to produce amphiphilic microgels, a hydrophobic monomer that is a protected form of AA, specifically tetrahydropyranyl acrylate (THPA), was synthesised in-house and then copolymerised with the hydrophobic crosslinker, EGDMA, in chloroform droplets that were formed using microfluidic devices. The protective group of tetrahydropyranyl was chosen because it is a good leaving group and it can be easily be hydrolysed even in macrogels.[28–31] The hydrolysis of the THPA groups produced anionic gels which we have chosen because they have shown promise in drug delivery[32] and protein delivery.[33] Specifically, in this study microgels of differing crosslinking ratios were produced, collected and polymerised off-the chip and then hydrolysed to produce AA based microgels. The pH responsiveness of the AA based microgels was evaluated by studying their swelling in different pH environments. Finally, to prove that the amphiphilic nature of the microgels assists in the delivery of drugs of different hydrophilicities, the encapsulation and delivery of both hydrophobic and hydrophilic moieties was investigated. To the best of our knowledge, this is the first report of covalently linked microgels of amphiphilic nature that have been prepared using a microfluidic platform.

Experimental

Materials and methods

Acrylic acid (AA, 99%), 2,3-dihydro-2*H*-pyran (DHP, 99%), ethylene glycol dimethacrylate (EGDMA), sodium dodecyl sulphate (SDS), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH, free radical inhibitor, 99%), 1-hydroxy-cyclohexyl phenyl ketone (HCPK, 99%) as the free-radical initiator, Sudan I, trypan blue, phenothiazine (98%), sulfuric acid, sodium bicarbonate ($NaHCO_3$), sodium sulphate (Na_2SO_4) hydrochloric acid, sodium hydroxide and basic alumina were purchased from Sigma-Aldrich (Dorset, UK). EGDMA was passed through a basic alumina column to remove the free radical initiator prior use. The organic solvent used to prepare the hydrophobic droplets was chloroform (99%), and was purchased from Fisher Scientific (Loughborough, UK). The chemical structure of the main reagents used (AA and EGDMA) and the in-house synthesized THPA monomer, synthesis described below, are shown in Fig. 1.

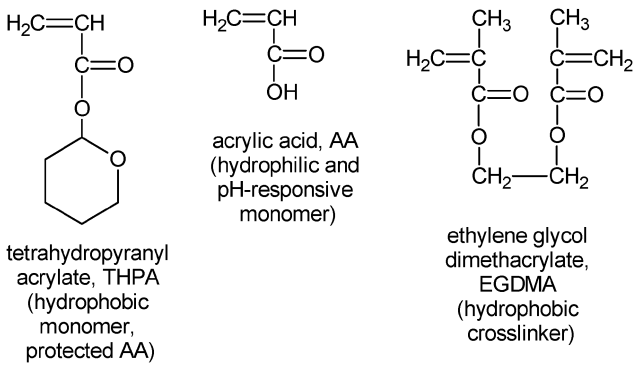

Fig. 1 Chemical structures of the monomers and crosslinker.

Synthesis of protected acrylic acid monomer, tetrahydropyranyl acrylate (THPA). The synthesis of THPA was similar to the previously described synthesis of tetrahydropyranyl methacrylate.[28–30,34,35] In a 500 mL three neck round bottom flask that containing 1 g of phenothiazine (free radical inhibitor) 125 mL of DHP (116.25 g, 1.38 mol), 20 drops of a 50% v/v sulfuric acid solution in water were added drop-wise. The colour should change to brown. Following this, a mixture of 125 mL DHP (116.25 g, 1.38 mol) and 94.6 mL AA (99.4 g, 1.38 mol) and 1 g phenothiazine (using a dropping funnel) was added and the round bottom flask was placed in an oil bath. The mixture was then left to react overnight at 50 °C.

The next day, once the mixture had cooled down, 10 g of $NaHCO_3$ and 40 g of Na_2SO_4 were added and allowed to stir for 2–3 h. The solids were then filtered out and the monomer mixture was passed through a basic alumina column twice (to remove the un-reacted AA). The removal of AA was confirmed by ^1H NMR. A free radical inhibitor, DPPH, was added into the THPA monomer and the monomer was stored in the fridge. It was distilled under vacuum prior to use.

Fabrication of microgels

Microfluidic chip fabrication and setup. Droplets containing THPA–EGDMA were were generated in a flow focusing chip geometry with a CP inlet, a dispersed phase (DP) inlet, and a single straight outlet channel (Fig. 2a). The channel structure was etched in glass to a depth of 50 μm and a width of 150 μm *via* photolithography and wet etching techniques.[36] Fused silica capillaries (150 μm i.d., 363 μm o.d., CM Scientific, UK) were glued into the inlet and outlet holes and connected to 500 μL glass syringes (SGE, Sigma-Aldrich, UK) with syringe adaptors (IDEX Health & Science, UK) (Fig. 2b). Two syringe pumps (PHD2000, Harvard Apparatus, UK) were used to control the flow rates of the CP and DP between 0.5 μL min^{-1} and 50 μL min^{-1}. A 5 cm long piece of Tygon tubing (254 μm i.d., 762 μm o.d., Cole-Parmer, UK) was attached to the outlet capillary at one end, while the other end was placed onto a microscope slide or a Petri dish for droplet collection. Droplets were photographed with a colour CCD camera (MTV-63V1N, Mintron, Taiwan) attached to an inverted microscope (Eclipse Ti, Nikon, UK), with images captured using WinDVD Creator 2 (Corel Ltd, UK) software. ImageJ freeware was used for the analysis of droplet size and colour

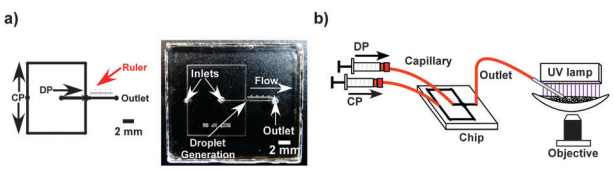

Fig. 2 (a) Layout and photograph of the flow focusing chip employed for microgel precursor droplet formation. The design featured a central channel for the oil based DP, containing the reagents, and outer channels for the CP, which merged into a 5 mm long outlet channel. (b) Experimental setup for on-chip droplet generation and collection of the droplets onto a slide or dish for UV initiated photopolymerisation.

intensity. Photopolymerisation was achieved using a 15 W UV light source (XX-15S, Ultra-Violet Products Ltd, UK). The entire setup was covered with a thick black cloth to in order to protect users from UV irradiation. Chips and syringes were covered with aluminum foil to prevent the reagents from gelating prior to their collection as droplets.

Formation of droplets and polymerisation of microgels. The DP was based on chloroform containing THPA and EGDMA with varying molar ratios (though the total concentration of the two reagents was always 30 wt%), and 4 wt% of HCPK (free radical photo-initiator). The CP was an aqueous solution of 0.1 wt% SDS. The concentration of SDS was below the critical micelle concentration.[37] The flow rates of the CP and DP were 5 μL min^{-1} and 0.5 μL min^{-1}, respectively, and were chosen following preliminary results which showed that stable droplets were formed at these flow rates. After droplet formation in the microfluidic device, the droplets were collected on a microscope slide or Petri dish and immediately polymerised by UV irradiation for 30 min. Three THPA–EGDMA microgels of differing compositions were formed, with molar ratios of 30 : 4, 50 : 4, and 70 : 4 THPA : EGDMA. The polymerised microgels were then hydrolysed in 1 M HCl overnight to produce pH responsive AA–EGDMA microgels. The hydrolysis was confirmed with FTIR spectroscopy.

Swelling studies in different pH environments

Aqueous solutions of NaOH (1 M) and HCl (1 M) were used to vary the pH of the AA–EGDMA microgel suspensions, from pH 1 to pH 14, in order to study the responsiveness of the microgel particles. The microgels were photographed with the colour CCD camera and inverted microscope setup described previously, with ImageJ used for image analysis. The extent of swelling and shrinking (relative size) was calculated by dividing the microgel size at a given pH by their original size measured at pH 7.

pK_a determination

The pK_a of the three synthesised microgels was determined by potentiometric titration. Specifically, 0.02 g of each microgel was dispersed in water, 1 M NaOH was added to increase the pH above 11 and then the titration was performed by repeatedly adding 0.05 mL of 0.1 M HCl and measuring the pH after each addition.

Dye encapsulation and release studies

Two types of dye were encapsulated in the microgels *via* two different methodologies, in order to determine the capability of the microgels to encapsulate and release both hydrophilic and hydrophobic moieties. 0.5 wt% hydrophobic Sudan I was mixed into the DP when the droplets were being prepared in the microfluidic channel, hence the dye was already present inside the microgel following the polymerisation step. 0.5 wt% hydrophilic trypan blue was loaded into already polymerised AA–EGDMA microgels in a pH 14 solution by diffusion with sonication. The chemical structures of both dyes are shown in Fig. 3. The model drug release was monitored using the colour camera of the microscope, in particular how the colour intensity of the microgels changed during release. Images were taken at regular time intervals (every 1 min or 5 min depending on how fast the release was). ImageJ freeware was used for the analysis of the colour intensity.

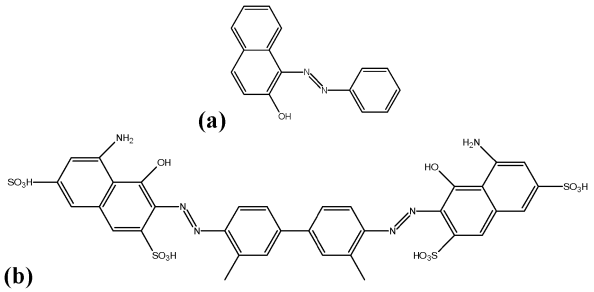

Fig. 3 Chemical structures of (a) hydrophobic Sudan I, and (b) hydrophilic trypan blue.

Results and discussion

Microgel fabrication and pH responsiveness studies

Three types of amphiphilic AA–EGDMA microgels of different crosslinking densities and hydrophobic contents were successfully fabricated *via* a microfluidic approach. Specifically, droplets of THPA:EGDMA in chloroform were formed in an aqueous CP and collected on a slide or dish, then polymerised *via* UV-initiated free radical polymerisation. Following polymerisation, these THPA–EGDMA microgels were hydrolysed by suspending them in a pH 1 solution to produce AA–EGDMA microgels. The hydrolysis step caused the microgels to shrink due to protonation of the ionisable AA units. For example, when the 50 : 4 THPA–EGDMA microgel (Fig. 4a) was hydrolysed to AA–EGDMA (Fig. 4b) the microgel shrank from 131 μm to 98 μm. However, when the AA–EGDMA microgel was then suspended in a pH 14 solution it swelled to 203 μm.

The pH responsiveness of each type of AA–EGDMA microgel formed with each molar ratio of THPA : EGDMA (30 : 4, 50 : 4, and 70 : 4) was studied by measuring the size of the microgels at different pH values. As can be observed in Fig. 5, the size of the microgels increased with the increase in the pH of the solution. This was expected and was due to the ionisable and hydrophilic AA units of the microgels, as it was confirmed by the microgels pK_as that were determined with potentiometric titration. Specifically the pK_as were found to be 6.1, 6.2 and 6.2 for the three microgels with different AA : EGDMA ratios, 30 : 4, 50 : 4, and 70 : 4, respectively, similar to that of pAA based stars.[38] Hence the AA units are negatively charged at high pH due to deprotonation,

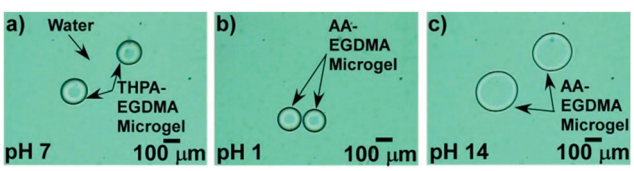

Fig. 4 THPA–EGDMA (50 : 4 molar ratio) microgels (a) before hydrolysis at pH 7, (b) after hydrolysis (forming AA–EGDMA microgels) at pH 1, and (c) at pH 14.

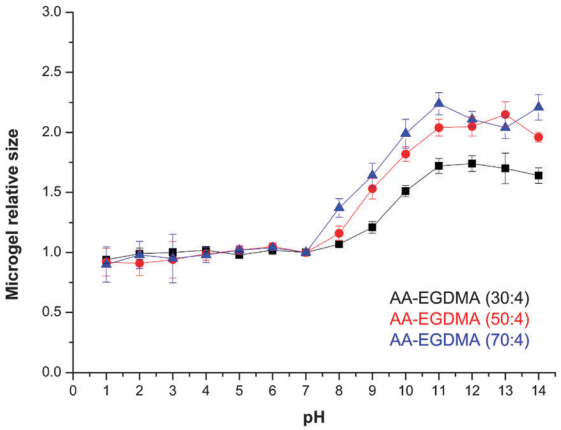

Fig. 5 Swelling ratios of the AA–EGDMA microgels in different pH environments. Microgels formed from THPA : EGDMA molar ratios of 30 : 4, 50 : 4 and 70 : 4 are represented as squares, circles and triangles.

while they are not ionised at low pH because they are protonated. Thus, at high pH values the polymer chains expand and extend due to the repulsive forces caused by the negatively charged AA units. When the polymer chains expand the entire microgel structure expands/swells. Returning the microgels to a lower pH environment causes them to shrink again as a result of deprotonation. This reversible pH responsive behaviour was expected, and has been observed previously for (meth)acrylic acid containing microgels[4,5,39] and macrogels,[29,30,34] and this property makes the AA–EGDMA microgels promising for encapsulation and release of various moieties. Note that the size of the microgels at very high pH reduces because of increased ionic strength due to the higher NaOH concentration, as it has been observed in macrogels.[29,30]

When comparing the three types of microgels in Fig. 5, it was apparent that by increasing the crosslinking density and the hydrophobic content, the size of the microgels decreased, as anticipated since the crosslinks avert the microgel from expanding. This expected trend, that has been observed before in microgels[4,39] as well as macrogels,[40] was only apparent in high pH solutions in which the AA units were ionised. At lower pH values, the polymer chains of the microgels were not extended, *i.e.* the microgels were in a collapsed (shrunken) state, so the crosslinks no longer interfered with the polymer chain movement and expansion.

Dye encapsulation and release studies

A hydrophobic and a hydrophilic dye were encapsulated and released in order to prove that the amphiphilic nature of the AA–EGDMA microgels could be utilised for the capture and delivery of moieties of varying hydrophobicity/hydrophilicity, similarly to amphiphilic macrogels.[41,42] The release of the model drugs was triggered by making the pH of the environment basic such that the AA units became ionised and the microgels swelled, allowing the dyes to diffuse out.

Hydrophobic dye – Sudan I. Sudan I (see the chemical structure in Fig. 3a) was encapsulated into the microgel by adding it to the oil based DP for droplet formation, meaning

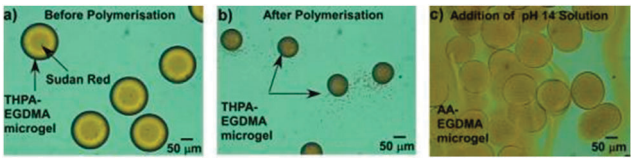

Fig. 6 (a) 50 : 4 THPA–EGDMA microgels with hydrophobic Sudan I dye encapsulated during droplet generation. (b) 50 : 4 THPA–EGDMA droplets after polymerisation (pH = 7). (c) Sudan I dye release from 50 : 4 AA–EGDMA microgels in pH 14 solution.

that the dye was already present in the microgels when they were then polymerised. THPA–EGDMA (50 : 4 molar ratio) droplets containing the Sudan I are shown in Fig. 6a, while Fig. 6b shows the microgels following polymerisation. The THPA–EGDMA microgels were hydrolysed in pH 1 solution to form AA–EGDMA microgels. When the 50 : 4 AA–EGDMA microgels were dispersed in pH 14 solution, as shown in Fig. 6c, the dye, which appears orange in the pictures although the colour is actually red, can clearly be seen diffusing out of the microgel. As described in the previous section, when suspended in basic solution the microgel swells due to extension of the polymer chains *via* repulsive ionic interactions, thereby enlarging the pores in the microgels and allowing the dye to be released. Furthermore, the AA units become more hydrophilic when they are transferred from acidic to basic pH, and so they are no longer able to retain the hydrophobic dye.

The release of the hydrophobic model drug was further evaluated by studying the release of Sudan I over time for all three types of microgels. The results are shown in Fig. 7. As can be clearly observed, the crosslinking density and hydrophobic content of the microgel strongly affects the release of Sudan I. Specifically, by increasing the crosslinking density (EGDMA content) the rate, as well as the amount, of hydrophobic dye released is reduced. This was expected, since by increasing the crosslinking density the mesh/pore size of the microgels decreases[43,44] so it is more difficult for the dye to diffuse out. In addition, by increasing the EGDMA (crosslinker) content,

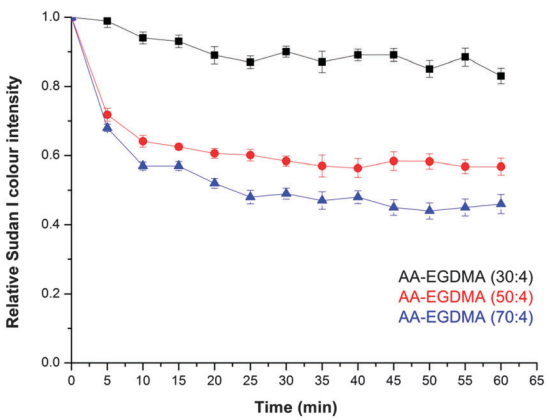

Fig. 7 Hydrophobic drug release at basic pH (pH > 11). The plots show the relative Sudan I colour intensity *versus* time for all three types of AA–EGDMA microgels with different crosslinking densities. Squares, circles and triangles represent the 30 : 4, 50 : 4 and 70 : 4 AA : EGDMA molar ratios, respectively.

which is itself hydrophobic, the hydrophobic dye will be retained in the microgel structure to a greater extent due to van der Waal's interactions.

Hydrophilic dye – trypan blue. A hydrophilic dye, trypan blue (see the chemical structure in Fig. 3b), was also investigated. In this case, 0.5 wt% trypan blue was loaded into the AA–EGDMA microgels after they had already been polymerised and hydrolysed. This was achieved by suspending the AA–EGDMA microgels into pH 14 solution of trypan blue in order to make them swell, allowing gradual diffusion of the trypan blue into the microgels which was assisted by sonication. Fig. 8a shows this process for 50 : 4 molar ratio AA–EGDMA microgels. As the pH was then reduced to pH 1, the microgels shrank and the trypan blue was encapsulated and concentrated within the gel particle. After purification, the pH was increased again to pH 7 (Fig. 8b), where it was found that the hydrophilic trypan blue was gradually released into the surrounding water. It can be seen that the blue colour intensity inside the microgel faded within an hour (Fig. 8c), indicating release of the dye from the microgel.

The release of the hydrophilic dye was further investigated over time for all three types of AA–EGDMA microgels in a basic pH solution (~pH 8.5). The results are shown in Fig. 9, and it is apparent that the release of trypan blue was influenced by the crosslinking ratio in a similar manner to the release of the hydrophobic dye (Sudan I). In particular, by increasing the crosslinking density and thus reducing the mesh size, the release of the hydrophilic dye was slower. This was expected since the higher mesh/pore size allows the dye to diffuse more easily and faster through the microgel structure.

Compared to the hydrophobic Sudan I release, the release of trypan blue from the AA–EGDMA microgels was much faster for all three types of microgel. For example, it took more than 25 min for the release of the hydrophobic Sudan I from the 70 : 4 AA–EGDMA microgel at basic pH to reach a plateau, while the plateau was reached in only 5 min when the hydrophilic trypan blue dye was being released. This can be attributed to the difficulty of the hydrophobic dye to diffuse into an aqueous environment compared to the hydrophilic dye as well as the fact that the hydrophobic domains of the microgels retain the hydrophobic dye for longer through van der Waals interactions.

In summary, the AA–EGDMA microgels were able to encapsulate and deliver both hydrophobic and hydrophilic dyes, and it has been demonstrated that the rate of release can be varied by adjusting the crosslinking density and hydrophobic content of the microgel.

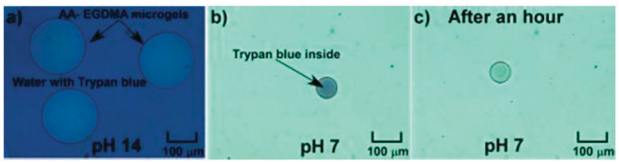

Fig. 8 Hydrophilic trypan blue encapsulation in, and release from, 50 : 4 AA–EGDMA microgels. (a) Encapsulation of the dye in pH 14 solution *via* diffusion. (b) Microgels containing the trypan blue immediately after their introduction into a pH 7 environment, and (c) after an hour of dye release at pH 7.

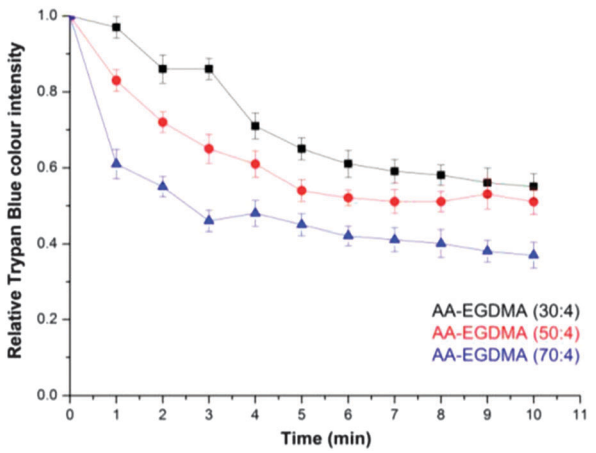

Fig. 9 Hydrophilic dye release in basic pH solution (~pH 8.5). The plots show the relative trypan blue colour intensity *versus* time for all three types of AA–EGDMA microgels with different crosslinking densities. Squares, circles and triangles represent the 30 : 4, 50 : 4 and 70 : 4 AA : EGDMA molar ratios, respectively.

Conclusions

Amphiphilic microgels based on hydrophilic, ionisable AA and the hydrophobic crosslinker EGDMA were successfully synthesised from droplets formed in a microfluidic platform. The use of microfluidics enabled the systematic variation of the crosslinking density and the synthesis of microgels with narrower size distributions than those prepared by conventional methods. The microgels were pH responsive. By increasing the pH of the solution, the size of the microgels increased due to ionisation of the AA units and the resultant electrostatic repulsion between them inside the gel particles. The swelling of the microgels increased by decreasing the crosslinking density. Finally, the encapsulation and release of a hydrophobic and a hydrophilic dye, which were used as model drugs, was investigated. The hydrophobic dye was incorporated during the droplet generation stage, while the hydrophilic dye was absorbed by microgels that had already been polymerised. The subsequent delivery was investigated and it was proven that the pH can be used to trigger the release. Thus, the amphiphilic nature of the AA–EGDMA microgels allowed the encapsulation and delivery of both types of model drugs, and as expected the hydrophilic dye was released faster than the hydrophobic dye since it was able to diffuse faster through the microgel and into the aqueous surroundings.

Acknowledgements

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