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## One-pot synthesis of responsive sulfobetaine nanoparticles by RAFT polymerisation: the effect of branching on the UCST cloud point

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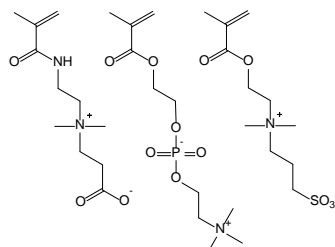
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We describe the one-pot synthesis of temperature-responsive branched polymer nanoparticles. Reversible addition fragmentation chain transfer (RAFT) polymerisation has been utilised to synthesise ultra-high molecular weight sulfobetaine polymers (up to ca. 500 kDa) with good control over molecular weight ( $M_n$ ) and dispersity ( $M_w/M_n$ ). The UCST cloud points of these linear polymers were found to increase with both  $M_n$  and concentration, and represent one of the few recent descriptions of polymers exhibiting UCST behaviour in aqueous solution. The incorporation of difunctional monomers results in branched polymers which display vastly reduced transition temperatures compared to their linear counterparts. Furthermore, the incorporation of a permanently hydrophilic monomer results in the formation of stable core-shell particles which no longer exhibit a cloud point in water, even at very high concentrations (ca. 50 mg/mL). The branched polymers are shown to form discrete well-defined nanoparticles in aqueous solution, and these have been characterised by DLS, SLS, TEM and DOSY. Their reversible swelling behaviour in response to temperature is also demonstrated.

### Introduction

Stimuli-responsive polymers, which exhibit conformational or phase transitions in response to external stimuli such as temperature, pH, light, electrolytes and reductant/oxidants,<sup>1, 2</sup> have been the focus of much attention in recent literature. The most promising and widely studied stimulus is temperature, the most common example of which is poly(*N*-isopropylacrylamide (PNIPAM), which demonstrates a lower critical solution temperature (LCST) in aqueous solutions.<sup>3</sup> Reviews specifically on temperature-responsive water-soluble polymers deal almost exclusively with polymers that show an LCST in water. In contrast, polymers exhibiting upper critical solution temperature (UCST) phase transitions in aqueous solution are few and far between, as demonstrated by the recent review from Agarwal *et al.*<sup>4</sup> The same group has reported several examples of the previously unnoticed UCST behaviour of well-known polymer systems such as poly(acrylamide-*co*-acrylonitrile)<sup>5</sup> and poly(*N*-acryloyl glycinamide);<sup>6</sup> however few other examples exist. Polybetaines are electrically neutral polymers that contain both cationic and anionic groups within a single monomer unit. There are several classes of betaines, differentiated by the nature of the cationic and anionic groups, including carboxybetaines,<sup>7</sup> phosphobetaines<sup>8</sup> and sulfobetaines<sup>9</sup> (Figure 1).



<sup>45</sup> Fig. 1 Examples of betaine monomers carboxybetaine (left),<sup>7</sup> phosphobetaine (middle)<sup>8</sup> and sulfobetaine *N,N'*-dimethyl(methacryloyl)ethylammonium propanesulfonate (DMAPS, right).<sup>9</sup>

<sup>50</sup> Polysulfobetaines are interesting for various reasons, including their antipolyelectrolyte behaviour in aqueous salt solution and bio- and haemo-compatible properties,<sup>10, 11</sup> making them suitable for many applications including dispersing agents, pharmaceuticals and viscosifying fluids. Several polysulfobetaines exhibit UCST behaviour in water including the methacrylate shown in Figure 1, the acrylamide<sup>12</sup> and the methacrylamide.<sup>13</sup> The methacrylate polymers were first synthesised directly by free radical polymerisation and their cloud point behaviour was studied.<sup>14, 15</sup> An alternative approach to direct polymerisation has been utilised by Armes and coworkers who have studied the selective betainisation of tertiary amine-containing polymers synthesised

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by group transfer polymerisation, resulting in the first example of polybetaines with narrow dispersity and controlled architecture.<sup>16</sup> The betainisation reaction has also been utilised for the incorporation of sulfobetaine monomers into doubly-responsive or so called “schizophrenic” AB diblock co-polymers. These polymers undergo transitions from A-core to unimers to B-core micelles on response to changing temperature.<sup>17,18</sup>

More recently, the direct controlled radical polymerisation of betaine monomers in aqueous solution has been reported,<sup>19</sup> including the polymerisation of the methacrylic sulfobetaine monomer *N,N'*-dimethyl(methacryloylethyl)ammonium propanesulfonate (DMAPS), utilising a water soluble chain transfer agent (CTA) and radical initiator. The polymerisations were carried out in aqueous salt solution (0.5 M NaBr), and aliquots analysed directly using size exclusion chromatography (SEC) (eluting with 80% 0.5 M NaBr solution/20% acetonitrile) and a refractive index (RI) detector. The polymerisations were carried out at a relatively low monomer concentration, seemingly to facilitate direct SEC characterisation of samples taken during the polymerisation.<sup>20</sup> Further examples of the RAFT-controlled polymerisation of betaine monomers including carboxybetaines,<sup>21</sup> have since been reported.

Controlled radical polymerisation techniques such as atom transfer radical polymerisation (ATRP) and RAFT offer good control over polymerisations, resulting in well-defined polymers that contain “living” end groups, which are available for chain extension or further chemistry.<sup>22</sup> A variety of methods are available to produce polymeric nanostructures with a huge variation in size, shape and chemical functionality. Self-assembly of amphiphilic diblock copolymers is one of the simplest and most convenient methods for the formation of nanostructured polymeric materials. Since the earlier report by Armes *et al.*<sup>17</sup> McCormick and coworkers have reported the direct synthesis of a near-monodisperse poly(dimethylacrylamide-*co*-sulfobetaine) block copolymer entirely in aqueous media. These polymers formed aggregates in pure water that could be dissociated by the addition of salt. Molecular weights of up to 20 kDa were achieved for the polybetaine block.<sup>23</sup>

The introduction of branching adds a further layer of complexity to polymeric nanostructures. Branched polymers, ranging from structurally precise dendrimers through to more disperse hyperbranched polymers, have been widely studied. The step-wise synthesis of dendrimers is notoriously difficult and their purification laborious, restricting their use and commercial interest.<sup>24</sup> Branched and hyperbranched polymers offer some of the advantages of dendrimers (increased solubility due to a higher proportion of end groups, and lower solution viscosity compared to their linear counterparts) whilst having significantly simpler syntheses. Sherrington and coworkers have been key in the development of versatile, scalable routes to branched and hyperbranched polymers, utilising conventional free radical polymerisation in the presence of mercaptans as chain transfer agents to prevent gelation,<sup>25</sup> a technique that has been modified for use in more complex systems such as emulsion polymerisation.<sup>26</sup> Using this approach, Weaver *et al.* recently reported an elegant route to pH-responsive branched copolymer nanoparticles in a one pot, single step synthesis. This involved the use of a pH-responsive core-forming monomer, a hydrophilic

macromonomer as the shell-forming block along with a crosslinker and chain transfer agent to prevent gelation, resulting in pH-responsive core-shell particles analogous to crosslinked micelles. The apparent  $pK_a$  of the tertiary amine residues could be tuned by varying the degree of branching.<sup>27</sup> Cross-linked particles offer a greater degree of stability in solution over micellar aggregates, as they are covalently linked and therefore do not disassemble below a critical aggregation concentration (CAC) as micellar systems would. They are therefore stable at ultra-low concentrations in solution, making them better suited to applications in which solution concentration is very difficult to control, such as drug delivery.

The use of RAFT polymerisation techniques in the synthesis of soluble, branched polymers has been reported by Perrier *et al.*, who have systematically studied the effects of changing the ratios of CTA, brancher (difunctional monomer) and radical initiator on the resulting polymer structure.<sup>28</sup> A comparable study on the development of branching in an ATRP system by Billingham, Armes and coworkers found that soluble, highly branched polymers were formed when there was less than one brancher per chain. In this case the monomer conversion could be pushed to near 100% without risk of gelation.<sup>29</sup>

We report here the synthesis and aqueous solution behaviour of temperature-responsive linear and branched DMAPS containing polymers and copolymers with polyethylene glycol methacrylate (PEGMA). The cloud point temperature of the linear PDMAPS homopolymer is shown to be dependent upon both molecular weight and concentration in solution (between 1–5 mg/mL), as expected. We show that at similar concentrations, branched DMAPS polymers exhibit no UCST cloud points; instead they form discrete particles that expand and contract in response to temperature changes. Only at much higher concentrations (~20 mg/mL) are any cloud points observed. Copolymerisation of the sulfobetaine monomer with the permanently hydrophilic macromonomer PEGMA affords the formation of core-shell particles that show a similar swelling/deswelling response to temperature. However, these PEGMA-coated branched particles exhibit no cloud points even at concentrations up to 50 mg/mL, indicating the PEG shell is shielding the responsive core from its surrounding environment. Particle size is measured by light scattering (DLS), diffusion ordered spectroscopy (DOSY) and transmission electron microscopy (TEM).

## Experimental

### Materials

Monomers and chain transfer agent (CTA) were purchased from Sigma-Aldrich and used as received. Radical initiators were purchased from Sigma Aldrich and purified by recrystallisation from methanol. Nanopure water (18 mΩ/cm) was obtained from a Millipore Simplicity system.

### Preparation of linear polymers

DMAPS monomer (18–1850 equiv.), CTA (1 equiv.), 4,4'-azobis(4-cyanovaleric acid) (ACVA) (0.2 equiv.) and PEGMA comonomer if required (5 equiv.) were dissolved in 0.5 M aqueous NaCl and the solution adjusted to pH 7 by the addition of dilute aqueous NaOH. After transferring to an ampoule fitted

with a stirrer bar, the solution was degassed by purging with nitrogen for 30 minutes (purging times were increased for larger volume polymerisations). The polymerisations were conducted at 65 °C and the conversion was monitored using <sup>1</sup>H NMR, by comparing the integration of the vinyl peaks (5.7 and 6.1 ppm) to the integration of the mid chain CH<sub>2</sub> group (2.2 ppm). The polymers were purified by extensive dialysis against deionised water (1–14 kDa MWCO dialysis tubing) with at least 6 water changes, and recovered as solids following freeze-drying.

#### 10 Preparation of branched polymers

DMAPS monomer (17–1760 equiv.), CTA (1 equiv.), 4,4'-azobis(4-cyanovaleric acid) (ACVA) (0.2 equiv.) and PEGMA comonomer (5 % equiv. w.r.t. DMAPS, 1-90 equiv.) were dissolved in 0.5 M aqueous NaCl and the solution adjusted to pH 7 by the addition of dilute aqueous NaOH. This solution was transferred to an ampoule fitted with a stirrer bar and EGDMA (0.9 equiv.) was added. The solution was degassed by purging with nitrogen for 30 minutes (purging times were increased for larger volume polymerisations). The polymerisations were conducted at 65 °C and the conversion was monitored using <sup>1</sup>H NMR, by comparing the integration of the vinyl peaks (5.7 and 6.1 ppm) to the integration of the mid-chain CH<sub>2</sub> group (2.2 ppm). The polymerisations were left to achieve high conversions (> 97 %) to ensure that branching occurred. The polymers were purified by extensive dialysis against deionised water (1–14 kDa MWCO dialysis tubing) with at least 6 water changes, and recovered as solids following freeze-drying.

#### Characterisation

The apparent molecular weight (compared to PEG standards) and dispersity of the polymers were determined by SEC, whilst more accurate molecular weight data was obtained by static light scattering (SLS) for selected polymers. SEC analysis was carried out on an Agilent 390-MDS system with 1 PL aquagel OH guard, 1 PL aquagel OH mixed M column and 1 PL aquagel OH 60 column. All SEC was carried out in phosphate buffer at pH 8.2 at a flow rate of 1 mL/min. Solutions of graphene oxide were synthesised as reported previously.<sup>30</sup> Aqueous solutions of graphene oxide (GO) (0.1 mg/mL) were sonicated for 5 min prior to use. Lacey carbon grids (400 Mesh, Cu) (Agar Scientific) were cleaned using air plasma from a glow-discharge system (2 min, 20 mA). The TEM grids were placed on a filter paper and one drop (*ca.* 0.08 mL) of the sonicated GO solution was deposited onto each grid from a height of 1 cm, allowing the filter paper to absorb the excess solution, and the grids were left to air-dry in a desiccator cabinet for 60 min. 4 μL of sample (0.1 mg/mL) was pipetted onto a GO grid and left for 2 minutes before blotting away. TEM images were obtained as described previously.<sup>31</sup> Bright field images were captured with a transmission electron microscope (JEOL TEM-2011), operating at 200 kV.

#### 50 UCST cloud point measurements

Turbidimetry by UV/vis spectroscopy was utilised to measure the UCST cloud points of the polymers in aqueous solution at various concentrations, using a Perkin-Elmer UV/vis Spectrometer (Lambda 35) equipped with a Peltier temperature controller. A wavelength of 500 nm with a heating/cooling rate of 1 °C/min was used. The cloud point temperatures were determined to be

the point at which the solution transmittance was 50 %. The heating and cooling cycles were carried out in triplicate.

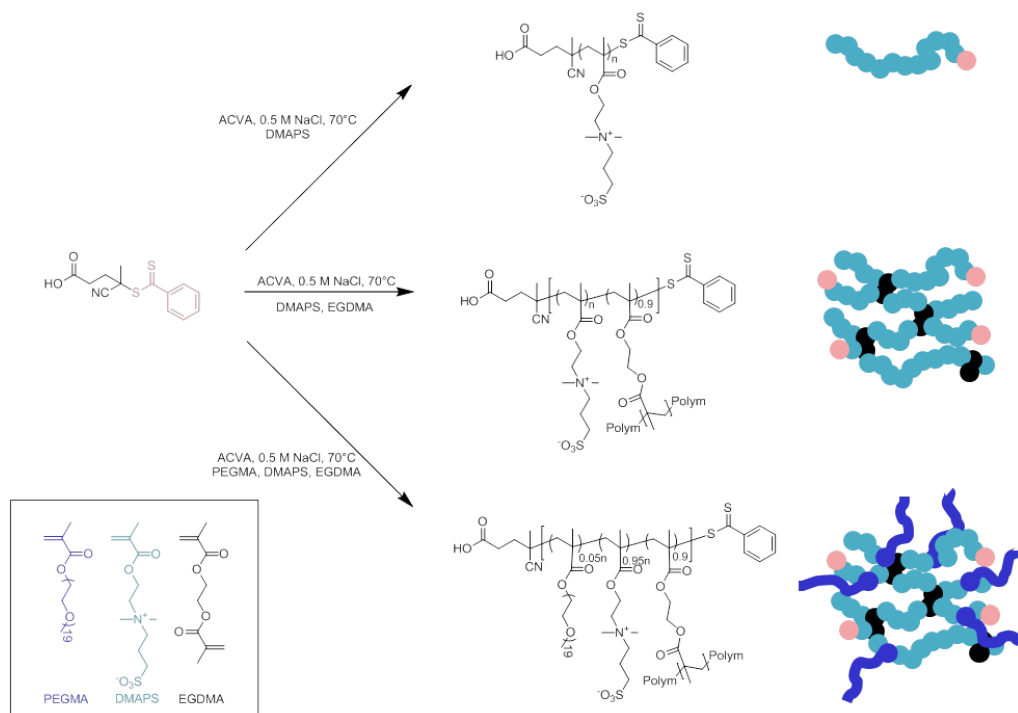
## Results and Discussion

60 The temperature-responsive behaviour of linear homopolymers of DMAPS is relatively well understood and the cloud point of PDMAPS has been shown to increase with both polymer molecular weight and concentration and be reduced by the addition of low molecular weight electrolytes such as NaCl.<sup>13</sup> However, the only systematic study of the UCST behaviour of PDMAPS has been on polymers synthesised by standard free radical polymerisation which typically have relatively broad dispersities, and as the cloud point is strongly dependent upon molecular weight, this is perhaps not an accurate representation of the cloud points.<sup>14</sup> Recently, PDMAPS with narrow dispersities and a range of molecular weights have been synthesised by direct and indirect controlled polymerisation methods; however, no cloud point data has been given for any of the polymers synthesised in these reports to allow comparison to the original data from 1980. This in part could be due to the sensitivity of PDMAPS to low molecular weight impurities, the cloud points significant molecular weight dependence, or indeed as discussed both here and in a recent review by Gibson and O'Reilly,<sup>32</sup> the challenges in understanding the complex behaviour of temperature-responsive polymers when chains are constrained by the particles' architecture. Conflicting reports of increases and decreases in size of polymer particles attributed either to swelling and contraction or macroscopic aggregation of responsive polymer particles in response to stimuli have been described, and it is difficult to ascertain which effect is most prominent. Descriptions of the temperature and salt response of both surface initiated PDMAPS<sup>33, 34</sup> and colloidal particles consisting of a polystyrene core with grafted PDMAPS chains on the surface<sup>35</sup> both demonstrate that reversible swelling of PDMAPS is possible. A recent report by the Haraguchi group, in which gels of sulfobetaine acrylamide polymers in water were formed, discusses the effect of crosslinking on their phase transition temperature. They found that as the crosslinking density increased, the phase transition temperature decreased. Their work differs from the work discussed here as they studied hydrogels rather than discreet branched particles, however the results support the results reported in our work.<sup>36</sup>

Inspired by recent work by Weaver *et. al.* in which pH-responsive polymer particles were synthesised utilising simple thiols as chain transfer agents,<sup>27</sup> we chose to synthesise branched PDMAPS and copolymers of DMAPS and PEGMA by RAFT polymerisation in order to form responsive nanoparticles in a facile one-pot procedure. RAFT was chosen as it has an excellent tolerance to functional groups, offers the ability to form well defined polymers directly in aqueous solution and results in polymers with well-defined structures. Our synthetic approach involved copolymerisation of DMAPS with PEGMA and difunctional monomers EGDMA and PEGDMA, controlled by the water soluble CTA 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPTA). The combination of monomers was chosen to allow us to determine the effect of both architecture and hydrophilicity on the solution properties of DMAPS polymers.

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**Scheme 1** Schematic representation of the synthesis of the linear and branched copolymers.

### Polymer synthesis

A typical synthetic procedure is depicted in Scheme 1 and involved the homo- and co-polymerisation of the functional monomers by RAFT in 0.5 M NaCl solution, adjusted to pH 7 by the addition of dilute NaOH. This resulted in deprotonation of the carboxylic acid groups of the CTA and initiator ensuring that they were soluble in the reaction medium. The experimental procedure was adapted from the report by McCormick *et. al.* in which DMAPS polymerisations were carried out in 0.5 M NaBr solution. Linear and branched polymers with a range of molecular weights from 5–500 kDa were targeted, both homopolymers of DMAPS and copolymers of DMAPS:PEGMA with a fixed % molar ratio of 95:5. In the branched polymer synthesis the ratio of CTA:branching agent was kept at 1:0.9 to ensure that soluble branched polymers were formed even when the monomer conversion was high.<sup>29</sup> Both linear and branched polymers are described by the target molecular weight of their linear component, for example polymer **6c** was synthesised with a feed ratio of DMAPS:PEGMA:CPTA:EGDMA of 1760:90:1:0.9; the linear portion has a target  $M_n$  of 500 kDa and is therefore described as 500 kDa branched PEG/DMAPS.

**Table 1** Characterisation of linear homopolymers of DMAPS and their cloud points in water.

#	Description	Target $M_n$ (kDa) <sup>a</sup>	$M_n^{SEC}$ (kDa) <sup>b</sup> $D$	$M_w^{SLS}$ (kDa) <sup>c</sup>	Cloud point (°C) <sup>d</sup>
<b>1a</b>	5 kDa linear PDMAPS	5	6 1.35	-	<sup>e</sup>
<b>2a</b>	20 kDa linear PDMAPS	20	10 1.62	29	<sup>e</sup>
<b>3a</b>	50 kDa linear PDMAPS	50	19.5 1.30	-	11
<b>4a</b>	100 kDa linear PDMAPS	100	35 1.42	-	23
<b>5a</b>	200 kDa linear PDMAPS	200	71 1.32	258	26
<b>6a</b>	500 kDa linear PDMAPS	500	129 1.47	448	43

<sup>a</sup>Target  $M_n$  based on ratio of CTA:monomer. <sup>b</sup> $M_n$  (SEC) based on PEG standards. <sup>c</sup>SLS measurements carried out between 0.125–2 mg/mL in 0.5 M NaCl. <sup>d</sup>Cloud point measurements carried out at 1 mg/mL [polymer] in water in triplicate. <sup>e</sup>No cloud points observed.

Initially linear homopolymers were synthesised (Table 1, **1a–6a**) by RAFT.  $M_n$  was controlled by altering the ratio of

CTA:DMAPS, with  $M_n$  from 5–500 kDa targeted. Molecular weight characterisation was performed by SEC with an eluent of phosphate buffer at pH 8.2 and is shown in Table 1. Although the apparent  $M_n$  values are grossly underestimated as they are compared to polyethylene glycol (PEG) standards, the control is evident from the relatively low dispersities and the trend of increasing molecular weight with decreasing equivalents of CPTA. The clear distinction between polymers of increasing molecular weight is demonstrated in Figure 2, where the molecular weight distributions of polymers **1a–6a** are overlaid. More accurate  $M_w$  data were obtained by static light scattering (SLS) of selected polymers in a good solvent, 0.5 M NaCl, at a total of 13 angles between 30 and 150 ° (see SI).

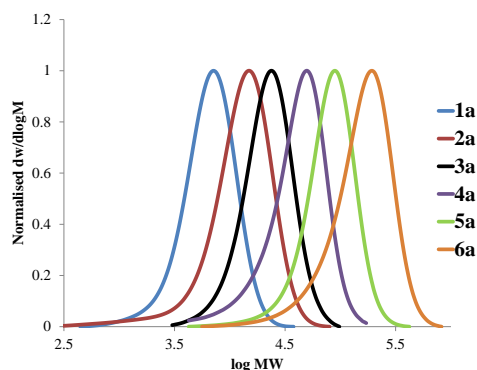


Fig. 2 SEC traces of linear homopolymers of DMAPS **1a–6a**.

A representative  $^1\text{H}$  NMR of the linear homopolymers is displayed in Figure 3 (see Figures S17 and S18 for branched polymer  $^1\text{H}$  NMR spectra), showing the RAFT end groups at 7.6–8.0 ppm, which are easily observed in the lower molecular weight polymers **1** and **2**. The RAFT end groups were maintained throughout the polymerisations as confirmed by the good correlation between the RI and UV (at 309 nm, the maximum absorbance value of the thiocarbonylthio group) traces in the SEC traces of polymer **3a** (see SI). Further evidence was given by a successful chain extension experiment, achieved by the use of 50 kDa DMAPS (polymer **3a**) as a macroCTA for the synthesis of 100 kDa DMAPS (polymer **4a**). A clear shift of molecular weight is demonstrated by SEC, as the overlaid molecular weight distributions show in Figure 2. The  $D$  values obtained for these polymerisations are either comparable to or an improvement upon those described for other polybetaines synthesised using RAFT.<sup>2337</sup>

A series of linear polymers with a 5 mol% incorporation of PEGMA were also synthesised for comparison.

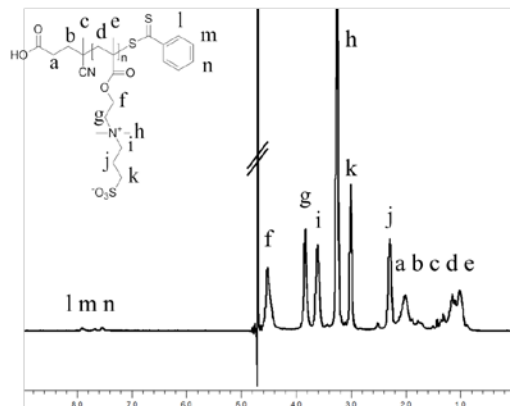


Fig. 3 Representative  $^1\text{H}$  NMR of PDMAPS showing RAFT end groups at 7.6–8.0 ppm (**1a** in 0.5 M NaCl in  $\text{D}_2\text{O}$ ).

Table 2 Branched polymers of DMAPS and their particle size.

#	Description	$M_n^{\text{SEC}}$ (kDa) <sup>b</sup>	$M_w^{\text{SLS}}$ (kDa) <sup>c</sup>	$D_h^{\text{DLS}}$ (nm) <sup>d</sup>	$D_w^{\text{TEM}}$ (nm) <sup>e</sup>
<b>1b</b>	5 kDa branched PDMAPS	8	-	-	-
<b>1c</b>	5 kDa branched PEG/PDMAPS	1.61	-	-	-
<b>2b</b>	20 kDa branched PDMAPS	8	135	-	-
<b>2c</b>	20 kDa branched PEG/PDMAPS	17	-	-	-
<b>3b</b>	50 kDa branched PDMAPS	2.12	-	-	-
<b>3c</b>	50 kDa branched PEG/PDMAPS	27	-	-	-
<b>4b</b>	100 kDa branched PDMAPS	4.91	267	10	6
<b>4c</b>	100 kDa branched PEG/PDMAPS	2.6	-	0.217	11
<b>5b</b>	200 kDa branched PDMAPS	69	750	15	9
<b>5c</b>	200 kDa branched PEG/PDMAPS	2.51	-	0.474	9
<b>6b</b>	500 kDa branched PDMAPS	2.30	106	0.322	14
<b>6c</b>	500 kDa branched PEG/PDMAPS	107	797	0.362	20
		142	20	0.246	14
		139	-	<sup>f</sup>	27 <sup>g</sup>
		3.09	-	-	-

<sup>a</sup>Target  $M_n$  of linear chains based on  $[\text{CTA}]:[\text{monomer}]$ ,

<sup>45</sup>  $[\text{EGDMA}]:[\text{CTA}]$  kept at 0.9:1. <sup>b</sup> $M_n^{\text{SEC}}$  based on PEG standards. <sup>c</sup>SLS measurements carried out between 0.125–2 mg/mL in 0.5 M NaCl.

<sup>d</sup> $D_h^{\text{DLS}}$  (number), 0.25 mg/mL in water, 25 °C, samples filtered through a 0.45  $\mu\text{m}$  nylon filter and allowed to equilibrate for 1 hour, average of 5 measurements taken. <sup>e</sup>Average size of 50 particles. <sup>f</sup>Sample too polydisperse for DLS analysis. <sup>g</sup>Significant aggregation observed (see SI10)

Branched equivalents of each of the linear polymers were then synthesised, by the addition of 0.9 equivalents of branching agent EGDMA wrt CTA into the polymerisations. The successful incorporation of brancher at high monomer conversions was

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observed by SEC (Figure 4), in which a high molecular weight shoulder can be observed when the polymerisations reach > 70 % conversion of monomer, and a broadening of the  $D$  (from ~1.4 in linear polymers to >2 in branched polymers) can be observed (Table 2). The significant broadening of  $D$  is taken as evidence of branching in numerous reports throughout the literature.<sup>27, 29, 38, 39</sup>

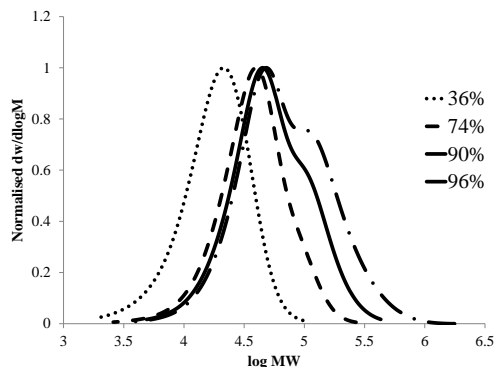


Fig. 4 SEC traces of samples at different percentage monomer conversion throughout the polymerisation of **3c** showing branching at high monomer conversion.

#### Solution properties.

The cloud points of the polymers in aqueous solution were measured by turbidimetry using a UV/vis spectrophotometer equipped with a Peltier temperature controller. A wavelength of 500 nm was used, and the cloud point was taken to be the point at which 50 % of light was transmitted through the solution. An average of three cycles was taken, and no hysteresis was observed (See SI).

Polybetaines can exhibit UCST behaviour in aqueous solution due to the Coulomb attractions between the opposite charges in the polymer.<sup>13</sup> Polymers dissolve in a solvent when  $\Delta G$  is negative, and exhibit UCST behaviour when both  $\Delta H$  and  $\Delta S$  are positive. At the phase transition temperature ( $T_p$ ) dissolution and phase separation are in equilibrium ( $\Delta G$  is zero and  $T_p = \Delta H/\Delta S$ ).  $T_p$  increases as  $\Delta H$  increases, and as the potential for Coulomb interactions between polymers increases with both molecular weight and concentration, so  $T_p$  increases.<sup>5</sup> This has previously been shown for PDMAPS synthesised by free radical polymerisation.<sup>14</sup> It was therefore expected that as the molecular weight of the polymers increased, the cloud point temperature would also increase. This trend is indeed observed for the linear homopolymers from 50–500 kDa (**3a–6a**, Table 1). As expected, polymers **1a** and **2a** exhibited no cloud points. This could be attributed to their low molecular weights – **1a** and **2a** are 5 and 20 kDa respectively and **3a**, a 50 kDa polymer has a cloud point of only 11 °C – or the presence of ionic end groups which has been reported to affect the cloud points of low molecular weight polymers.<sup>40</sup> The cloud point temperature of **4a** at increased concentrations of 2.5 and 10 mg/mL was measured, and found to be 29 and 40 °C respectively; this shows that, as expected, increasing the concentration of polymer increased the cloud point temperature.

Copolymerisation with acrylamide has been shown to increase

the UCST cloud point of polyacrylamide due to its increased hydrophobicity,<sup>3</sup> therefore conversely we expect the incorporation of hydrophilic groups into PDMAPS to decrease its cloud point relative to homopolymer analogues. However, the presence of 5 mol% PEGMA resulted in soluble polymers from 5–500 kDa exhibiting no measureable UCST cloud points.

The effect of incorporating the branching group EGDMA into the polymers was next studied. As expected from the results obtained for the linear copolymers of DMAPS/PEGMA, the branched copolymers also observed no UCST cloud points over the range of temperatures (4–90 °C) and concentrations (1–55 mg/mL) measured. More surprisingly however, the branched homopolymers of DMAPS, even the higher molecular weight samples (500 kDa), did not exhibit UCST cloud points at 1–5 mg/mL. At very high concentrations (20–50 mg/mL) some evidence of aggregation upon cooling was observed for the branched homopolymers, but not for the branched copolymers of DMAPS/PEGMA (see SI Table S2 for more details). This is attributed to the permanently hydrophilic PEG shell shielding the DMAPS core and preventing aggregation.

To the authors' knowledge, the effect of branching on the UCST cloud point of PDMAPS has not been previously reported, although in a related study by the Haraguchi group, it was reported that as the crosslinking density of hydrogels of an acrylamide betaine were formed was increased, the phase transition temperature decreased.<sup>36</sup> Here it is proposed that the homopolymer of PDMAPS forms a core-shell structure in response to decreased temperature, with the shorter chains of PDMAPS solubilising the branched core in solution. At elevated temperatures the core swells, but the branching groups constrain the polymer structure, resulting in a particle collapse upon response to decreased temperature rather than macroscopic precipitation (Figure 5).

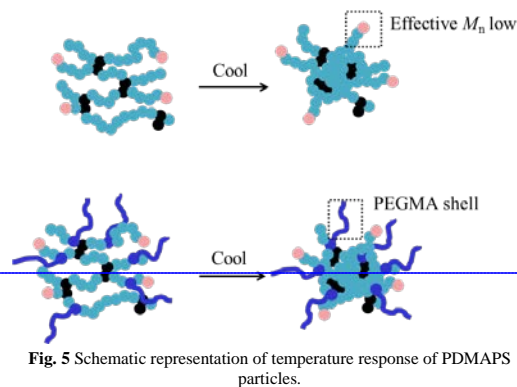


Fig. 5 Schematic representation of temperature response of PDMAPS particles.

The  $D_h$  of the branched polymer nanoparticles in aqueous solution was obtained by dynamic light scattering (DLS) (Table 2). Data for the lower molecular weight particles were not conclusive, however for the higher molecular weight species the size of the nanoparticles increases with molecular weight, as expected. The  $D_h$  of these unimolecular particles correlates with recent reports of polymer particles of similar molecular weight.<sup>41</sup> The size of nanoparticles was also confirmed by TEM analysis (Table 2 and SI) and comparison of the particle sizes of **4b** and

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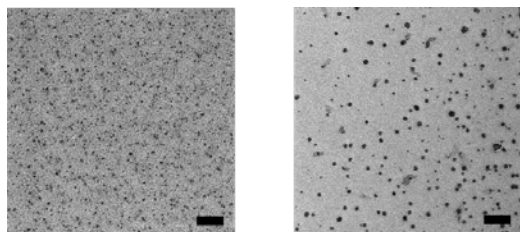
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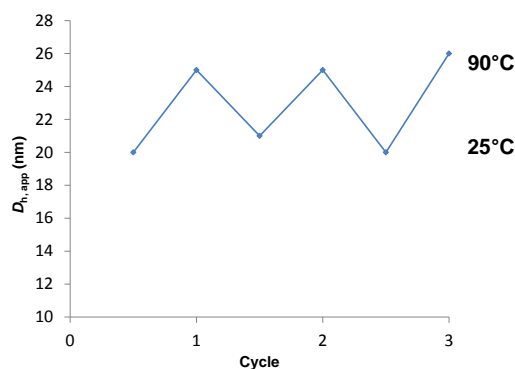


**6b** (Figure 6) clearly demonstrates the increase in size with increasing molecular weight.



**Fig. 6** Representative TEM images of **4b** (left) and **6b** (right). Scale bar 100 nm.

Further evidence of particle size in solution was obtained by diffusion ordered spectroscopy (DOSY) NMR.<sup>42</sup> This technique measures the diffusion of particles in solution and using the Stokes-Einstein equation, relates this diffusion to the particle diameter. Measurement of **6b** using this method gave a  $D_h$  of 20 nm (see SI), which correlates well with the size obtained by DLS.



**Fig. 7** Reversible swelling of **6b** over 3 temperature cycles from 25–90 °C (in H<sub>2</sub>O at 0.25 mg/mL by DLS).

DLS analysis at variable temperatures clearly shows the reversible swelling of the nanoparticles in response to temperature (Figure 7). Repeated heating and cooling cycles and dilution of the polymer solutions have no effect on particle size by DLS or TEM, confirming that nanoparticles are in fact single polymer particles and not the product of aggregation.

## Conclusions

In conclusion, the controlled polymerisation of the DMAPS monomer was achieved using RAFT polymerisation, resulting in ultra-high molecular weights PDMAPS. The cloud point was observed to increase with both  $M_n$  and concentration of polymer in solution, as expected. Copolymers of DMAPS with permanently hydrophilic PEGMA were no longer found to exhibit UCST cloud points due to their increased hydrophilicity. Branched polymers were also synthesised *via* RAFT, and interestingly branched PDMAPS homopolymers were found to exhibit a cloud point only at very high concentrations. The

incorporation of a permanently hydrophilic PEGMA into these branched systems prevented any aggregation upon cooling; therefore the branched copolymers exhibited no cloud points at any concentration. These branched polymers were found to exist as discrete nanoparticles in solution, and displayed reversible swelling behaviour in response to temperature. Their particle size was measured by DLS, TEM and DOSY NMR and was found to increase with increasing molecular weight. This is the first example of the synthesis of branched PDMAPS by controlled radical polymerisation techniques and offers a valuable insight into the effect of branching upon the aggregation behaviour of responsive polymers.

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## Notes and references

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- † Electronic Supplementary Information (ESI) available: [UV GPC data, UV-vis cloud point measurements, light scattering data (DLS and SLS), TEM images of branched particles and DOSY NMR data]. See DOI: 10.1039/b000000x/
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