

# Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter?

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Multicompartment thermoresponsive gels are novel materials with fascinating self-assembly and interesting applications. The aim of this study was to investigate for the first time the effect of the length of the alkyl side group of a hydrophobic monomer on the thermoresponsive and self-assembly behaviour of terpolymers. Specifically twelve well-defined terpolymers based on the hydrophilic monomers 2-(dimethylamino)ethyl methacrylate (DMAEMA) and poly(ethylene glycol) methyl ether methacrylate (PEGMA), and on the hydrophobic monomer ethyl-, *n*-butyl or *n*-hexyl methacrylate (EtMA, BuMA or HMA) of varying architectures (ABC, ACB, BAC and statistical) were synthesised using Group Transfer Polymerisation. The A, B and C blocks were based on PEGMA, the alkyl containing methacrylate monomer, and DMAEMA, respectively. The molecular weights (MWs) and compositions of the polymers were kept the same. The polymers and their precursors were characterised in terms of their MWs, MW distributions and compositions. Aqueous solutions of the polymers were studied by turbidimetry, hydrogen ion titration, light scattering and rheology to determine their cloud points,  $pK_{a,s}$ , hydrodynamic diameters and thermoresponsive behaviour and investigate the effect of the architecture and the hydrophobic alkyl side group of the terpolymers. It was found that the  $pK_{a,s}$  and the  $T_g$ s were mostly affected by the hydrophobicity of the side groups and not by the architecture, while the cloud points and the sol-gel transition of the polymers were affected by both the length of the alkyl side group and the polymer architecture. Interestingly the sharpest sol-gel transitions and stable multicompartment hydrogels were observed for the ABC triblock copolymers with the short alkyl-side groups even though the sol-gel transition occurred at higher temperatures.

## Introduction

“Smart” polymeric materials are materials that respond to an external stimulus, like ionic species, pH, temperature, electromagnetic radiation and sound with changes in volume, solubility, conformation and configuration.<sup>1-7</sup> The ability of these materials to respond to a stimulus makes them promising for a vast variety of applications from biomedical applications, such as genes, proteins, radionuclides, protein and drug delivery,<sup>1,8-13</sup> tissue engineering (tissue regeneration),<sup>1,14-24</sup> and wound dressings,<sup>25</sup> to industrial applications, such as surface modification,<sup>26</sup> colloid stabilisation,<sup>27</sup> water remediation,<sup>28</sup> and oil recovery.<sup>29</sup>

Our group focuses on thermoresponsive polymers and their possible application in tissue engineering. Tissue engineering involves the use of a scaffold/material with the appropriate 3-dimensional (3D) structure that will provide sufficient mechanical support and has the ability to transport both nutrients and growth factors to encapsulated cells.<sup>30,31</sup> The use of synthetic polymers as this matrix forming material has received a lot of

interest mainly due to their advantages, such as easy tailoring of their mechanical and chemical properties, compared to natural products.<sup>15,16</sup> Thermoresponsive polymers in particular are being studied as injectable gels for tissue engineering.<sup>1,14-16,20,32</sup>

This application involves the encapsulation of cells in a 3D structure in the body. Specifically, the thermoresponsive polymer is mixed at room temperature with the cells and then injected into the body. Upon injection the polymer presents a sol-gel transition and forms a physical gel, due to the temperature increase (to 37 °C) that is above the lower critical solution temperature (LCST) of the polymer. The cells are encapsulated within the 3D structure of the physical gel.

For this type of application there are several parameters to consider. These parameters should systematically be investigated in order to produce the ultimate injectable gel. The injectable gel should be biocompatible, readily available and relatively inexpensive. It should also provide the right mechanical support and respond fast to changes in temperature. These criteria are affected by the chemical structure as well as physical characteristics of the polymer, such as molecular weight (MW) and architecture.

Our studies focus on thermoresponsive triblock terpolymers. Thermoresponsive triblock terpolymers have gained a lot of

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interest because they have fascinating self-assembly behaviour and form micelles with complex structures like core-shell micelles.<sup>33–50</sup> Furthermore at the right concentration and temperature they form multicompartment injectable gels.<sup>36,39,43–50</sup> The gel formation has been shown to be affected by many parameters like the MW, composition, block length, chemistry of the polymer as well as the polymer architecture. For example in some studies ABC triblock copolymers were compared to ABA triblock copolymers with contradictory results. Armes *et al.* reported that an ABC triblock, where A was hydrophilic and thermoresponsive, B was hydrophilic and C was hydrophobic and thermoresponsive, formed gels but at higher concentrations compared to an ABA triblock based on the same A and B blocks.<sup>39</sup> On the other hand Lodge *et al.* reported the opposite when comparing an ABA triblock with an ABC triblock copolymer with again A being hydrophilic and thermoresponsive, B being hydrophilic, and C being hydrophobic.<sup>36</sup> However, it should be pointed out that even though block A was the same for both studies based on *N*-isopropylacrylamide, NIPAm blocks B and C were based on different monomers. However these observations reveal how complex the self-assembly behaviour of triblock copolymers is and that only through systematic investigation clear trends can be established.

In our studies we have systematically investigated the effect of the architecture<sup>50</sup> (ABC, ACB, BAC and statistical), the composition<sup>49,50</sup> as well as the molecular weight<sup>49</sup> (MW) of the terpolymers. The aim of this study was to investigate the effect of the hydrophobic alkyl side group on self-assembly and sol-gel transition. Twelve terpolymers were synthesised. The C block was based on the thermo- and pH-responsive monomer 2-(dimethylamino)ethyl methacrylate (DMAEMA) used in many biological related studies like gene delivery,<sup>51–56</sup> and the A block was based on the non-ionic hydrophilic monomer methoxy poly(ethylene glycol). polyPEGMA is also thermoresponsive but at higher temperatures than polyDMAEMA.<sup>57–61</sup> The B block was varied. Specifically it was based on three different alkyl containing non-ionic hydrophobic methacrylate monomers: ethyl-, butyl- and hexyl-methacrylate (EtMA, BuMA and HMA). Thus, four polymers of different architectures (ABC, ACB, BAC and statistical) were synthesised based on DMAEMA, PEGMA and one hydrophobic monomer (EtMA, BuMA or HMA) so the effect of the alkyl side group could systematically be investigated for the architecture of each polymer.

The polymers were synthesised with a “living” polymerisation technique, specifically Group Transfer Polymerisation (GTP),<sup>62–65</sup> in order to produce polymers of narrow MW distribution (MWD)<sup>63</sup> and be able to vary the architecture of the triblock copolymers easily by using sequential polymerisation. GTP was chosen because, compared to the conventional anionic polymerisation, it is less time consuming and more cost-effective.<sup>64</sup> When compared to controlled free radical polymerisation methods, GTP is also less time consuming (typical reaction time ~10 min over several hours to 24 h) and ideal for sequential polymerisation because it is fast and the monomer to polymer conversion is 100% in most cases.<sup>64</sup>

## Experimental

### Materials and methods

1-Methoxy-1-(trimethylsiloxy)-2-methyl propene (MTS, initiator, 99%), sodium metal, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH, free radical inhibitor, 99%), EtMA (99%), HMA (99%) and PEGMA (MW = 300 g mol<sup>-1</sup>, monomer) were purchased from Aldrich, UK. The chemical structures of the initiator and the monomers are shown in Fig. 1. Tetrabutylammonium hydroxide (40% in water), basic alumina (Al<sub>2</sub>O<sub>3</sub>, 95%), potassium metal, DMAEMA (monomer, 99%) and BuMA (monomer, 99%) were purchased from Acros Organics, UK. Tetrahydrofuran (THF, polymerisation solvent, 95%) and *n*-hexane (precipitation solvent, 95%) were purchased from Fisher Scientific.

DMAEMA, EtMA, BuMA and HMA monomers were passed twice through basic alumina to remove inhibitors and protic impurities and stirred over CaH<sub>2</sub> for 3 hours in the presence of DPPH. Both monomers were kept refrigerated until distillation before use. PEGMA was passed twice through basic alumina as a 50% v/v solution in THF and stirred overnight over CaH<sub>2</sub>. No DPPH was added to the PEGMA monomer solution due to its inability to distil PEGMA prior to use. The solution was refrigerated until the polymerisation and it was filtered directly into the reaction flask with a 0.45 μm syringe filter.

The initiator was distilled once before polymerisation and kept sealed under argon until use. Tetrabutylammonium bibenzoate (TBABB) was the catalyst for polymerisation and was synthesised by the reaction of tetrabutylammonium hydroxide and benzoic acid, as described by Dicker *et al.*<sup>65</sup> The catalyst was dried and stored under vacuum until use. THF was refluxed over a potassium/sodium amalgam for 3 days to dry before polymerisation. All glassware was dried overnight at 140 °C and assembled hot under dynamic vacuum before use.

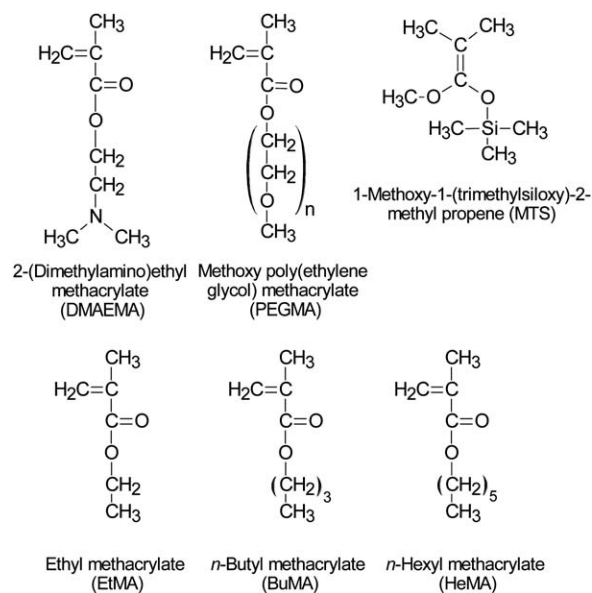


Fig. 1 Chemical structures of the monomers and the initiator.

## Triblock copolymer synthesis

The synthetic procedure was the same for all polymers. Specifically a typical sequential GTP procedure was followed.<sup>24,51–53,62,66–70</sup> The synthesis of BuMA<sub>14</sub>-*b*-PEGMA<sub>2</sub>-*b*-DMAEMA<sub>16</sub> is given below as an example.

Freshly distilled THF (78 mL) and MTS (0.80 mL, 0.69 g, 3.94 mmol) were syringed into a 250 mL round bottom flask containing TBABB (~10 mg) previously sealed with a septum and purged with argon. Then BuMA was added (8.8 mL, 7.9 g, 55.4 mmol) using a syringe. The temperature rose from 20.8 to 31.7 °C. After 10 minutes the exothermic reaction had abated and two 0.1 mL aliquots of the reaction solution were extracted for GPC and <sup>1</sup>H NMR analysis. Then a PEGMA solution in THF (3.8 mL of a 50 vol% solution, 2.0 g, 6.6 mmol) was added using a syringe and filter and the temperature increased from 25.5 to 26.2 °C. Subsequently two more 0.1 mL aliquots were collected for GPC and <sup>1</sup>H NMR analysis and DMAEMA (10.6 mL, 9.8 g, 62.6 mmol) was added. A final temperature increase was observed from 22.5 to 33.3 °C and final GPC and <sup>1</sup>H NMR samples were obtained.

All the synthesised copolymers were recovered by precipitation through *n*-hexane and dried at room temperature in a vacuum oven for two days. In total, nine ABC triblock copolymers of the same MW but of different architectures and hydrophobic monomers were prepared by varying the sequence of the monomer additions and the hydrophobic monomer (EtMA or BuMA or HMA). Three statistical terpolymers of the same MW based on the three different hydrophobic monomers were also synthesised by polymerising all three monomers (DMAEMA, PEGMA with EtMA or BuMA or HMA) simultaneously.

At this point it should be pointed out that the synthesised polymers can be described as oligomers because of the presence of PEGMA. As defined by IUPAC: an oligomer is a molecule of intermediate relative molecular mass, the structure of which essentially comprises a small plurality of units derived, actually or conceptually, from molecules of lower relative molecular mass. Note that:

(1) A molecule is regarded as having an intermediate relative molecular mass if it has properties which vary significantly with the removal of one or a few of the units.

(2) If a part or the whole of the molecule has an intermediate relative molecular mass and essentially comprises a small plurality of units derived, actually or conceptually, from molecules of lower relative molecular mass, it may be described as oligomeric, or by oligomer used adjectivally.

The synthesised polymers fall in the second category because of the presence of the macromonomer PEGMA block (and the small degree of polymerisation (DP) of the PEGMA block), so they are classified as oligomers. However because the authors thought it would be confusing to the reader to refer to the prepared molecules as ABC triblock oligomers they are referred to as polymers and copolymers, similar to other studies where a macromonomer was used.<sup>49–51,67–69,71,72</sup>

## Characterisation in organic solvents

**Gel permeation chromatography.** The MWs and the MWDs of all the linear precursors to the copolymers and all the

copolymers were determined by gel permeation chromatography (GPC) using a single PL-Mixed “E” Polymer Laboratories column. THF containing 5% triethylamine was the mobile phase and was pumped with a flow rate of 1 mL min<sup>-1</sup> using a Viscotek vt7510 pump. A Viscotek 3580 differential refractometer was used to measure the refractive index signal. The calibration curve was based on nine narrow MW linear poly(methyl methacrylate)s (PMMA)s with MWs of 690, 5720, 1020, 1200, 1960, 4000, 8000, 13 300 and 20 010 g mol<sup>-1</sup>.

**Proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H NMR).** A JEOL 400 MHz spectrometer instrument was used to acquire the proton NMR spectra of the copolymers and their precursors in CDCl<sub>3</sub>.

## Characterisation in bulk

**Differential scanning calorimetry.** A Perkin-Elmer differential scanning calorimeter (DSC) was used to measure the glass transition temperatures (*T*<sub>g</sub>s) of all the copolymers at a heating rate of 50 °C min<sup>-1</sup>. Each sample was scanned twice between -70 °C and +60 °C. The second heat was used for data analysis.

## Characterisation in aqueous solution

1% w/w aqueous solutions of the copolymers were characterised in terms of their effective p*K*<sub>a</sub>s, hydrodynamic diameters using hydrogen ion titration and light scattering respectively. The thermoresponsive behaviour was investigated in 1 and 20 wt% polymer solutions in phosphate buffered saline (PBS) for cloud and gel points.

**Hydrogen ion titrations.** Polymer solutions were titrated between pH 2 and pH 12 using a standard NaOH 0.75 M solution under continuous stirring. The pH was measured using a Fisherbrand Hydrus 400 pH meter. The p*K*<sub>a</sub>s were calculated as the pH at 50% ionisation.

**Dynamic light scattering.** A Malvern Photon Correlation Spectrometer equipped with a 70 mW blue laser operating at 470 nm was used for the dynamic light scattering (DLS) measurements at an angle of 90° and at room temperature to determine the hydrodynamic diameters of the copolymers in aqueous solution. Five 2 minute runs were performed for each polymer in aqueous solution and the data were averaged. Prior to the DLS measurements, the 1% w/w polymer aqueous solutions (with the pH adjusted to the physiological pH ~7.4) were filtered through 0.45 μm PTFE syringe filters, and were left to rest so that any air bubbles present could escape.

The diameters of the polymers in water were also calculated theoretically. Three models were used: (1) when a random coil configuration was assumed the formula:  $\langle dg^2 \rangle^{1/2} = 2 \times (2 \times 2.20 \times DP/3)^{1/2} \times 0.154 \text{ nm}$  was used, whereas, (2) when a spherical micelle based on ABC triblock copolymers were assumed the diameter was calculated *via* adding the DP of the hydrophobic block and twice the DP of the longest hydrophilic block (DMAEMA block) and multiplying by the projected length of one monomer unit (0.254 nm), and (3) when a spherical micelle that will have almost double the length of the fully stretched polymer chain was assumed (ACB and BAC), the diameter was calculated by adding twice the DP of the two

hydrophilic blocks and the DP of the hydrophobic block and then multiplying by the projected length of one monomer unit. It should be noted that the DPs were adjusted to the GPC and  $^1\text{H}$  NMR experimental data.

**Visual thermoresponse: cloud and gel points.** An IKA stirrer hotplate, a VWR VT-5 temperature controller and a water bath were used. 1 and 20 wt% polymer–PBS solutions (buffer pH = 7.2 to 7.4) were prepared in glass vials, suspended in the temperature-controlled water bath. The solution was heated at a rate of  $1\text{ }^\circ\text{C min}^{-1}$  over a range of  $20\text{ }^\circ\text{C}$  to  $90\text{ }^\circ\text{C}$  and observed for a visual indication of both gel and cloud points. The tube was inverted in order to determine if a physical gel was formed.

**Rheological thermoresponse.** Rheology measurements of 20 wt% polymer–PBS solutions (pH = 7.2–7.4) were undertaken using a Bohlin VOR rheometer with a 1/40 cone geometry. Solutions were cooled to  $5\text{ }^\circ\text{C}$ , presheared for 1 minute at  $1\text{ s}^{-1}$  and equilibrated at  $5\text{ }^\circ\text{C}$  for 15 minutes. Measurements of complex viscosity ( $\eta^*$ ), viscous modulus ( $G'$ ) and elastic modulus ( $G''$ ) were taken over a range of  $5\text{ }^\circ\text{C}$  to  $65\text{ }^\circ\text{C}$  at a heating rate of  $1\text{ }^\circ\text{C min}^{-1}$ .

## Results and discussion

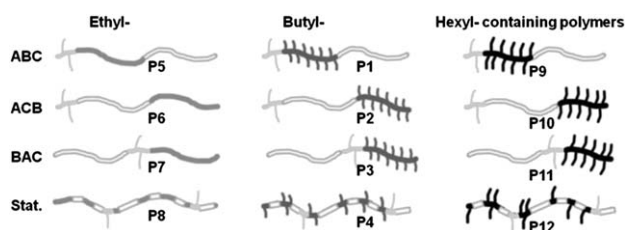
### Synthetic strategy

Polymers of constant MW and composition were synthesised by GTP using the PEGMA, EtMA, BuMA, HMA and DMAEMA monomers. The two hydrophilic monomers, PEGMA and DMAEMA, were always used while the hydrophobic monomer was varied so 4 terpolymers for each hydrophobic monomer of different alkyl group lengths on the side chain (ethyl, butyl and hexyl) were synthesised. These 4 terpolymers had different polymer architectures, ABC, ACB, BAC and statistical. All the polymers are shown in Fig. 2.

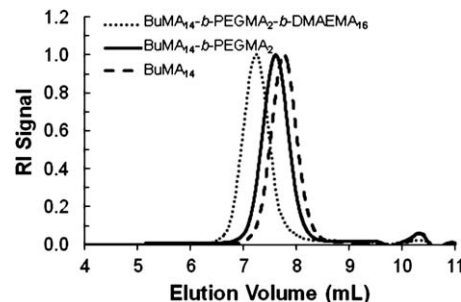
### Molecular weight and compositions

Table 1 shows the theoretical and experimental MWs, MW distributions (polydispersity indices, PDIs) and compositions for all of the polymers and their precursors as determined by GPC and  $^1\text{H}$  NMR.

All of the polymers and their precursors had narrow MWDs with PDIs, below 1.20, indicating a successful living polymerisation, similar to other GTP syntheses using the PEGMA macromonomer.<sup>49,50,52,66,67,70</sup> The only exceptions were the PEGMA oligomers that are expected to have broad PDIs.



**Fig. 2** Schematic representation of the synthesised copolymers. HMA, BuMA, EtMA, PEGMA and DMAEMA are denoted in black, dark gray, gray, light gray and white, respectively.



**Fig. 3** Gel permeation chromatograms of BuMA<sub>14</sub>-*b*-PEGMA<sub>2</sub>-*b*-DMAEMA<sub>16</sub> and its precursors.

Furthermore the GPC chromatograms clearly show a successful sequential polymerisation. In particular, with the addition of the second and third monomer the peak shifts to the left, at higher MWs and without the presence of any other peaks that could indicate unreacted monomers, partial deactivation of polymer chains, and/or unsuccessful addition of the second or the third monomer. A typical GPC chromatogram is shown in Fig. 3. The first peak from the right represents the first block, BuMA<sub>14</sub>, the second peak represents the diblock copolymer, BuMA<sub>14</sub>-*b*-PEGMA<sub>2</sub> and the third peak represents the resulting triblock copolymer BuMA<sub>14</sub>-*b*-PEGMA<sub>2</sub>-*b*-DMAEMA<sub>16</sub>.

As can be seen in Table 1 the  $M_n$ s of the polymers and the precursors to the polymers were either close to or slightly higher than the theoretical MWs. This is attributed to partial deactivation of the MTS initiator due to impurities, similar to other GTP studies.<sup>50,67–70</sup>

The theoretical (expected) compositions and the experimental compositions as determined by  $^1\text{H}$  NMR are also listed in Table 1. The compositions were determined by the integral ratio of the signals from the six methyl protons next to the amine of DMAEMA (2.3 ppm), the three methyl protons at the end of the PEG chain (3.4 ppm) and the two methyl protons next to the ester bond of BuMA (3.95 ppm). The compositions of the terpolymers were very close to the expected value (with  $\pm 3\text{ wt}\%$ ), which supports a successful synthesis.

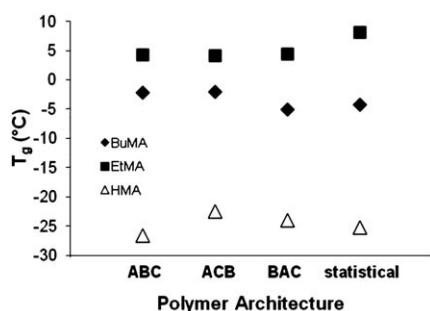
### Differential scanning calorimetry

The DSC results for all triblock copolymers are shown in Table 2. All of the polymers present only one  $T_g$  as expected, due to the short polymer blocks and thus low  $\chi$ -parameter, demonstrating that no microphase separation is occurring in the bulk. This is in agreement with our previous study.<sup>49,50</sup> Specifically only one transition is observed and not three at  $-41\text{ }^\circ\text{C}$  (corresponding to PEGMA),  $+10\text{ }^\circ\text{C}$  (corresponding to DMAEMA) and  $+65$  or  $+20$  or  $+5\text{ }^\circ\text{C}$  that would correspond to EtMA, BuMA, HMA, respectively. However as shown in Fig. 4 an increase in the  $T_g$  is observed as the alkyl chain of the hydrophobic monomer decreases for all polymers with similar MW and architecture, as expected. For example for ABC triblock copolymers, polymers 5, 1 and 9, the  $T_g$  increases from  $-26$  to  $4\text{ }^\circ\text{C}$  as the alkyl group changes from hexyl to butyl to ethyl. This is expected since the longer alkyl groups increase the free

**Table 1** Molecular weights and compositions of ABC triblock and statistical copolymers and their precursors<sup>a</sup>

Polymer number	Theoretical structure <sup>b</sup>	wt% PEGMA-(Et/Bu/He)-MA-DMAEMA		Theoretical MW <sup>c</sup> g mol <sup>-1</sup>	GPC results	
		Theoretical	<sup>1</sup> H NMR		$M_n$	$M_w/M_n$
1	PEGMA <sub>2</sub>	100-00-00	100-00-00	600	1000	1.40
	PEGMA <sub>2</sub> - <i>b</i> -BuMA <sub>14</sub>	20-80-00	24-76-00	2600	3400	1.10
	PEGMA <sub>2</sub> - <i>b</i> -BuMA <sub>14</sub> - <i>b</i> -DMAEMA <sub>16</sub>	10-40-50	14-30-56	5100	5300	1.14
2	PEGMA <sub>2</sub>	100-00-00	100-00-00	600	1000	1.35
	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	17-00-83	19-00-81	3100	3400	1.12
	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub> - <i>b</i> -BuMA <sub>14</sub>	10-40-50	11-39-50	5100	5200	1.15
3	BuMA <sub>14</sub>	00-100-00	00-100-00	2100	2600	1.11
	BuMA <sub>14</sub> - <i>b</i> -PEGMA <sub>2</sub>	20-80-00	23-77-00	2600	3294	1.11
	BuMA <sub>14</sub> - <i>b</i> -PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	10-40-50	11-42-47	5100	5200	1.15
4	PEGMA <sub>2</sub> - <i>co</i> -BuMA <sub>14</sub> - <i>co</i> -DMAEMA <sub>16</sub>	10-40-50	13-35-52	5100	5200	1.18
5	PEGMA <sub>2</sub>	100-00-00	100-00-00	600	900	1.34
	PEGMA <sub>2</sub> - <i>b</i> -EtMA <sub>18</sub>	20-80-00	21-79-00	2600	3300	1.11
	PEGMA <sub>2</sub> - <i>b</i> -EtMA <sub>18</sub> - <i>b</i> -DMAEMA <sub>16</sub>	10-40-50	10-42-48	5100	5700	1.13
6	PEGMA <sub>2</sub>	100-00-00	100-00-00	600	1000	1.31
	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	17-00-83	19-00-81	3100	3400	1.12
	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub> - <i>b</i> -EtMA <sub>18</sub>	10-40-50	12-41-47	5100	5300	1.17
7	EtMA <sub>18</sub>	00-100-00	00-100-00	2100	2500	1.10
	EtMA <sub>18</sub> - <i>b</i> -PEGMA <sub>2</sub>	20-80-00	22-78-00	2600	2900	1.11
	EtMA <sub>18</sub> - <i>b</i> -PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	10-40-50	10-45-45	5100	5400	1.18
8	PEGMA <sub>2</sub> - <i>co</i> -EtMA <sub>18</sub> - <i>co</i> -DMAEMA <sub>16</sub>	10-40-50	13-41-46	5100	5300	1.19
9	PEGMA <sub>2</sub>	100-00-00	100-00-00	600	1200	1.26
	PEGMA <sub>2</sub> - <i>b</i> -HMA <sub>12</sub>	20-80-00	21-79-00	2600	3300	1.13
	PEGMA <sub>2</sub> - <i>b</i> -HMA <sub>12</sub> - <i>b</i> -DMAEMA <sub>16</sub>	10-40-50	12-39-49	5100	5700	1.15
10	PEGMA <sub>2</sub>	100-00-00	100-00-00	600	1200	1.15
	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	17-00-83	26-00-74	3100	3400	1.13
	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub> - <i>b</i> -HMA <sub>12</sub>	10-40-50	12-35-53	5100	5600	1.14
11	HMA <sub>12</sub>	00-100-00	00-100-00	2100	2400	1.10
	HMA <sub>12</sub> - <i>b</i> -PEGMA <sub>2</sub>	20-80-00	21-79-00	2600	3000	1.11
	HMA <sub>12</sub> - <i>b</i> -PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	10-40-50	13-43-44	5100	5200	1.13
12	PEGMA <sub>2</sub> - <i>co</i> -HMA <sub>12</sub> - <i>co</i> -DMAEMA <sub>16</sub>	10-40-50	13-35-52	5100	5800	1.15

<sup>a</sup> PEGMA, EtMA, BuMA, HMA and DMAEMA are abbreviations for methoxy poly(ethylene glycol) methacrylate, ethyl methacrylate, *n*-butyl methacrylate, *n*-hexyl methacrylate and 2-(dimethylamino)ethyl methacrylate, respectively. <sup>b</sup> Theoretical and expected molecular weights of the polymer were calculated using this formula:  $MW_{\text{polymer}} = DP_A \times MW_A + DP_B \times MW_B + DP_C \times MW_C + 100 \text{ g mol}^{-1}$ , where MW and DP stand for molecular weight and degree of polymerisation, and A, B, C correspond to the three different monomers used. Note that  $100 \text{ g mol}^{-1}$  is the MW of the initiator fragment that is attached to the end of each polymer chain. <sup>c</sup> The calibration curve was based on nine narrow molecular weight linear poly(methyl methacrylate)s (PMMA)s with MWs of 690, 5720, 1020, 1200, 1960, 4000, 8000, 13 300 and  $20\,010 \text{ g mol}^{-1}$ .



**Fig. 4** Glass transition temperature,  $T_g$  vs. polymer architecture. The HMA-, BuMA-, and EtMA-containing polymers are represented with triangles, diamonds and squares, respectively.

volume of the polymers and thus lower the  $T_g$ . Finally since the block lengths are not long enough to microphase separate the polymer architecture does not seem to influence the  $T_g$  as has been reported in previous studies.<sup>49,50</sup>

## Solution properties

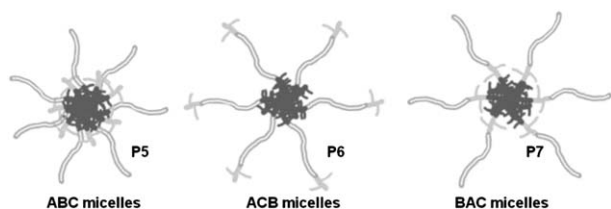
**Hydrodynamic diameters.** The experimental and theoretical hydrodynamic diameters of the polymers in aqueous solution are shown in Table 2. The theoretical values for the triblock copolymers are the upper limits of the hydrodynamic diameters and were calculated assuming fully stretched chains and the formation of micelles which have a configuration that is schematically illustrated in Fig. 5. For the statistical copolymers the theoretical values assume a random coil configuration.

The experimental values of the hydrodynamic diameters confirmed that all of the triblock polymers formed micelles while the statistical copolymers did not. Furthermore, the experimentally determined hydrodynamic diameters followed the same trend as their theoretically predicted values. It can also be observed that the diameters of the micelles formed are lower than their theoretical values. This was expected since the theoretical calculation assumes a fully extended conformation of the chains that is not the case, especially for the BuMA block,

**Table 2** Theoretical and experimentally determined hydrodynamic diameters,  $T_g$ s, effective  $pK_a$ s and cloud points (in PBS) of the copolymers

Polymer number	Theoretical structure <sup>a</sup>	Hydrodynamic diameter (nm)		$T_g$ (°C) $\pm$ 1 °C	Effective $pK_a$ s $\pm$ 0.2	Cloud points (°C) $\pm$ 1 °C
		Theoretical <sup>b</sup>	Experimental $\pm$ 0.5			
1	PEGMA <sub>2</sub> - <i>b</i> -BuMA <sub>14</sub> - <i>b</i> -DMAEMA <sub>16</sub>	14.2	10.9	-2.2	6.9	53
2	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub> - <i>b</i> -BuMA <sub>14</sub>	15.0	11.1	-2.0	6.8	53
3	BuMA <sub>14</sub> - <i>b</i> -PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	14.7	10.7	-5.0	6.9	54
4	PEGMA <sub>2</sub> - <i>co</i> -BuMA <sub>14</sub> - <i>co</i> -DMAEMA <sub>16</sub>	2.42	6.1	-4.2	6.9	44
5	PEGMA <sub>2</sub> - <i>b</i> -EtMA <sub>18</sub> - <i>b</i> -DMAEMA <sub>16</sub>	16.0	11.7	4.3	6.8	—
6	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub> - <i>b</i> -EtMA <sub>18</sub>	16.3	13.2	4.2	6.8	—
7	EtMA <sub>18</sub> - <i>b</i> -PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	17.7	11.8	4.5	6.8	—
8	PEGMA <sub>2</sub> - <i>co</i> -EtMA <sub>18</sub> - <i>co</i> -DMAEMA <sub>16</sub>	2.72	7.1	8.2	6.8	57
9	PEGMA <sub>2</sub> - <i>b</i> -HMA <sub>12</sub> - <i>b</i> -DMAEMA <sub>16</sub>	14.2	11.6	-26.5	6.8	48
10	PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub> - <i>b</i> -HMA <sub>12</sub>	15.6	15.2	-22.5	6.7	46
11	HMA <sub>12</sub> - <i>b</i> -PEGMA <sub>2</sub> - <i>b</i> -DMAEMA <sub>16</sub>	13.4	11.4	-24.0	6.8	46
12	PEGMA <sub>2</sub> - <i>co</i> -HMA <sub>12</sub> - <i>co</i> -DMAEMA <sub>16</sub>	2.43	7.7	-25.2	6.4	29

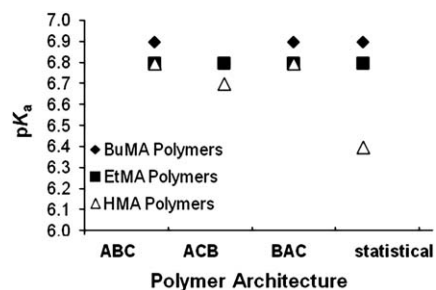
<sup>a</sup> PEGMA, EtMA, BuMA, HMA and DMAEMA are abbreviations for methoxy poly(ethylene glycol) methacrylate, ethyl methacrylate, *n*-butyl methacrylate, *n*-hexyl methacrylate and 2-(dimethylamino)ethyl methacrylate, respectively. <sup>b</sup> The theoretical upper limit of the diameters of the micelles formed by the triblock copolymers. For the theoretical calculation of the diameter of the statistical copolymers a random coil configuration was assumed.



**Fig. 5** Schematic representation of the micelles being formed by ABC, ACB and BAC triblock copolymers where A, B and C are PEGMA, BuMA and HMA, respectively.

which is in a collapsed state. This is in agreement with previous studies.<sup>49,50,67</sup> On the other hand the size of the statistical copolymers is higher than the theoretically predicted values, which assume a random coil configuration which indicates that water is a good solvent (at least for the PEGMA and DMAEMA units) and this leads to more extended chains than the random coil configuration. The value is small enough to confirm that no micelles or aggregates were present as expected and observed before.<sup>50</sup>

**Effective  $pK_a$ s.** The effective  $pK_a$ s of the polymer DMAEMA units are shown in Table 2 and in Fig. 6. The  $pK_a$  values for all synthesised polymers were between 6.7 and 6.9 which are in agreement with previous studies on DMAEMA containing polymers.<sup>49-53,73</sup> The only polymer that presented a lower  $pK_a$  was the HMA containing statistical terpolymer, the polymer with the most hydrophobic monomer that was not as soluble as the rest of the polymers in water and precipitated during the titration. In general, statistical copolymers are less soluble than block copolymers because they cannot form micelles and stabilise themselves in the solvent. Finally, no significant differences were observed between the  $pK_a$  values for polymers of different architectures, indicating that the polymer architectures do not affect the  $pK_a$  value, similar to what we have



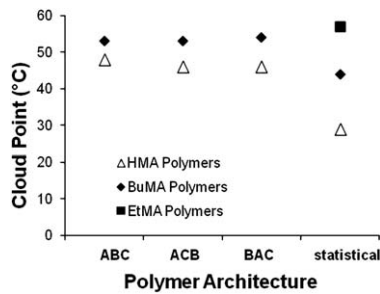
**Fig. 6** Effective  $pK_a$  vs. polymer architecture. The HMA-, BuMA-, and EtMA-containing polymers are represented by triangles, diamonds and squares, respectively.

observed before for linear polymers,<sup>49,50</sup> star<sup>51</sup> polymers and polymeric networks.<sup>68,69,71,72</sup>

**Cloud points.** In Table 2 the cloud points of 1 wt% polymer in PBS solutions are listed and represented in Fig. 7. As the temperature increases the clear polymer solution becomes cloudy due to the precipitation of the polymer. This is attributed to the well-known thermoresponsive ability of both DMAEMA<sup>50,52,57,74</sup> and PEG.<sup>6,57-61,67</sup> However it should be pointed out that the cloud point of a polymer solution is strongly influenced by the polymer's MW,<sup>24,67,74-76</sup> composition,<sup>9,24,49,50,75,77-80</sup> architecture<sup>50,80</sup> and grafting density<sup>81,82</sup> as well as the solvent,<sup>9,83</sup> the solution pH<sup>57,74</sup> and ionic strength.<sup>9,59,84,85</sup> The cloud point of a DMAEMA homopolymer with a MW around 5000 g mol<sup>-1</sup> is around 43 °C and decreases by increasing the MW,<sup>24</sup> while the cloud point of PEG containing homopolymers is influenced by both the MW and the number of PEG units of the PEGMA macromonomer.<sup>58</sup>

In this study all the BuMA- and HMA-containing polymers presented a cloud point, while from the EtMA based copolymers only the statistical one presented a cloud point. The cloud points of the HMA based triblock copolymers were always at a

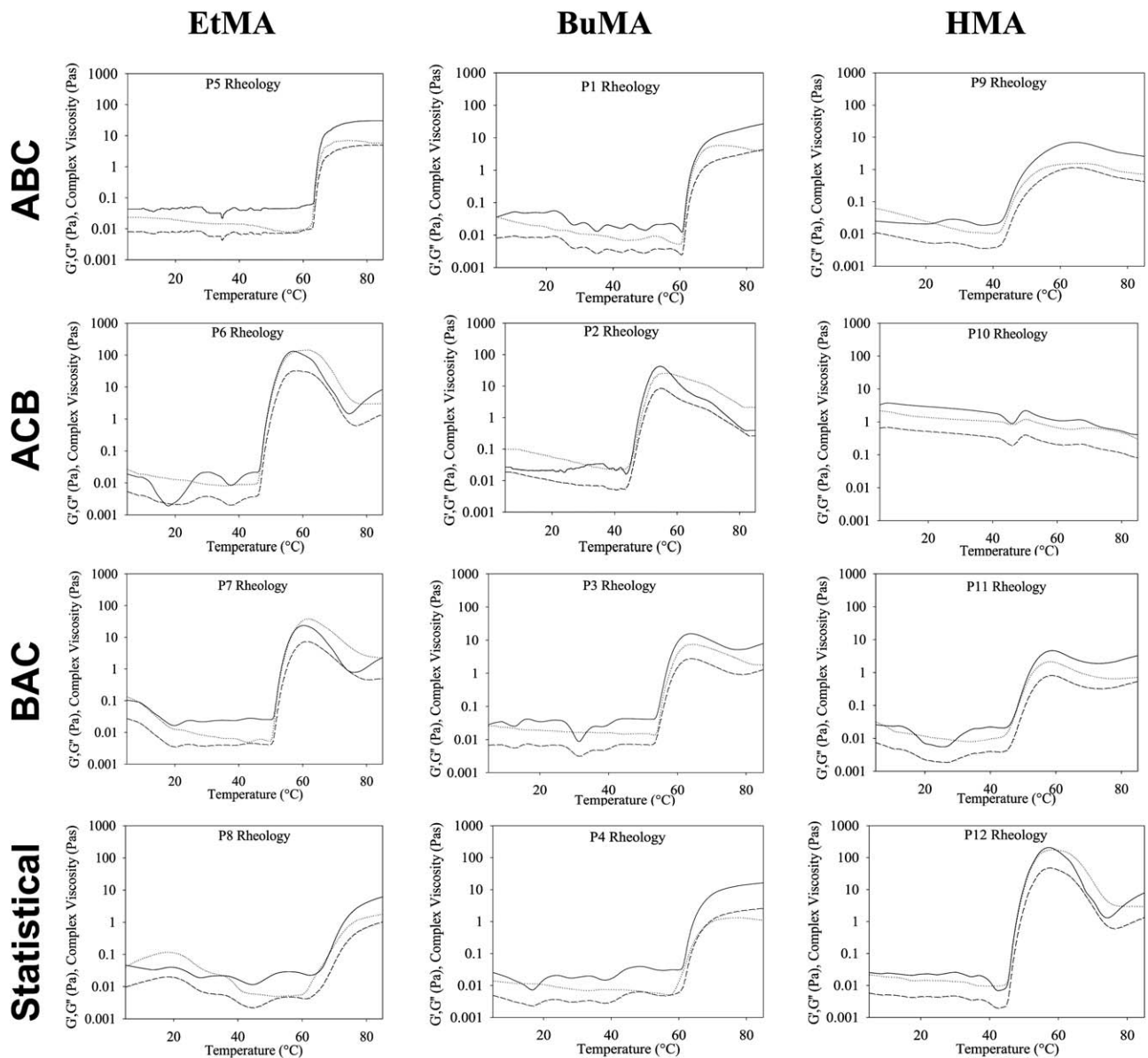




**Fig. 7** Cloud points of the ABC triblock copolymers in 1 wt% PBS solutions vs. polymer architecture. The HMA-, BuMA-, and EtMA-containing polymers are represented by triangles, diamonds and squares, respectively.

lower temperature when compared to the corresponding BuMA based polymers, while the EtMA based triblock terpolymers did not even present a cloud point. This is because the hydrophobicity is increased by increasing the alkyl side group and by increasing the hydrophobic character of the polymer the cloud point decreases. This is in agreement with previous studies<sup>9,24,50,78,85</sup> and is a similar observation to the study where the PEG units of PEGMA macromonomer were found to affect the cloud point.<sup>58</sup>

When comparing the three triblock copolymer architectures no clear trend is observed and all the triblocks have the same cloud point within experimental error. However the statistical copolymers always present a lower cloud point when compared



**Fig. 8** Rheology curves of 20% solutions of polymers 1–12 in PBS. Results arranged into columns for each hydrophobic monomer, EtMA, BuMA and HMA, and rows for each architecture: ABC, ACB, BAC and statistical copolymer. The full, dotted and dashed lines correspond to the elastic modulus ( $G'$ ), viscous modulus ( $G''$ ) and complex viscosity, respectively.

to the triblock copolymers based on the same monomer. This is because the triblock copolymers form micelles that they have to destabilise and aggregate further before the polymer precipitates while the statistical copolymer cannot form micelles and thus precipitate easier at lower temperatures. This is supported by previous studies.<sup>50,83</sup> In fact this effect is so dominant for the EtMA containing polymers that only the statistical copolymer presented a cloud point (shown as a black square) while none of the triblock copolymers presented a cloud point at the tested temperatures.

**Gel points by rheology and visual tests.** The gel points of 20 wt% polymer–PBS solutions were tested for their rheological characteristics in response to temperature as shown in Fig. 8. Specifically the rheology of the nine ABC triblock copolymers was investigated from 25 to 85 °C. The dependence on the architecture (ABC, ACB, BAC and statistical) is shown when moving from top to bottom, while from the left to right the dependence on the hydrophobic monomer is shown (EtMA, BuMA, HMA).

It should be noted that in terms of the rheology results the definition of a “hard gel” for the gel point was used.<sup>86</sup> So the gel point is taken as the instant when the elastic modulus  $G'$  exceeds the viscous modulus  $G''$  (*i.e.* the solid phase becomes dominant).<sup>86</sup> Therefore the rheology results in Fig. 8 show that only some of the polymers form thermoresponsive gels even though the viscosity increases by increasing the temperature. Two main observations can be made:

(1) The architecture of the polymer has a significant effect on the rheological thermoresponsive behaviour. ABC triblock copolymers always present a sharp sol–gel transition and form stable gels. On the other hand the statistical copolymers do not. The viscosity increases but the statistical copolymers either present a sol–gel transition but not as strong (lower modulus values – BuMA based polymer) or not a very stable gel (HMA based polymer). The BAC triblock copolymers present sol–gel transitions but not as stable or as strong as the ABC triblock copolymers. The ACB triblock copolymers do not show a sol–gel transition. Specifically the viscosity increases for the EtMA- and the BuMA-based ACB triblock polymers while the HMA-based ACB triblock was a gel at all temperatures. These observations are supported from previous studies and confirm the ABC architecture where the hydrophobic block is in the middle forms the best thermoresponsive gels, with the sharper sol–gel transition.<sup>50</sup>

(2) By increasing the length of the hydrophobic monomer the temperature where the sol–gel transition (or the viscosity increases significantly even if no stable gel is formed) decreases, with the exception of the HMA based ACB triblock copolymers which were a gel at all temperatures. This was expected as it has been shown before that by increasing the hydrophobicity of the polymers the gel point decreases.<sup>49,50</sup> However what can be described as surprising is that by increasing the alkyl side group and thus the hydrophobicity of the polymer the stability of the forming gels reduces. For example for ABC triblocks, even though the gel point (the temperature at which the gel forms) is reducing as the alkyl side group increases, the most stable gels are formed when the alkyl groups are short, thus the gels with

the most hydrophobic monomer form less stable gels. This is similar to Papadakis *et al.* observations for ABA triblock copolymers where different hydrophobic monomers comprised the A blocks.<sup>87</sup> This phenomenon was explained with the help of the  $T_{g,s}$ . Although the longer alkyl side groups increase the hydrophobicity of the polymer they also lower the  $T_{g,s}$  and thus offer more elasticity. It should be noted that even though the  $T_{g,s}$  were determined in bulk conditions they offer an indication of how glassy the hydrophobic core is. The more “glassy” (or less elastic) the hydrophobic core of the micelle the more stable the gels that are formed from the micelles. Thus more “glassy” domains in the multicompartments gels form more stable gels.

It should be mentioned that visual tests of the gel formation were also performed. The statistical copolymer solutions presented a cloud point and not a gel point. On the other hand none of the triblock copolymers presented a cloud point but some presented a gel point. The most “stable” gels, where the gel did not flow when inverting the tube fully, were formed by the ABC EtMA- and BuMA containing triblocks. The HMA ABC triblock formed a gel but when the tube remained inverted for a longer time the gel collapsed which indicates the lower stability of the gel as this was observed by rheology. All the other triblock copolymers, ACB and BAC did not form stable gels with the exception of the ACB HMA containing triblock that was a gel at all temperatures, as this was also supported by rheology.

## Conclusions

Twelve well-defined terpolymers based on one hydrophilic, pH and thermo-responsive monomer (C block), one non-ionic hydrophilic monomer (A block) and non-ionic hydrophobic monomer (B block) were successfully synthesised by GTP. Specifically, polymers of different architectures (triblock and statistical copolymers) and of different hydrophobic monomers with different lengths of the side alkyl groups were prepared and characterised in terms of their aqueous and thermoresponsive properties. The  $pK_a$ s were affected by the hydrophobicity of the polymers but not by the polymer architecture while the thermoresponsive behaviour was affected by both the architecture and the alkyl side group. Specifically lower cloud points were observed for the statistical copolymers over the triblock copolymers and when the polymers had a longer alkyl side group. Finally the sharpest sol–gel transition was observed by the ABC triblock copolymers and interestingly more stable gels were formed for the polymers with a short alkyl side group (less elastic).

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