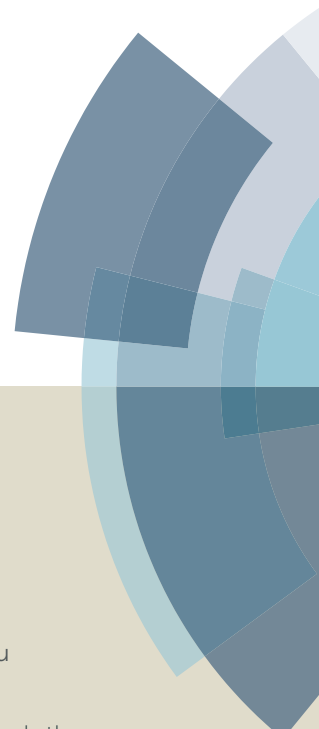


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ARTICLE

Thermoresponsive Gels based on ABC Triblock Copolymers: Effect of the Length of the PEG Side Group

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In this study, one statistical and nine well-defined ABC triblock thermoresponsive terpolymers were synthesised via group transfer polymerisation (GTP). The A, B, and C blocks were based on poly(ethylene glycol) (PEG) based methacrylate, *n*-butyl methacrylate (BuMA), and 2-(dimethylamino)ethyl methacrylate (DMAEMA), respectively. The length of the PEG side group was varied. Specifically, three different PEG based monomers were used; methoxy di-, penta-, and nona(ethylene glycol) methacrylate (DEGMA, PEGMA, and NEGMA, respectively). Along with the length of PEG side group, the composition of the terpolymers was also systematically varied in order to investigate the effect of both these parameters on the thermoresponsive behaviour of the polymers. The molar mass (MM) and the architecture were kept the same. Their hydrodynamic diameters, the effective pK_s s, and the cloud points of aqueous copolymer solutions were determined by dynamic light scattering (DLS), potentiometric titrations, and visual tests, respectively. Micelle formation was observed for all the copolymers and the pK_s s were influenced by the hydrophobic content but not by the PEG side length. On the other hand both the composition and the PEG side chain length affected the cloud points and the sol-gel transition. In summary, it was demonstrated the sol-gel transition can be tailored by varying both the PEG length as well as the composition of the polymers.

Introduction

Materials that are able to respond to external stimuli like ionic species, pH, temperature, electromagnetic radiation and sound with changes in volume, solubility, conformation and configuration are called “smart” materials,¹⁻⁷ and have gained significant scientific attention due to their wide range of applications. Such materials can find purpose in the biomedical field like gene, protein, radionuclide and drug delivery,^{1, 8-13} tissue engineering (tissue regeneration),^{1, 14-24} as wound dressings²⁵ and to industrial applications such as surface modification,²⁶ colloid stabilisation,²⁷ water remediation,²⁸ and oil recovery.²⁹

Our interest lies in thermoresponsive polymers and their possible application in tissue engineering^{30, 31} as well as in 3-D printing^{32, 33} and the combination of the two to print 3-D scaffolds for tissue engineering.^{32, 34-37} Traditionally thermoresponsive polymers in tissue engineering have been used as injectable gels that involves the encapsulation of cells in a 3-D structure in the body.^{1, 14-16, 20, 38, 39} In particular, in this application the thermoresponsive polymer is mixed at room temperature with the cells and then injected into the body. Upon injection, due to the temperature increase (to 37 °C) which is above the lower critical solution temperature (LCST) of

the polymer, the polymer presents a sol-gel transition and forms a physical gel. The cells are encapsulated within the 3-D structure of the physical gel. However with the recent development of 3-D printing now thermoresponsive polymers have started to attract attention in bioprinting where the polymer/cell mixture is printed to form a 3-D scaffold.^{6-8, 46}

In both applications what is crucial is to identify the design criteria of the thermo-responsive gel; such as polymer chemistry, composition, molar mass (MM), architecture, concentration etc. The polymer solution should have a sharp thermoresponsive transition and be able to form a stable gel with the cells encapsulated inside. In order to investigate all the design criteria in a systematic manner, well-defined polymers have to be used where there will be control over the molar mass distribution (MMD), the composition as well as the architecture. Such polymers can only be produced using living or controlled polymerisation techniques.⁴⁰ Group Transfer Polymerisation or GTP, that is a living polymerisation method, was chosen as the polymerisation method for the present study,⁴¹⁻⁴³ since it is more cost effective compared to living anionic polymerisation because it is performed at room temperature and at higher concentrations and it is much faster than controlled free radical polymerisation techniques and easier to scale up.⁴³⁻⁴⁵ Specifically, one-pot block copolymer synthesis can easily be achieved because each polymerisation step takes around ~15 min and the monomer is fully converted to the polymers.^{44, 46, 47} Thus, this method allows the fast and easy synthesis of many triblock copolymers on the same day and it allows the structural parameters of the polymers to be easily and controllably tailored like MM, composition and architecture.

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In our previous studies we have systematically investigated the effect of the architecture^{48, 49} (ABC, ACB, BAC and statistical), the symmetry,⁵⁰ the composition,^{45, 48} the alkyl side group length,⁴⁹ as well as the MM⁴⁵ of the triblock copolymers on their thermoresponsive ability and in particular their sol-gel transition. All of these polymers were based on the ionic hydrophilic pH- and thermoresponsive 2-(dimethylamino)ethyl methacrylate (DMAEMA), and the non-ionic hydrophobic *n*-butyl methacrylate (BuMA) and most of them also on the non-ionic hydrophilic poly(ethylene glycol) (PEG) methacrylate. In summary, we have proven that statistical copolymers have a poor ability to form gels and if they do form gels these are not very stable and in terms of the triblock copolymers the best architecture, with the most clear sol-gel transition is the ABC architecture where the hydrophobic block is the middle, B block.^{48, 49} With regard to the MM,⁴⁵ an optimum MM was observed around 7000 – 10000 g mol⁻¹.

The aim of this study was to systematically investigate how the length of the PEG side group, i.e. the PEG macromonomer, affects the thermoresponsive behaviour of the gels. So we have kept the other two monomers the same, DMAEMA and BuMA, chosen the optimum architecture ABC with the B being the BuMA and aimed for a MM within the optimum range. The composition (BuMA-PEG macromonomer ratio) was slightly varied for each PEG macromonomer used close to what is believed to be the optimum 30-35 wt%, because by varying the PEG length the hydrophilicity of the overall polymer also changes and we wanted to ensure the optimum hydrophilic-hydrophobic ratio will be investigated to achieve the best sol-gel transition. Thus, three PEG macromonomer were used; methoxy di-, penta-, and nona (ethylene glycol) methacrylate (DEGMA, PEGMA, and NEGMA, respectively) and for each of those three ABC triblock copolymers of varying compositions were synthesised. A statistical copolymer of the intermediate PEG length, PEGMA and composition was also synthesised for comparison. The successful syntheses of all the polymers were confirmed and aqueous based solutions of these polymers were thoroughly investigated with emphasis on their thermoresponsive and rheological properties.

Experimental

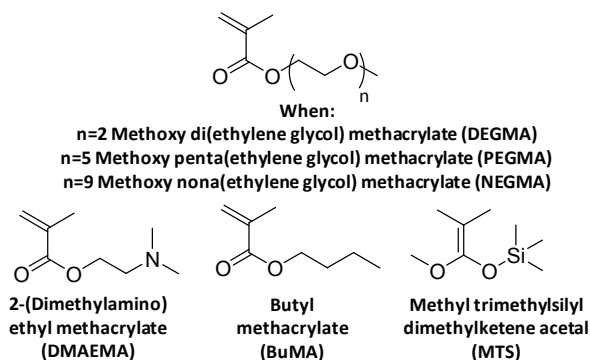


Fig. 1 Chemical structures of the monomers and the initiator.

Materials and methods

DMAEMA (monomer, 98%), BuMA (monomer, 99%), DEGMA (monomer, MM=188.22 g mol⁻¹, 95%), PEGMA (monomer,

MM=300 g mol⁻¹), NEGMA (monomer, MM=500 g mol⁻¹), calcium hydride (CaH₂, ≥90%), aluminium oxide activated basic (Al₂O₃•KOH), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH, free radical inhibitor), methyl trimethylsilyl dimethylketene acetal (MTS, initiator, 95%), potassium metal, sodium metal, and tetrahydrofuran (THF, HPLC grade, polymerisation solvent, ≥99.9%) were purchased from Aldrich, UK. Tetrahydrofuran (THF, GPC grade, mobile phase in chromatography) and *n*-hexane (precipitation solvent) were purchased from Fischer Scientific and VWR chemicals, respectively. Tetrabutylammonium hydroxide (40% in water) was purchased from Acros Organics, UK. Fig. 1 shows the chemical structures of the monomers and the initiator.

The low MM monomers, DMAEMA, BuMA, and DEGMA, were passed twice through basic alumina to remove the inhibitor and the acidic impurities. Concerning the higher MM monomers, PEGMA, and NEGMA, 50% v/v solutions in freshly distilled THF were passed twice through basic alumina. All the monomers were stirred for 3 hours over CaH₂ for water removal. DPPH was only added in DMAEMA, BuMA and DEGMA monomers which were kept refrigerated and distilled under vacuum directly prior their use. DPPH was not added in PEGMA and NEGMA solutions, due to their inability of being distilled. Therefore, they were kept refrigerated and filtered, using 0.45 μm syringe filters, directly into the reaction flask. The polymerisation solvent, THF, was dried prior to use by refluxing for 3 days using a sodium-potassium alloy. MTS was freshly distilled directly before the polymerisation and kept under an inert argon atmosphere until use. The catalyst, tetrabutylammonium bibenzoate (TBABB) was synthesised from benzoic acid and tetrabutylammonium hydroxide, according to the procedure reported by Dicker et al.,⁵¹ and it was purified by recrystallisation in dried diethyl ether prior to use. The catalyst was then dried and kept under vacuum until use. All glassware used for the distillations and the polymerisations were dried overnight at 140 °C and assembled hot under dynamic vacuum before use.

Triblock copolymer synthesis

The triblock copolymers were synthesised via sequential GTP, and the detailed synthesis of DEGMA₈-*b*-BuMA₂₁-*b*-DMAEMA₁₉ follows: TBABB (~10 mg) was added in a 250 mL round-bottom flask which was then sealed using a rubber septum and purged with inert argon gas. Freshly distilled THF (60 mL) and MTS (0.4 mL, 0.34 g, 1.97 mmol) were syringed into the reaction flask. Then, 2.9 mL of DEGMA was added (2.95 g, 16 mmol) and the temperature was increased from 25.4 to 31.1°C. After the completion of the exothermic reaction, two 0.1 mL samples were taken out of the flask for GPC and ¹H NMR analysis. Subsequently, BuMA (6.6 mL, 5.91 g, 42 mmol) was added and the temperature rose from 27.5 to 36.9 °C. Two 0.1 mL aliquots were extracted for GPC and ¹H NMR analysis. DMAEMA monomer was added last (6.3, 5.91 g, 38 mmol) and a temperature increase from 28.5 to 38 °C was observed. Two samples were obtained (0.1 mL each) for GPC and ¹H NMR analysis. All the copolymers were recovered by precipitation in cool *n*-hexane and were dried in a vacuum oven at room temperature. In total, nine ABC triblock copolymers having the same target MM but different composition and PEG side group length were synthesised by varying the molar ratio amounts and the PEG based monomer, respectively. Also, a statistical copolymer based on PEGMA-BuMA-

DMAEMA and having the intermediate composition was also synthesised for comparison by simultaneous addition of the monomers into the reaction flask before the addition of the MTS.

Characterisation in organic solvents

Gel permeation chromatography. The MMs and the MMDs of all the copolymers and their linear precursors (first block and diblock) were determined by GPC using an Agilent, SECcurity GPC system, with a Polymer Standard Service (PSS) SDV analytical linear M column (SDA083005LIM). THF containing 5 vol. % triethylamine was used as the mobile phase and was pumped with a flow rate of 1 mL min⁻¹ using a "1260 Iso" isocratic pump. An Agilent 1260 RI detector was used to measure the refractive index (RI) signal. The calibration curve was based on six narrow MM linear poly(methyl methacrylate) (PMMA) standard samples (2000, 4000, 8000, 20000, 50000, 100000 g mol⁻¹).

Proton nuclear magnetic resonance spectroscopy (¹H NMR). All the ¹H NMR spectra of the copolymers and their linear precursors in CDCl₃ were obtained using a 400 MHz Avance Bruker NMR spectrometer instrument.

Characterisation in aqueous solution

The effective pK_as, the hydrodynamic diameters and the cloud points of 1% w/w of all the linear copolymer aqueous solutions were determined by potentiometric titrations, dynamic light scattering and visual observations. The thermoresponsive behaviour of 1, 2, 5, 10, 15 and 20 % w/w copolymer solutions in phosphate buffered saline (PBS) was also investigated.

Potentiometric titrations. 1% w/w aqueous polymer solutions were titrated from pH 2 to 12 using a standard 0.25 M NaOH aqueous solution. The pH change was monitored by using a portable HI98103 pH checker from Hanna instruments. The pK_a was determined as the pH at 50% protonation of DMAEMA units.^{48, 52, 53}

Dynamic light scattering. The hydrodynamic diameters of 1% w/w aqueous polymer solutions (pH adjusted to 6.7 to 7.0) were determined by using a Zetasizer Nano ZSP (Malvern, UK) instrument. The measurements were taken at room temperature, using a backscatter angle of 173°. Three runs were performed for each sample and the results reported are the mean values. Before the DLS measurements, the solutions were filtered through nylon 0.45 μm PTFE syringe filters and they were left to rest to remove the bubbles.

The theoretical hydrodynamic diameters were also calculated and compared with the corresponding experimental ones. The calculations were based on two different models. (1) In the case of random coil configuration the following formula was used: $\langle dg^2 \rangle^{1/2} = 2 * (2 * 2.20 * DP/3)^{1/2} * 0.154 \text{ nm}$.⁵⁴ (2) The hydrodynamic diameter of the spherical micelles formed by the ABC triblock copolymers was calculated 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). For the calculations, the experimental DPs were adjusted to GPC and ¹H NMR results.

Cloud point. An IKA RCT basic stirrer hotplate, equipped with an IKA ETS-D5 temperature controller, and a continuously stirred water

bath were used for visually observing the cloud points of 1% w/w aqueous polymer solutions in glass vials. The solutions were heated from 20 to 80 °C and a visual observation was taken every one degree. The pH of all the polymer solutions was around 8.8 to 9.5. The statistical terpolymer (Polymer 10) was completely insoluble at this pH.

Visual gel point. The gelation of 1, 2, 5, 10, 15 and 20% w/w copolymer solutions in PBS was tested by using an IKA RCT basic stirrer hotplate, an IKA ETS-D5 temperature controller, and a water bath. The glass vials were suspended in the water bath and the thermal response was visually observed (cloud and gel points) from 20 to 80 °C. The gel point was taken as the temperature at which a stable physical gel was formed upon tube inversion. All the statistical terpolymer solutions were insoluble at this pH.

Rheology. The rheological behaviour of 15% w/w polymer/PBS solutions was investigated using a TA Discovery HR-1 hybrid rheometer equipped with a 40 mm parallel Peltier steel plate (996921), following a similar procedure as the one reported by Ge et al. (2011).⁵⁵ The solutions were cooled to 20 °C, presheared for 1 min at 1 s⁻¹ and equilibrated at 20 °C for 15min. Temperature ramp tests were carried out from 20 to 65 °C with a ramp rate of 1 °C min⁻¹, recording the changes in shear storage modulus (elastic modulus, G') and shear loss modulus (viscous modulus, G''). The strain was set at 1% and the angular frequency at 1 rad s⁻¹. The complex viscosity (η*) was calculated according to the following equation: $\eta^* = [(G''/\omega)^2 + (G'/\omega)^2]^{1/2}$; where ω is the angular frequency.⁵⁶

Results and Discussion

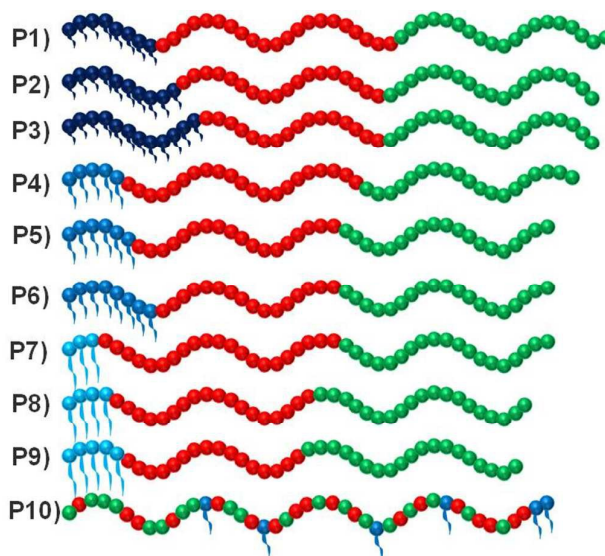


Fig. 2 Schematic representation of the ABC triblock copolymers (P1-P9) and the statistical terpolymer (P10). The DEGMA, PEGMA, NEGMA, BuMA and DMAEMA repeated units are coloured in dark blue, blue, light blue, red and green, respectively.

Table 1 Molar masses and compositions of ABC triblock copolymers and statistical copolymer and their precursors.

Polymer No.	Theoretical structure ^a	wt% (D/P/N)EGMA-BuMA-DMAEMA		Theoretical MM ^b g mol ⁻¹	GPC results ^c	
		Theoretical	¹ H NMR		<i>M_n</i>	<i>M_w/M_n</i>
1	DEGMA ₈	100-00-00	100-00-00	1600	2430	1.12
	DEGMA ₈ - <i>b</i> -BuMA ₂₁	33-67-00	36-64-00	4600	6140	1.10
	DEGMA ₈ - <i>b</i> -BuMA ₂₁ - <i>b</i> -DMAEMA ₁₉	20-40-40	21-38-41	7600	9980	1.07
2	DEGMA ₁₀	100-00-00	100-00-00	1975	2710	1.11
	DEGMA ₁₀ - <i>b</i> -BuMA ₁₈	42-58-00	43-57-00	4600	6080	1.09
	DEGMA ₁₀ - <i>b</i> -BuMA ₁₈ - <i>b</i> -DMAEMA ₁₉	25-35-40	25-35-40	7600	10000	1.08
3	DEGMA ₁₂	100-00-00	100-00-00	2350	2950	1.12
	DEGMA ₁₂ - <i>b</i> -BuMA ₁₆	50-50-00	50-50-00	4600	5730	1.09
	DEGMA ₁₂ - <i>b</i> -BuMA ₁₆ - <i>b</i> -DMAEMA ₁₉	30-30-40	27-32-41	7600	9590	1.07
4	PEGMA ₅	100-00-00	100-00-00	1600	2740	1.13
	PEGMA ₅ - <i>b</i> -BuMA ₂₁	33-67-00	36-64-00	4600	6860	1.08
	PEGMA ₅ - <i>b</i> -BuMA ₂₁ - <i>b</i> -DMAEMA ₁₉	20-40-40	21-39-40	7600	10340	1.08
5	PEGMA ₆	100-00-00	100-00-00	1975	3030	1.12
	PEGMA ₆ - <i>b</i> -BuMA ₁₈	42-58-00	43-57-00	4600	6330	1.09
	PEGMA ₆ - <i>b</i> -BuMA ₁₈ - <i>b</i> -DMAEMA ₁₉	25-35-40	26-34-40	7600	10400	1.07
6	PEGMA ₈	100-00-00	100-00-00	2350	3480	1.12
	PEGMA ₈ - <i>b</i> -BuMA ₁₆	50-50-00	55-45-00	4600	6320	1.10
	PEGMA ₈ - <i>b</i> -BuMA ₁₆ - <i>b</i> -DMAEMA ₁₉	30-30-40	34-28-38	7600	9680	1.11
7	NEGMA ₃	100-00-00	100-00-00	1600	2830	1.21
	NEGMA ₃ - <i>b</i> -BuMA ₂₁	33-67-00	37-63-00	4600	7090	1.10
	NEGMA ₃ - <i>b</i> -BuMA ₂₁ - <i>b</i> -DMAEMA ₁₉	20-40-40	23-38-39	7600	11900	1.09
8	NEGMA ₄	100-00-00	100-00-00	1975	2850	1.24
	NEGMA ₄ - <i>b</i> -BuMA ₁₈	42-58-00	45-55-00	4600	6410	1.20
	NEGMA ₄ - <i>b</i> -BuMA ₁₈ - <i>b</i> -DMAEMA ₁₉	25-35-40	27-35-38	7600	10300	1.15
9	NEGMA ₅	100-00-00	100-00-00	2350	3450	1.16
	NEGMA ₅ - <i>b</i> -BuMA ₁₆	50-50-00	54-46-00	4600	5710	1.15
	NEGMA ₅ - <i>b</i> -BuMA ₁₆ - <i>b</i> -DMAEMA ₁₉	30-30-40	33-28-39	7600	9650	1.15
10	PEGMA ₆ - <i>co</i> -BuMA ₁₈ - <i>co</i> -DMAEMA ₁₉	25-35-40	27-33-40	7600	9790	1.10

^a DEGMA, PEGMA, NEGMA, BuMA, and DMAEMA are the abbreviations for methoxy di(ethylene glycol) methacrylate, methoxy penta(ethylene glycol) methacrylate, methoxy nona(ethylene glycol) methacrylate, *n*-butyl methacrylate, and 2-(dimethylamino) ethyl methacrylate, respectively.

^b The theoretical MM was calculated as the sum of the multiplication of the MM of each repeated unit by the corresponding DP plus 100 g mol⁻¹; where MM and DP are the abbreviations of molar mass and degree of polymerisation, respectively. 100 g mol⁻¹ was added because it is the fragment of the GTP initiator that remains on the polymer backbone.

^c The *M_n* and *Đ* were determined by GPC using linear poly(methyl methacrylate) (pMMA) standards of MW equal to 2000, 4000, 8000, 20000, 50000, 100000 g mol⁻¹.

Synthetic strategy

The ABC triblock copolymers and the statistical terpolymer were synthesised via sequential and simultaneous GTP, respectively. In total, nine ABC triblock copolymers were synthesised by systematically varying the composition and the PEG side group length. The A block consists of a PEG based monomer (DEGMA, PEGMA, or NEGMA) whereas the B and C blocks consist of BuMA and DMAEMA units, respectively. Three different compositions of PEG based-BuMA-DMAEMA were targeted; 20-40-40 wt%, 25-35-40 wt%, and 30-30-40 wt%, respectively. The statistical terpolymer was based on PEGMA and possessed intermediate composition of 25-35-40 wt% for PEGMA-BuMA-DMAEMA, respectively. All the copolymers are shown in Fig. 2.

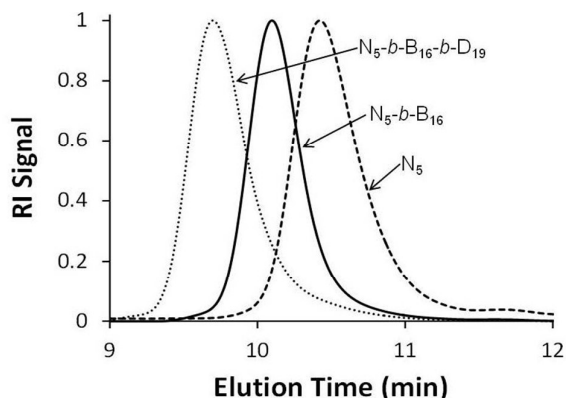
Molar mass and compositions

The theoretical and experimental MMs, the MMDs (dispersity indices, *Đ*), the theoretical and experimental compositions of all the terpolymers and their linear precursors (homopolymer and diblock), determined by GPC and ¹H NMR, are shown in Table 1.

As it can be observed in Table 1, the *Đ* values varied from 1.07 to 1.24, indicating the successful 'living' polymerisation; similar to other published studies on GTP.^{45, 48-50} The *Đ* decreased as the molar mass of the polymers increased, as is expected for a living polymerisation method. The polymers that had the highest *Đ* values corresponded to the PEG based homopolymers, and especially to NEGMA as expected and observed before. Specifically, this was previously reported in studies on PEG based monomers and was

attributed to: (1) the PEG based monomers are macromonomers having average MM and therefore, wider MMD and/or (2) the impurities contained in the high MM PEG based monomers which were not able to be purified via distillation.^{45, 48, 49, 57}

Fig. 3 GPC traces of the Polymer 1: DEGMA₈-*b*-BuMA₂₁-*b*-DMAEMA₁₉ and its



precursors.

The \bar{D} of the statistical copolymer was also higher than the \bar{D} s of the triblock copolymers; with the exception of the two NEGMA based triblock copolymers, which were based on the higher MM PEG macromonomer, which has a wider MMD. This is in agreement with previous studies and it was attributed to the fact that the temperature and the polymerisation cannot be controlled as well since the monomers were not added in the reaction drop-wise but were already in the flask when the initiator was added.⁵⁰

The M_n values listed in Table 1 were slightly higher than the expected ones. This is attributed to the partial deactivation of the initiator caused by the impurities and moisture present in the reaction flask; similarly to other GTP studies^{58, 59} as well as due to the fact the GPC calibration is based on PMMA standards.

The GPC traces that correspond to DEGMA₈-*b*-BuMA₂₁-*b*-DMAEMA₁₉ (Polymer 1) and its precursors are shown in Fig. 3. It can be seen that the peaks of the first block, diblock, and triblock copolymers shifted to lower elution times, and no peaks related to the first block and diblock were observed, thus indicating the successful sequential polymerisation. The same observations were made for all triblock copolymers (see GPC traces in Supporting Information).

The theoretical and experimental monomer compositions of the polymers and their precursors are also listed in Table 1. The experimental compositions were calculated as the integral ratio of the three peaks corresponding to three repeated units in the ¹H NMR spectra (see Supporting Information). The peak of DMAEMA unit is the one appearing at 2.25 ppm which corresponds to the six methyl protons next to the amine group. The peak at 3.35 ppm which corresponds to the three methoxy protons of PEG based monomers and the peak at 3.9 ppm which belongs to the two methyl protons next to the BuMA ester were also used to determine the experimental compositions. The theoretical and experimental weight percentages are in a good agreement, thus supporting a successful polymerisation.

Aqueous solution properties

Hydrodynamic diameters. The experimental and theoretical hydrodynamic diameters of all the terpolymers in aqueous solution are listed in Table 2. The theoretical hydrodynamic diameters of the triblock copolymers are the maximum values calculated by assuming micelle formation of fully stretched polymer chains. The theoretical value of the statistical copolymer was based on random coil configuration and it was found to be around 3 nm. It can be observed that all the copolymers formed micelles since their experimental hydrodynamic diameters are higher than the one corresponding to the random coil (the diameter would have been close to 3 nm according to their MM).

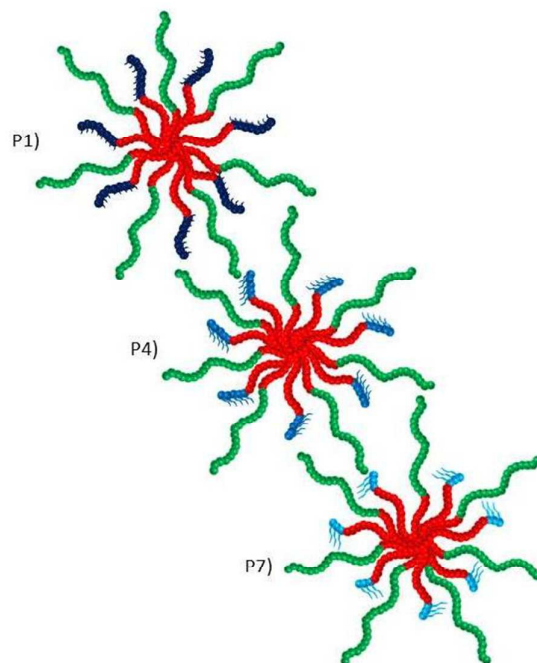


Fig. 4 Schematic representation of micelles formed by Polymer 1, 4, and 7, which are DEGMA, PEGMA, and NEGMA based, respectively. The DEGMA, PEGMA, NEGMA, BuMA, and DMAEMA units are shown in dark grey, grey, light grey, black, and white, respectively.

The experimental hydrodynamic diameters of the ABC triblock copolymers were lower than the theoretical ones; as expected and previously reported.^{45, 48, 49, 57, 60} This was due to the two main assumptions made by the theoretical model used for the calculations: (1) the hydrophobic blocks (BuMA part) fully overlap and (2) the polymer chain is fully extended (both hydrophobic and hydrophilic parts). However, in reality, the hydrophobic part is in a collapsed state, thus decreasing the micelle size. The assumed micelle configuration adopted by the ABC triblock copolymers is schematically illustrated in Fig. 4 for Polymers 1, 4, and 7; DEGMA₈-*b*-BuMA₂₁-*b*-DMAEMA₁₉, PEGMA₅-*b*-BuMA₂₁-*b*-DMAEMA₁₉, and NEGMA₃-*b*-BuMA₂₁-*b*-DMAEMA₁₉, respectively. The DEGMA, PEGMA, NEGMA, BuMA, and DMAEMA units are coloured in dark grey, grey, light grey, black, and white, respectively.

Table 2 Hydrodynamic Diameters, Effective pK_a s and Cloud points of 1% w/w Aqueous Solutions of the Copolymers.

Polymer No.	Theoretical structure ^a	Hydrodynamic diameter (nm)		Effective pK_a s	Cloud points
		Theoretical	Experimental ± 0.5	± 0.1	± 2 °C
1	DEGMA ₈ - <i>b</i> -BuMA ₂₁ - <i>b</i> -DMAEMA ₁₉	20.0 ^b	10.1	6.8	43
2	DEGMA ₁₀ - <i>b</i> -BuMA ₁₈ - <i>b</i> -DMAEMA ₁₉	19.2 ^b	11.7	6.9	45
3	DEGMA ₁₂ - <i>b</i> -BuMA ₁₆ - <i>b</i> -DMAEMA ₁₉	18.2 ^b	11.7	7.0	43
4	PEGMA ₅ - <i>b</i> -BuMA ₂₁ - <i>b</i> -DMAEMA ₁₉	20.6 ^b	11.7	6.9	45
5	PEGMA ₆ - <i>b</i> -BuMA ₁₈ - <i>b</i> -DMAEMA ₁₉	19.8 ^b	11.7	6.9	51
6	PEGMA ₈ - <i>b</i> -BuMA ₁₆ - <i>b</i> -DMAEMA ₁₉	16.7 ^b	10.1	6.9	52
7	NEGMA ₃ - <i>b</i> -BuMA ₂₁ - <i>b</i> -DMAEMA ₁₉	23.1 ^b	10.1	6.9	51
8	NEGMA ₄ - <i>b</i> -BuMA ₁₈ - <i>b</i> -DMAEMA ₁₉	19.1 ^b	11.7	7.0	57
9	NEGMA ₅ - <i>b</i> -BuMA ₁₆ - <i>b</i> -DMAEMA ₁₉	17.0 ^b	15.7	7.0	62
10	PEGMA ₆ - <i>co</i> -BuMA ₁₈ - <i>co</i> -DMAEMA ₁₉	2.8 ^c	11.7	6.7	^d

^aDEGMA, PEGMA, NEGMA, BuMA, and DMAEMA are the abbreviations for methoxy di(ethylene glycol) methacrylate, methoxy penta(ethylene glycol) methacrylate, methoxy nona(ethylene glycol) methacrylate, *n*-butyl methacrylate, and 2-(dimethylamino) ethyl methacrylate, respectively.

^b The theoretical values of triblock copolymers were calculated as $(2 * DP_{DMAEMA} + DP_{BuMA}) * 0.254$ nm; where DP is the degree of polymerisation; based on GPC and ¹H NMR results. This calculation assumes fully stretched polymer chains.

^c The theoretical value of the statistical copolymer was calculated by assuming a random coil configuration ($\langle d_g^2 \rangle^{1/2} = 2 * (2 * 2.20 * DP/3)^{1/2} * 0.154$ nm); where DP is the total degree of polymerisation as resulted from GPC and ¹H-NMR analysis.

^d The determination of the cloud point of the statistical copolymer was not possible because the polymer was insoluble at the pH tested.

The experimental hydrodynamic diameters of the triblock copolymers did not follow the expected theoretical trend; in contrast with previous studies.^{45, 48, 49} This was attributed to two factors: (1) the BuMA content which is the one affecting the theoretical value (since the DP of the DMAEMA is constant) was in a collapsed state; thus slight variations of the BuMA block will not affect the size of the micelle, and (2) the PEG side groups were not taken into account in the calculations. However, PEG side groups they were present in the polymer chain, and possibly affect the micelle configuration. The effect of side group length on the micelle size was previously reported.⁵⁷ The longer the PEG side chain and/or the higher the DP of the PEG based unit, the more the DMAEMA block is forced to extend, thus increasing the micelle size; as in the case of Polymer 9.

Also, it can be observed that the statistical terpolymer (Polymer 10) forms some kind of aggregates, which is not in agreement with our previous studies where the diameter was closer to that of a random coil,⁴⁸⁻⁵⁰ but not surprising since random copolymers can form aggregates if they contain lengthy side groups. Specifically, the aggregation behaviour of amphiphilic random copolymers has been previously reported in the literature, and it depends on several parameters such as the MM and the hydrophobic-hydrophilic balance.⁶¹⁻⁶³ As reported by Laskar et al., random copolymers based on PEGMA and dodecyl methacrylate (DMA) formed micelles.⁶¹ Also, micelle formation of random copolymers based on 2-(1-imidazolyl) ethyl methacrylate (ImEMA) and methacrylic acid (MAA) was observed by Hadjikallis et al.⁶⁴

Hadjiyannakou et al. reported the increased aggregation tendency of diblock copolymers based on benzyl methacrylate (BzMA) and methoxy hexa(ethylene glycol) methacrylate (HEGMA), which was attributed to the increased hydrophobicity of the HEGMA backbone.⁶⁵

Effective pK_a s. The effective pK_a s of DMAEMA units, listed in Table 2, range from 6.7 to 7; which is consistent with previous published studies on DMAEMA polymers.^{45, 48-50, 66} The pK_a decreased by increasing the hydrophobic BuMA content for all block based terpolymers, within experimental error. This trend was previously observed and it was attributed to the decreased dielectric constant by increasing hydrophobicity.^{45, 48, 50, 58, 67}

Interestingly, the DMAEMA units of the statistical copolymer showed a lower pK_a . At this point, it should be noted that the statistical copolymer was overall the least soluble and precipitated at higher pH values, where the DMAEMA units are not protonated. The fact that the random copolymer makes aggregates but not the traditional core-corona micelles where the DMAEMA block will be in the corona may affect the DMAEMA protonation due to steric hindrance; thus lower pH is needed to protonate the DMAEMA groups on the polymer compared to the block based counterparts. It should be noted that this observation is similar to one of the statistical polymers that also had reduced solubility in our previous studies where the pK_a was 6.4, lower than the corresponding triblock based counterparts that had pK_a s between 6.7 and 6.8 and this was attributed to the poor solubility of the statistical copolymer and its inability to form micelles to stabilise itself in

solution,⁴⁹ similarly to the present study. Furthermore in another recent study it was demonstrated that pK_a varies is the polymer is above or below the LCST (so if it is insoluble) that also supports this finding.⁶⁸

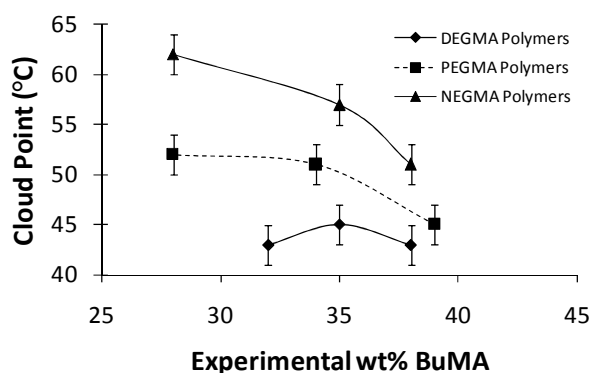


Fig. 5 Cloud points of the 1% w/w ABC triblock copolymers aqueous solutions as a function of the hydrophobic BuMA content. The diamonds, squares, and triangles correspond to the DEGMA, PEGMA, and NEGMA based units.

Cloud points. The cloud points of 1% w/w polymer solutions in DI water (pH~8 where DMAEMA units were not protonated) are listed in Table 2. The determination of the cloud point of the statistical copolymer was not feasible due to its reduced solubility at the pH tested. All the triblock copolymers studied presented a cloud point within the temperature range tested. The thermoresponsive behaviour of these polymers can be attributed to both DMAEMA^{48, 69-71} and PEG^{6, 57, 70, 72-75} units of the terpolymers. However, it should be pointed out that the cloud point of a polymer solution is strongly influenced by the polymer's MM,^{24, 57, 71, 76, 77} composition,^{9, 24, 45, 48, 76, 78-81} architecture^{48, 81} and grafting density^{82, 83} as well as the solvent,^{9, 84} the solution pH^{70, 71} and the ionic strength.^{9, 73, 85, 86} The cloud point of a DMAEMA homopolymer with a DP \approx 20, similar to the DMAEMA block in the present study, is around 43 °C and decreased by increasing the MM,²⁴ while the cloud point of PEG containing homopolymers is influenced by both the MM but also by the number of PEG units of the PEGMA macromonomer.⁷² PEG macromonomer based copolymers demonstrated cloud points that were strongly affected by the content in the copolymer as well as the side chain length of the PEG based methacrylate units.^{70, 74, 87, 88}

Thus, the cloud points of the ABC triblock terpolymers that are listed in Table 2 and shown in Fig. 5 can be explained by the two varied design factors, the polymer hydrophobic content and the length of the PEG group. By increasing the BuMA content and reducing the length of the PEG based monomer the polymers become hydrophobic. However since the PEG based monomer versus the BuMA monomer ratio was varied when the BuMA content was increased the hydrophobicity did not necessarily increase, at least in the case of the shortest PEG based monomer, DEGMA, because DEGMA is also hydrophobic.

Thus as it can be seen in Fig 5 when increasing the hydrophobic BuMA content the cloud points remain constant because the overall hydrophobicity of the polymers does not increase. In the case of the NEGMA based polymers where NEGMA is the most hydrophilic monomer we see a clear trend; the cloud point of copolymer aqueous solutions, decreased by increasing the BuMA content, as expected and reported previously.^{45, 48-50, 89} This trend is less pronounced by still visible for the PEGMA based polymers where the cloud points decreased from 52, to 51 and to 45 °C by increasing the BuMA content from 28, to 34 and to 39 wt%, respectively. The only 1 °C difference for the first two polymers is possibly due to the fact that the first polymer PEGMA₅-b-BuMA₂₁-b-DMAEMA₁₉ had a bit higher PEGMA content than it expected.

It should be noted that the strong influence of the pendant group length of the PEG based units on the cloud point was expected. Specifically, when comparing the three triblock copolymers with BuMA wt% content around 35%, the cloud point increased from 45 to 51 to 57 °C when the length of the PEG macromonomer increased from 2 to 5 to 9 repeating ethylene glycol units, similarly to previously reported studies on PEG based homopolymers and double hydrophilic diblock copolymers.⁸⁸

Gel point by rheology. The gel points of 15% w/w triblock copolymer solutions in PBS were tested by rheology from 20 to 65 °C and the results are shown in Fig. 6. The dependence of the PEG side chain length is shown from top to bottom; DEGMA, to PEGMA, to NEGMA. The effect of the composition is shown from left to right; where the hydrophobic content, BuMA is decreased from 40 to 35 to 30 wt%. Both the hydrophobic content and the PEG side group chain length did strongly affect the thermoresponsive behaviour; as expected and observed previously for the hydrophobic content.^{45, 48}

In rheological terms, the gel point is defined as the point where the storage modulus exceeds the loss modulus.⁹⁰ A clear increase of both moduli and viscosity is observed for all the polymers with exception of Polymer 3. More specifically, the rheology curve of Polymer 3 denotes increased viscosity in the whole temperature range, which is in agreement with the visual observation; which will be discussed later. Two trends are generally observed in the rheology curves. Firstly, the sol-gel transition temperature is clearly decreased by decreasing the PEG side chain length from NEGMA, to PEGMA, to DEGMA, respectively. Secondly, the sol-gel transition is affected by the hydrophobic content; the temperature of abrupt increase is decreased by increasing the BuMA content. The effect of the polymer composition is in agreement with our previous studies on ABC triblock terpolymers, where the intermediate hydrophobic content showed the best sol-gel transition.^{45, 48, 50}

It should be noted that for the DEGMA and the PEGMA based polymers, viscosity of the solution appeared to be decreasing as the temperature increased, so a de-gelling behaviour was observed. However, this was attributed to the decreased solubility of these polymers and it was observed with the visual tests that this is due to syneresis and towards higher temperatures complete phase separation i.e. precipitation of the polymer.

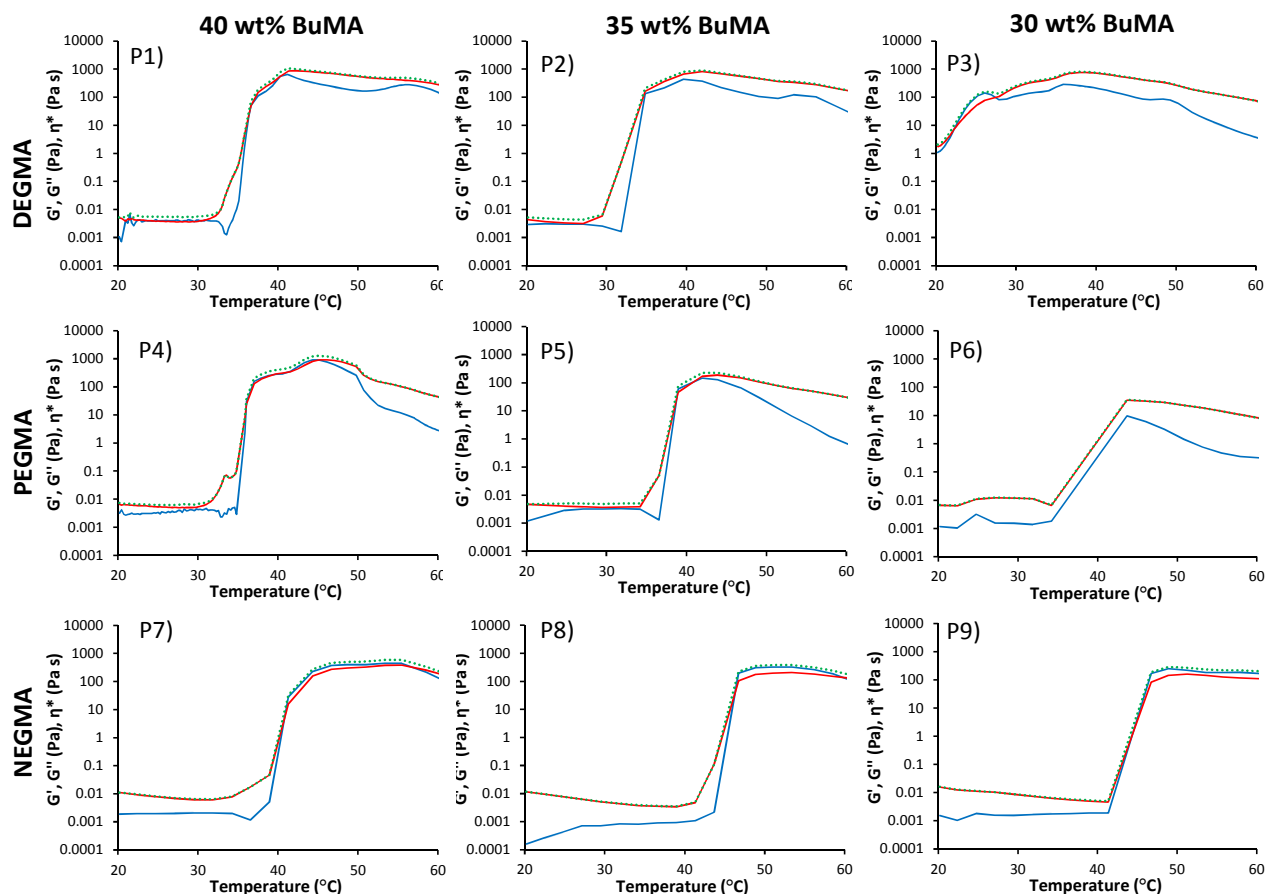


Fig. 6 Temperature ramp rheology curves of 15% w/w ABC copolymer solutions in PBS with varying compositions (left to right) and PEG side chain length (top to bottom). The solid blue, solid red and dotted green lines correspond to the storage modulus, loss modulus, and complex viscosity, respectively.

Visual gel point. All the triblock copolymers were tested visually for gelation over a temperature range of 20 to 80 °C and concentration range from 1 to 20 % w/w in PBS. The phase diagrams of the ABC triblock copolymer solutions in PBS are shown in Fig. 7. The statistical copolymer was not soluble in PBS, even at the lowest concentration and temperature investigated, and therefore it was not able to be tested.

It was observed that all the 1 % w/w copolymer solutions in PBS showed a cloud point; with the exception of Polymer 1 which demonstrated some solubility issues (most hydrophobic triblock copolymer out of the whole series). A trend was observed for the cloud points in PBS which was similar with the one observed for the cloud points in DI water. More specifically, the cloud point decreased by increasing the hydrophobicity; as expected.^{48-50, 89} By increasing the PEG length the cloud points in PBS also increased, similarly to the ones in water, as expected. At 2 % w/w the observations were similar but the cloud points were at lower temperatures as expected. At higher polymer concentrations (> 5 %

w/w) the thermoresponsive behaviour of the triblock was different depending on the composition and PEG length.

What was of special interest was if the triblock copolymer formed physical gels or not and interestingly only some of them did. Specifically, a clearly defined region where the polymer solution was in a gel-state was observed for Polymer 1-5 and Polymer 7, which were the most hydrophobic (both combination of hydrophobic content and PEG based macromonomer chain length). This is in agreement with previous studies where by increasing the hydrophobic content the gelation was facilitated.^{45, 48, 49, 91-94} This region is demonstrated with the dotted line on the phase diagrams and with the light blue and blue circles that denote cloudy and transparent stable gel, respectively.

It should be pointed out that the phase diagrams of the polymers whose solutions did form stable gels were similar. Specifically (i) a transparent or slightly cloudy polymer solution was observed at low concentrations and temperatures and (ii) as the concentration and

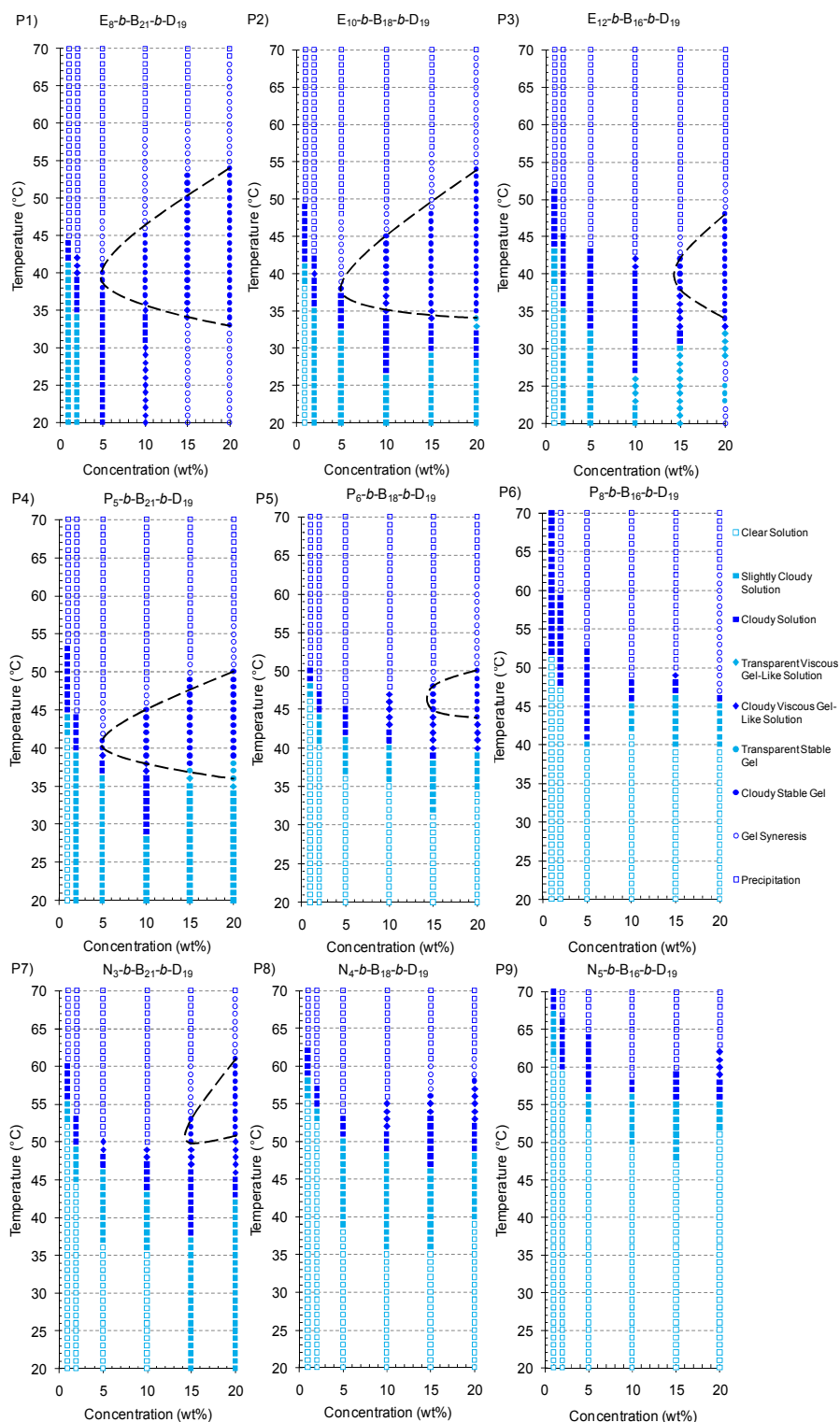


Fig. 7 Phase diagrams of the nine ABC triblock copolymer solutions in PBS with varying compositions (left to right) and PEG side chain length (top to bottom). The clear, slightly cloudy and cloudy solutions are indicated by white, light blue and blue squares. The light blue and blue diamonds correspond to transparent and cloudy viscous gel-like solutions, respectively. The transparent and cloudy stable gels, as well as the gel syneresis are shown by light blue, blue, and both blue and white circles, respectively. The square coloured in both blue and white corresponds to the precipitation. An approximation of the gelation region is denoted by black dashed line.

the temperature increased a viscous, gel-like solution was observed, (iii) then a stable gel and (iv) finally the polymer precipitated out and a phase separation was observed. The gelation temperature and concentration (the minimum temperature and minimum concentration where a stable gel was first observed) decreased as the hydrophobicity of the polymer decreased i.e. PEG length decreased and BuMA content increased, as expected. It should be noted that most of the observed gels were cloudy with the exception of Polymer 4, a PEGMA based polymer where transparent gels were observed at higher concentrations (15 and 20 % w/w) and this could be of interest for potential applications where transparency is important.

On the other hand, Polymers 6, 8, and 9 did not demonstrate a clear region where stable gels were presented, which was attributed to the increased hydrophilicity of these polymers. Thus, as the temperature or/and concentration was increased the viscosity of these polymer solutions increased but then the polymer became completely insoluble and phase separation was observed.

Attempting to compare the rheological with the visual test results is quite challenging. The rheological results clearly demonstrate an increase of viscosity at a temperature but which temperature that corresponds to, in terms of the visual results, is not easy to establish. In terms of the polymers that do form a physical gel visually, Polymers 1, 2, 3, 4, 5 and 7, the temperature that corresponds to a visual stable gel is always higher than the rheological one that just corresponds to a viscosity increase. Thus, the viscosity increase does not necessarily denote the formation of a stable gel visually. This was observed before and it was attributed to the fact that the visually obtained gel corresponds to a mechanically stronger gel than that when the corresponding rheological transition occurs.⁴⁸ When comparing the rheological results with the visual results for the polymers that did not visually present a stable physical gel but only a gel-like, viscous solution or a gel with syneresis, again, the rheological transition seems to happen at lower temperatures and it takes a couple of degrees for that transition to be visually observed.

Conclusions

The syntheses of nine well-defined ABC triblock copolymers and one statistical terpolymer were successfully performed by GTP. The copolymers were based on the ionic hydrophilic pH- and thermoresponsive DMAEMA, the non-ionic PEG based methacrylate (DEGMA, PEGMA, and NEGMA), and the hydrophobic BuMA. Three different compositions as well as the PEG side chain length were systematically varied in order to investigate their effect on the thermoresponsive behaviour of the copolymers. Micelle formation was observed for all the terpolymers and the effective pK_a s were affected by the hydrophobic BuMA content and the architecture. Interestingly, the cloud points were affected by both the composition (BuMA content) and the PEG side group length and increases as the hydrophilic content and the PEG length increased. The gel points were investigated over a wide range of temperatures and concentrations and found to be influenced

by both the composition and the PEG side chain length. Stable gels were formed by the most hydrophobic and with the shortest PEG length macromonomers.

Acknowledgements

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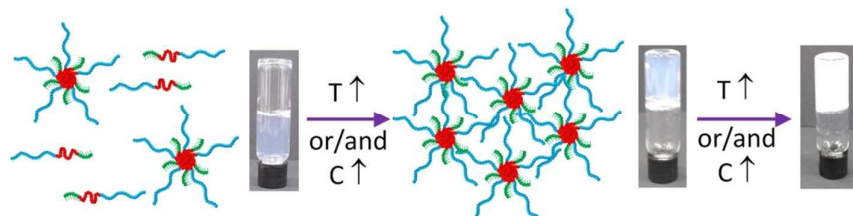
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Thermoresponsive Gels based on ABC Triblock Copolymers: Effect of the Length of the PEG Side Group

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ABC triblock copolymers of varying compositions and length of the PEG side groups were fabricated and their thermoresponsive behaviour was thoroughly investigated.