Polymer architecture dictates thermoreversible gelation in engineered emulsions stabilised with branched copolymer surfactants

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ABSTRACT

The generation of materials which switch from a liquid to gel state upon warming can enable new healthcare technologies with improved functionality, such as in situ gel-forming materials for drug delivery to topical or parenteral sites. The majority of these materials are aqueous polymer solutions, which then suffer from an inability to solubilise hydrophobic drugs. This study investigates the generation of thermoresponsive "engineered emulsions" which are low viscosity emulsions at low temperature but switch to a gel state upon warming. This is achieved by the synthesis of novel branched copolymers containing diethylene glycol methyl ether methacrylate (DEGMA) as a thermoresponsive component giving an LCST. The effect of polymer architecture is shown to be intimately linked to the rheology of these systems, where branching, elevation of molecular weight, and the presence of hydrophobic end groups is demonstrated to be commensurate with gel formation upon heating. Mechanisms of gel formation were probed by small-angle neutron scattering, which demonstrated that the branched copolymer surfactants formed oblate ellipsoids in solution which grew anisotropically with temperature, forming larger disk-like nanoparticles. The formation of these elongated particles leads to thickening of the systems, whilst connectivity of the aggregates and BCS at the oil-water interface is required for gel formation to occur. Overall, the study indicates design principles for this novel class of thermoresponsive material with great potential in healthcare, cosmetic, and energy applications.

KEYWORDS

Small-angle neutron scattering, rheology, gel, thermoresponsive, thermosensitive.

Introduction

Emulsions are metastable colloids made up of at least two immiscible phases in which one phase is dispersed in another and forms droplets.^{1,2} The immiscible fluids in emulsion systems may be kinetically stabilised by lowering interfacial tension,³ traditionally achieved using small-molecule or polymeric surfactants which adsorb at the liquid-liquid interface.¹ The use of macromolecules at the interface offers manipulation of chemistry such that in addition to stabilising the emulsion system, they impart stimuli-responsive – so-called "smart" – functionality.^{4,5} These stimuli-responsive polymeric materials undergo change in their physical and chemical properties in response to changes in their environment, such as mechanical stress, pH, light, temperature, and biological stimuli.^{6–9}

Thermoresponsive polymers modify their physical properties in response to temperature. Typically this manifests as an alteration of the solubility of the constituent units with temperature which can be coupled to the intrinsic ability to control polymer properties through modification of architecture and inclusion of co-monomers to lead to complex materials.¹⁰ Thermoresponsive materials are being widely employed in healthcare research, enabling novel and improved therapies and diagnostics, amongst a plethora of applications such as drug delivery platform, additive manufacturing and tissue engineering ¹¹. Polymer solutions that are capable of transforming a gel state with elevation of temperature on a critical point are termed as "Thermoreversible gels" or "Thermogelling materials".¹¹ A sol-gel transition occurs after heating a thermoreversible gel above a critical temperature, affected by an overall increase in hydrophobicity above the lower critical solution temperatures (LCST) triggering self-assembly processes and physical interaction which increases the viscosity of the system. ¹² These materials could transition from liquid to gel at body temperature, enabling *in situ* gelation and thus enhanced retention and therapeutic effect.¹¹ However, the vast majority of these materials are aqueous polymer solutions which often limits the application of the materials to hydrophilic encapsulants.

In 2008, Weaver introduced the term "emulsion engineering"¹³ to describe a droplet trapping concept in which the droplet surface functionality is designed to kinetically trap the droplets giving a percolating network structure in the material, resulting in solidification. Droplet assembly and dispersion were dependent on inter-droplet hydrogen bonding, controlled by acidic or basic pH conditions.¹³ Weaver and colleagues achieved these phenomena by stabilising emulsions with branched copolymer surfactants (BCSs) composed of a pH responsive monomer, a hydrophilic macromonomer for steric stabilisation, a crosslinker for branching and a chain transfer agent to introduce hydrophobic alkyl chain ends.^{14,15} The hydrophobic chain ends of the branched copolymers offers excellent attachment to the surface of the oil droplets and the capacity to alter the stabilisation and surface functionality of the droplet in the continuous aqueous phase.¹⁶ These features are the essentials of "engineered emulsions". This concept was demonstrated with weakly acidic polymeric surfactants that were able to cooperatively form hydrogen-bond between emulsion droplets in the unionised state, imparting a pH-dependent sol-gel behaviour. As is typical of emulsions, these systems are complex and polymers imparting stimuli-responsive behaviour are required to stabilise emulsions with oils of widely varied polarity, droplet size and oil/water ratio as well as varied emulsification technique ³. Thus, the field is both promising, and highly challenging. Our group has recently demonstrated for the first time that thermoresponsive engineered emulsions may be generated from poly(N-isopropylacrylamide) BCSs, in which poly(N-isopropyl acrylamide) exhibits an LCST. These emulsions successfully exhibited thermoreversible gelation, however the gel state was weak (G' of ca 30 Pa) and existed over a narrow temperature range.¹⁷ There is a need to generate thermoresponsive engineered emulsions of higher performance, which requires an understanding of how polymer architecture links to rheology.

This study reports the first thermoresponsive engineered emulsions from di(ethylene glycol) methyl ether methacrylate (DEGMA) branched copolymer surfactants. DEGMA, as a member of the highly versatile and biocompatible oligoethylene glycol methacrylate class,¹⁸ offers a promising blueprint for the generation of these materials. Polymer architecture will be varied to probe links with the temperature-dependent rheological behaviour of these systems, where there is no knowledge around how branching degree or hydrophobic chain ends affect thermoresponse, nor how molecular weight affects these DEGMA systems. The nanoscale

processes underpinning thermoresponse will be determined by small-angle neutron scattering. This approach will generate novel materials for healthcare applications along with design principles for these advanced functional materials.

Materials and Methods

Materials

Di(ethylene glycol) methyl ether methacrylate (DEGMA, 95%), Poly(ethylene glycol) methyl ether methacrylate (PEGMA, Mn 950 gmol⁻¹), Ethylene glycol dimethacrylate (EGDMA, 98%), and 1-Dodecanethiol (DDT, 99%), dodecane anhydrous (99%) were purchased from Sigma-Aldrich (UK). Alpha, alpha-azoisobutyronitrile (AIBN, >99%) was obtained from Molekula (UK). 1-Hexadedecanethiol (HDT, 97%) and 1-tetradecanethol (TDT, 94%) were purchased from Alfa Aesar (UK). Ethanol and dimethylformamide (DMF) were supplied by VWR (UK). Lithium bromide (99%) and 2-mercaptoethanol (ME, 99%) were purchased from Acros Organics (UK). Dialysis tubing with molecular weight cut off (MWCO) of 14 kDa was purchased from Sigma Aldrich (UK). GPC EasiVial poly(methyl methacrylate) mixed standards and a poly(methyl methacrylate) single standard (72 kDa) were procured from Agilent (UK). Deionised H₂O was employed in all experiments. All chemicals were used as received.

Synthesis of PDEGMA-co-PEGMA branched copolymer surfactant by free radical polymerisation

A series of thermoresponsive branched copolymer surfactants (BCSs) were synthesized by free radical polymerisation. In a general synthesis, DEGMA, PEGMA, cross-linker (EGDMA) and chain transfer agent (DDT/HDT/TDT/ME) were dissolved in 190 mL ethanol and bubbled with nitrogen gas. After 1 h of nitrogen purging, an ethanolic solution of AIBN (10 mL) was added to the solution. The apparatus was set at 70 °C for 48 h for polymerisation to proceed. After 48 h, the synthesised polymer was distilled at 80 °C to remove excess ethanol. The resultant crude polymer was then dissolved in water and transferred to a pre-soaked dialysis bag. The dialysis bag was immersed in a beaker containing de-ionised water for 7 to 10 days and the

water was replaced at regular intervals to facilitate the purification process. The resultant polymer solution was subjected to lyophilisation for 48 h to obtain a freeze-dried product. The yields for all the lyophilised polymers were above 85% calculated to the total mass of the raw materials.

A library of 9 polymers were produced, labelled P1-P9. P1-P3 explored the effect of branching degree by variation of cross-linker in feed (table 1) and P4-P6 explored the effect of Mn by control of initiator/chain transfer agent:monomer ratio (table 1). P7-P9 explored the effect of hydrophobic end group using the feed for P1 (table 1) but switching DDT for TDT (P7), HDT (P8) and ME (P9).

Sample ID:	P1	P2	Р3	P4	Р5	P6
DEGMA (mmol)	174	174	174	174	174	174
PEGMA (mmol)	6	6	6	6	6	6
EGDMA (mmol)	12	6	0	12	12	12
DDT (mmol)	12	12	12	8	6	3
AIBN (mmol)	1.2	1.2	1.2	0.8	0.6	0.3

Table 1. Reagent quantities for the synthesis of BCS P1-P6.

Characterisation of thermoresponsive BCSs

¹H NMR was used to characterise the BCS on Bruker Advance AM 600 NMR instrument using CDCl₃ as a solvent at ambient temperature. As an internal standard, the residual solvent peak was used. Delta 5.3.1 NMR software was used to process the data.

The number average molecular weight (Mn) and polydispersity of synthesised BCSs were characterised using an Agilent 12600 Infinity II GPC equipped with a refractive index (RI) detector. An Agilent Varian PLGel 5 μ m mixed D column was equipped to the system. 0.1% w/v lithium bromide in dimethylformamide was used as eluent, at a flow rate of 0.8 mL/min with the column temperature set at 30 °C. All samples were prepared in dimethylformamide

at a concentration level of 2 mg/mL prior to analysis. Dynamic light scattering was conducted on 1 mg/mL BCS samples in deionised water without filtration using a Malvern Nano-ZS instrument from 20-60 °C at 1 °C intervals.

Emulsion Preparation

A series of 1:1 w/w oil in water emulsions were prepared to study the thermoresponsive behaviour by rheology. 2.5 g Polymer solutions of 2.5, 5 and 10 wt% were prepared in ice cold water with stirring in a 30 mL glass vial. 2.5 g of dodecane oil phase was added to the polymer solution which was then homogenised for 2 min at 2,4000 rpm to obtain a 1:1 w/w oil in water emulsion using a Silverson L4R Heavy Duty Mixer Emulsifier (US). The resulting emulsions were kept undisturbed for 36 h at ambient condition. Approximately 1.1 g of the water phase separated at the lower phase of the creamed emulsion was withdrawn and the emulsion cream was studied for rheological behaviour with respect to its response to temperature using a rheometer. This gives an approximate oil phase volume of 0.57 in the emulsions studied.

Rheology of thermoresponsive emulsions

Rheology experiments were performed on an AR 1500ex rheometer by TA instruments (USA) equipped with a Peltier temperature control unit and a 40 mm parallel plate geometry with a specified gap distance of 500-750 μ m at oscillating stress of 1 Pa and frequency of 6.3 rad/s. The change in storage modulus (G') and loss modulus (G") as a function of temperature was recorded. Each emulsion's creamed layer was placed on the rheometer lower plate prior to the measurement after which temperature sweeps were performed. Temperature ramps were performed in the range 20 to 60 °C at 1 °C per minute intervals.

SANS studies on BCS solutions with variation of temperature

SANS experiments were performed on the D22 instrument at the Institut Laue-Langevin (Grenoble, France). The neutron wavelength was 6 Å, the sample-detector distances were 2, 5.6, and 17.6 m, and the collimation distances were 2.8, 8, and 17.6 m, respectively. The

detector offset was 300 mm. These settings gave a wave vector range $2.7 \times 10^{-3} \le q \le 0.45$ Å⁻¹. Rectangular quartz cuvettes with a thickness of 1 mm were used for all samples. Measurements were performed at 25, 40, and 50 °C with a minimum equilibration time of 15 min prior to sample run. All samples (P1-6) were prepared at 20 wt% in D₂O prior to measurement. Data stitching was performed on Igor Pro (Wavemetrics, USA)¹⁹ and data fitting was conducted using SasView 4.2.2. The following factors were set *a priori* to reduce the number of fitting parameters: scale/volume fraction (0.2), power law exponent (-4), and solvent SLD (6.37×10^{-6} Å⁻²). The remaining parameters were fit by the software. When discussing degrees of hydration, scattering length densities (SLDs) were calculated from the monomeric unit using the NIST Neutron activation and scattering calculator.²⁰ Rheological analysis of these samples was also conducted, using the protocols described above.

Results and Discussion

Synthesis of thermoresponsive BCS by free radical polymerisation

A modified one-pot synthesis was employed to generate an initial thermoresponsive branched copolymer surfactant (P1), informed by Weaver and co-worker's studies on pH-responsive Engineered Emulsions.^{13,14,21} P1 was synthesised using DEGMA as the thermoresponsive unit, PEGMA-950 as the hydrophilic macromonomer, EGDMA as the cross-linker, AIBN as the initiator and DDT as the chain-transfer agent (Figure 1i) in ratios guided by previous publications.²² DEGMA, a monomer which imparts temperature sensitivity with an LCST of 26 °C,²³ is expected to transition from a hydrophilic to a relatively hydrophobic state upon warming that was intended to trigger self-assembly processes leading to gelation. PEGMA, a hydrophilic macromonomer for stabilisation, is expected to preferably be solubilised in the water phase at the liquid-liquid interface and offer steric stabilisation over the measured temperature range. EGDMA is a widely used cross-linker in free radical polymerization which provides branching in the system. DDT was chosen as a chain transfer agent to interact favourably with the dodecane oil phase due to the hydrophobicity of the C12 alkyl chain, aiming to impart thermodynamic stability to the emulsion.



Figure 1: Free radical polymerisation of PDEGMA-PEGMA-EGDMA-DDT and theoretical structure of branched copolymer surfactant (i), exemplar GPC chromatogram of P4 (ii) and exemplar 1H NMR spectra (iii).

Following the synthesis of P1, a library of thermoresponsive BCSs were synthesised by varying composition of cross-linker, molecular weight and chain-transfer agents of longer and shorter carbon chain lengths, relative to P1, with the overall aim of linking polymer architecture to emulsion behaviour (table 2). The successful synthesis of these copolymers was confirmed by GPC and ¹H NMR (Figure 1ii and iii). P2 and P3 were based on the initial synthesis (i.e., P1) with half the quantity of crosslinker (EGDMA) and with no cross-linker, i.e. 6 and 0 mmol

respectively, with the intention of exploring the effect, and necessity, of branching. P4, P5 and P6 were variations of P1 with reduced levels of initiator (AIBN) and chain-transfer agent (DDT) at 0.8/8 mmol AIBN/DDT, 0.6/6 mmol AIBN/DDT and 0.4/4 mmol AIBN/DDT, respectively. As chains may be propagated from either AIBN-derived radicals or those transferred to DDT, a reduction in the quantity of these elements whilst retaining their ratio offers control of molecular weight by increasing the average degree of polymerisation per chain. P7, P8 and P9 were synthesised with varying carbon length of the chain transfer-agents whilst keeping the composition of DEGMA, PEGMA and EGDMA constant as per P1. P7, P8 and P9 were synthesised with TDT, HDT and mercaptoethanol respectively, giving chain ends of C-14, C-16, and a "zero-length" ethyl alcohol group. This series will evaluate the impact of the polymer chain-end hydrophobicity on the branched copolymer's aqueous solution characteristics, as well as exploring behaviour without hydrophobic termini (P9).

The ¹H NMR spectra (Figure 1iii) confirmed the polymerisation of branched copolymers with no evidence of vinylic protons in the 6-7 ppm region (P1-P9 spectra given in SI). GPC was employed to determine molecular weights and polydispersity indices (PDIs) of the BCSs, as well as confirming that distributions were monomodal. Indeed, GPC chromatograms (exemplar data in Figure 1ii) for all the polymers P1 to P9 displayed monomodal distribution of molecular weight. The GPC data recorded for such complex and amphiphilic architectures are likely to deviate from the true values of molecular weight.²⁴ As such, recorded molecular weight parameters are not absolute, but expressed relative to PMMA standards, they do indicate that the polymerizations were successful, followed expected trends, and allow determination of polydispersity indices (Đ). The polymers exhibited consistent and systematic compositional variation throughout the series.

Study	Sample	Composition / Variable	Mn (kg/mol)	Mw (kg/mol)	Ð
Effect of architecture & crosslinker	P1	Full cross-linker (12 mMol)	7.7	19.2	2.5
	P2	Half cross-linker (6 mMol)	5.9	11.7	2.0
	P3	No cross-linker (0 mMol)	5.2	8.3	1.6

Table 2: Composition of thermoresponsive BCSs and their characterisation by GPC

Effect of molecular	P4	0.8/8 mmol AIBN/DDT	10.5	33.3	3.2
weight	P5	0.6/6 mmol AIBN/DDT	13.6	68.0	5.0
	P6	0.4/4 mmol AIBN/DDT	20.6	181.7	8.8
Effect of chain-transfer	P7	Tetradecanethiol (C14)	8.8	24.4	2.8
agent (CTA)		Hexadecanethiol (C16)	8.9	25.1	2.8
	Р9	Mercaptoethanol (CO)	15.9	30.3	1.9

P1 to P3 evaluates the impact of cross-linker for thermoresponsive behaviour of the emulsion systems. GPC analyses for the branched copolymers P1, P2 and P3 revealed the number average molecular weight (Mn) of 7.7, 5.9 and 5.2 kg/mol respectively. A decrease in Mn and D was observed with lowering the cross-linker concentration (Table 1) whilst keeping the stoichiometry of the monomer to chain-transfer agent constant. Thus, it is intuitive that P1 with the highest concentration of cross-linker displays the greatest degree of branching and thus the highest molecular weight, with the Mn decreasing as cross-linker is reduced. P4 to P6 demonstrate that Mn may be tailored by AIBN/DDT in the feed. An increase in molecular weight and polydispersity is observed with reduced concentration of chain initiator and chaintransfer agent, allowing clear control over this parameter and granularity between Mn in emulsifiers. P7, P8, P9 are synthesized with varying carbon length chain-transfer agents. P7 is synthesized with TDT (C14), P8 with HDT (C16) and P9 with ME (C0). Comparing P9 (i.e., synthesis with ME) with P7 and 8, an apparent increase in Mn is observed though ME has shorter carbon chain length than DDT. This is attributed to the hydrophobic P7 and 8 interacting with column packing to increase elution times and reducing apparent Mn,²⁴ as well as the possibility of intramolecular interaction of DDT, both common limitations in GPC of amphiphilic copolymers.

All BCS were capable of stabilizing dodecane-in-water emulsions, giving rise to a creamed phase which was isolated for analysis. Droplet size analysis by light microscopy (figure S10) revealed mean droplet radii between 7.5 and 11 microns which gave rise to comparable levels of creaming between emulsions.

Effect of polymer branching on thermoresponse of BCS-stabilised emulsions

Rheology was used to measure temperature-induced changes in viscoelasticity of the creamed phase of BCS-stabilised emulsions. Viscoelasticity of emulsions is characterisation by the storage (or "elastic") modulus (G') and loss (or "viscous") modulus (G") with temperature. It is typically considered that the emulsion behaves solid-like when G'>G" and liquid-like when G'<G". In this work, the intersection of the G' and G" is considered as the gel point.



Figure 2: Rheological behaviour of emulsions stabilised by thermoresponsive BCSs where P1 is the sample with "full cross-linker", P2 is with "half cross-linker" and P3 has no cross-linker. Emulsions were explored at 2.5, 5 and 10 wt% polymer concentration. G' is shown in red and G" is shown in blue. Dark colours show the 'up' heating ramp whilst light colours show the subsequent 'down' cooling ramp.

BCS concentration had a large effect on emulsion behaviour with temperature (Figure 2). For the P1 system with the highest branching degree, all emulsions showed evidence of thermoresponse. At 2.5 wt% polymer concentration, a slight rise in G' and G" was observed at 45 °C which represents an overall increase in viscosity and was termed "thermo-thickening" behaviour. However, gelation, defined as a point where G' exceeds G", did not occur. A similar behaviour was observed with a polymer concentration of 5 wt% where the temperatureinduced thickening behaviour started at approximately 40 °C which increased with temperature up to 55 °C, however the emulsion again remained predominantly liquid-like at all temperatures (G''>G'). The magnitude of G' and G'' increase with temperature was greater in the 5 wt% emulsion than in the 2.5 wt% system. For emulsion systems with 10 wt% polymer concentration, a significant rise in G' and G" was observed at 40 °C followed by a cross-over point (i.e., when solid-like behaviour was first observed, as G' > G") at ca 48 °C, defined as the gelation temperature (Tgel). This Tgel is believed to correspond to the establishment of an inter-droplet percolating gel-network, as hypothesised in prior studies on responsive emulsions ^{13,14,17}. As aggregation progresses, both G' and G'' increased up to 55 °C. Beyond this temperature, the network giving rise to the gel state appeared to break down, due to a reduction in both G' and G". Hence, the study showed that the polymer concentration is an important factor that could be manipulated in an emulsion system to increase the thermal response and/or trigger thermo-gelation. Indeed, the high concentrations required for gelation suggest that polymer is likely to be spatially distributed both at the droplet interface and in the bulk, where both may be necessary for gelation to occur. All systems demonstrated reversibility of the rheological transitions, desirable to retain functionality after heating/cooling, barring the small increase in viscosity (G") noticeable in the "down" curve of the P1 emulsions at 2.5 and 5 %w/w. Overall P1 demonstrates that the polymerisation of DEGMA, presenting a LCST, and the hydrophilic macromonomer PEGMA in the BCS architecture demonstrate the desired thermoreversible gelation behaviour.

Variation of EGDMA in monomer feed allowed control of branching and its exploration as a factor in emulsion thermoresponse. P1 is most highly branched, followed by P2, then P3 has no branching present (table 1). In different systems, cross-linker to polymer ratio has previously allowed tuning of the LCST, polymer size and colloidal stability ^{25,26}, and thus was hypothesized to affect BCS performance. The effect of varying cross-linker (EGDMA)

concentrations on the rheology of emulsions stabilised with BCSs is shown in Figure 2. As discussed previously, thermoresponsive emulsions stabilized by P1 (full cross-linker) showed thermo-thickening behaviour at 2.5 and 5 wt% and thermoreversible gelation at a concentration of 10 wt%. The rheology of the emulsions stabilised by the BCS synthesised with half the quantity of cross-linker (P2) was also assessed at polymer concentration of 2.5, 5 and 10 wt%. At 2.5 wt% BCS concentration, emulsion thinning was observed at 23 °C which was stable until 47 °C and a small rise in G" was observed at 50 °C. Similar behaviour was observed with 5 wt% polymer concentration, however, the increase seen in G' and G" observed at 47 °C was greater in magnitude. After this point, the thickening event collapsed. Emulsions stabilised at 10 wt% polymer concentration showed two thermal events, which occurred at 25 and 50 °C. A second event of thermo-thickening is observed with the rise in temperature at 40 °C with Tgel occurring at 50 °C. The gel state was then stable up to 60 °C, highest temperature assayed.

BCS P3 contained no cross-linker and is expected to have a linear structure of PDEGMA-co-PEGMA with dodecyl chain ends. Temperature induced thinning was observed at 25 °C and no sign of thickening behaviour was observed until 60 °C. Despite gradual increase in polymer concentration at 2.5, 5 and 10 wt%, no significant changes to the thermal response were observed. This indicated that the presence of cross-linker to form BCSs is essential in the design of these emulsions. This highlights the importance of the branched structure in enabling "engineered emulsions". This finding is consistent with Weaver and co-workers' study of pH-responsive emulsions which attributed this effect to the presence of multiple hydrophobic DDT domains per polymer chain in the branched systems, allowing stronger tethering to the oil phase and ultimately the ability to form elastically active bridges between emulsion droplets ^{14,27}.

SANS of BCS solutions was conducted to explore mechanisms of gelation. For these synthesized BCS, three temperatures were investigated to capture the behavior across the gelation process namely: 25, 40 and 50, where 25 lies below the LCST, 40°C around the expected onset of transition and 50°C above the transition. 20 %w/v solutions were explored for each temperature, which is equivalent to the aqueous phase used in the generation of 10 wt% emulsion systems. The data were fitted with the combination of form factors, P(q), and structure factors, S(q). The form factors describe the morphology of the scattering object and

the structure factor describes the interaction between the particles. The SANS data were fitted with a constant scattering length density (SLD) of 6.37 for the D₂O solvent whereas the SLD for the BCS were varied where there is uncertainty around the degree of hydration of the polymers. Volume fractions were approximated *a priori* to 0.2. Form factors required for fitting were ellipsoids (El) and a power law (PL), whilst hard sphere (HS) and sticky hard sphere (SHS) structure factors were required in some instances.



Figure 3. SANS data (circles) with fits (lines) of 20 wt% BCS solutions in D_2O with variation of cross-linking and temperature. Legend inset with sample id and cross-linker feed. Models used to fit data inserted top right.

Initially, the BCS series exploring branching density was examined by SANS. This series had cross-linker feed at 12, 6, and 0 mmol, for P1, P2, and P3, respectively (Figure 3). At 25 °C (below LCST), the 20% w/v solutions were best described by an ellipsoid form factor with polar radius greater than equatorial radius, indicating an oblate spheroid. Fitting was attempted with spherical and prolate spheroid models, but adequate fits could not typically be achieved. Cylinder form factors resulted in disk-like objects of large radii and short length, giving poorer fits than the oblate spheroids. The fitting required a contribution from a hard-sphere structure factor in most instances. Moreover, a power-law form factor was added to the model to fit the low q region, believed to arise from larger aggregates or clusters of polymer, as observed in PEO homopolymers.²⁸ The precise nature of these objects cannot be determined reasonably within this Q range but fit to a Q⁻⁴ decay, indicative of a sharp interface. Upon warming to 40 and 50 °C, the oblate ellipsoid model gave strong fits to the data but the power law contribution was greatly reduced and was no longer required to fit the data in branched systems (P1,P2) which can be interpreted as a reduced fraction of polymer chains in solution which gave rise to this clustering.

Without steric constraints surfactant molecules tend to form spherical micelles, however it is known that chemical structure may disallow the surfactant to take the required curvature for this shape.²⁹ In particular, when the tail-group of the surfactant is large compared the head-group, the surfactant will tend to form anisotropic structures such as cylinders, ellipsoids, or lamellae, based on the "critical packing parameter". This theory has also been applied with great success to block copolymers.³⁰ In these BCS it may be rationalized that, particularly above LCST, the volume of the "hydrophobic" moieties (DEGMA above LCST and DDT chainends) will be large relative to the PEG headgroups given the excess DEGMA in the feed, which may disallow spherical micelles to be formed. Furthermore, given the presence of multiple DDT chain ends per BCS and steric constraints from the branched structure, it is plausible that the macromolecules cannot take the spherical conformation and instead tend towards objects of lower curvature, such as these oblate ellipsoids.

The dimensions of the oblate ellipsoidal particles formed by P1-P3 solutions are given in Figure 4. Strikingly, all ellipsoids increase in dimensions and become increasingly oblate (equatorial radius > polar radius) with heating. For example, P1 has an elliptical form which

upon heating increases approximately 11.5-fold in volume and becomes more disk-like in shape, with the ratio of equatorial to polar radius increasing from 3.5 to 6.3. Whilst P1-P3 have similar polar radii, the equatorial radii are greatly increased with branching, indicating increased oblate character in the order P1>P2>P3. It is believed that this is a result of the greater restrictions on acquiring high curvature as branching is introduced. For example, P3 at 25 °C has the lowest degree of ellipsoidal character and could be fitted as a polydisperse sphere but is kept as an ellipsoid for consistency.



Figure 4. a. Dimensions of BCS ellipsoidal particles with variation of cross-linking and temperature, derived from SANS fitting and b. degree of hydration of BCS particles estimated from SLD.

Furthermore, the systems appear to become increasingly dehydrated as they are heated. Hydration of the BCS particles was estimated using SLD from the fits against SLD for pure DEGMA $(0.620 \times 10^{-6} \text{Å}^{-2})^{20}$ and pure D₂O $(6.37 \times 10^{-6} \text{Å}^{-2})$. It can be seen in Figure 4 that all BCS particles reduce their D₂O content with heating. This is consistent with the thermoresponsive DEGMA component undergoing a desolvation when heated above LCST and expelling D₂O. Given the potentially counterintuitive combination of particle growth and desolvation, the hypothesized process occurring during heating is the combination of ellipsoidal species as DEGMA desolvates, resulting in growth. P1 and P2 had lower degrees of hydration than P3, which is attributed to the dense branching structure hindering solvation of the inner moieties of the BCS.

Overall, the BCS formed ellipsoids which grew anisotropically and became less solvated with temperature. The nature of thermothickening (P1,P2) vs thermothinning (P3) (figure 2) from this data may be prescribed to differing nature of aggregates, with P1 and P2 forming larger, more oblate, particles facilitating jamming and connectivity in the system. Indeed, P1 at 50 °C, which gave the strongest gel-like rheology, was fit with a sticky hard-sphere structure factor, indicative of inter-ellipsoid attraction. An additional factor in gel formation which may not be observed by SANS is the interfacial characteristics of the BCS. It has previously been postulated that pH-responsive BCS exhibited multiple tethering points to oil droplets when a branched structure with multiple dodecyl- chain ends was present.²² This strength of tethering to the oil phase could be essential to enabling droplet-droplet connectivity to allow gel formation, providing rationale for the thermothickening behavior of the branched (P1,P2) vs non-branched (P3) polymers, given that the branched (P1,P2) systems have multiple hydrophobic chain ends per macromolecule.

Effect of polymer molecular weight on the rheology of BCS-stabilised emulsions

The effect of molecular weight on the rheological behaviour of thermoresponsive emulsions was then studied. Figure 5 shows the rheology of thermoresponsive emulsions stabilised with P1, P4, P5 and P6 (increasing rank order of Mn, table 1) at concentrations 2.5, 5 and 10 %w/w. The rheological behaviour of P1 has been discussed previously, and this data is included as a reference point for the additional BCSs. At 2.5 wt%, the rheological behaviour of emulsion stabilised with P4, P5 and P6 did not show clear differences to P1, with thermal response weak in these emulsions. The thermo-thickening was enhanced relative to P1 at 5 wt% emulsion stabilised with P4, however, gelation did not occur (G' < G''). Emulsions stabilised with 5 wt% of P5 demonstrated Tgel at ca. 45 °C. However, this gelation was not stable over a broad range of temperature exhibiting breaking of the gel at 50 °C. Similarly, the emulsion stabilised with the highest molecular weight polymer, P6, at 5 wt% exhibited Tgel at 45 °C which was stable until 55 °C. Thus, greater molecular weights appear to favour gel formation, shifting the concentrations required for gel formation to lower values. This effect may be

attributed in part to longer polymer chains being more likely to be able to form physical connection to other polymer chains. With increase in polymer concentration to 10 wt%, the emulsions stabilised with P4, P5, and P6 exhibited Tgel at 46, 45, and 40 °C while heating and showed similar thermo-reversible behaviour while cooling. Moreover, the length of gelation plateau was stable to the highest temperature assayed 60 °C indicating the gel stability over a broad range of temperature. The storage moduli (G') of P5 and P6 was ca ten-fold greater in the gel state (up to ca 300 Pa) than the previously reported PNIPAM BCS systems (ca 30 Pa).¹⁷ Furthermore, the onset of thickening for these two constructs was ca 35 °C, such that hardening could be triggered upon exposure to the body's heat, which is advantageous for drug delivery.³¹ Thus, this new blueprint for BCS is able to generate materials with much greater rheological properties for applications requiring resistance to shear, such as drug delivery to topical sites or depot injections.



Figure 5. Effect of molecular weight and polymer concentration on thermo-gelation activity of emulsion. Molecular weight of thermoresponsive BCSs P1<P4<P5<P6.



Figure 6. SANS data (circles) with fits (lines) of 20 wt% BCS solutions in D_2O with variation of molecular weight and temperature. Legend inset with sample id and Mn determined by GPC. Models used to fit data inserted top right.

The effect of molecular weight of BCS on nanostructure was then evaluated by SANS (Figure 6). This series explored Mn at 7.7, 10.5, 13.6, and 20.6 for P1, P4, P5, and P6 respectively. Please note that polydispersity also increased with Mn (Table 1). At 25 °C the P4 data could again be fit to ellipsoids with a power law contribution, however the power law was not needed at P5. Once samples were heated to 40 and 50 °C, SANS data for P1, P4 and P5 showed only subtle variation in structure, in-line with their comparable rheology at these temperatures. Parameters extracted from the fitting again reflect this subtlety, with only small deviations in hydration and nanoparticle dimensions (Figure 7). P6 (ESI) SANS experiments showed vastly different scattering profiles which have been tentatively assigned to multiple ellipsoids at 25 and 40 °C, before forming an ellipsoid of equivalent dimensions to the other Mn samples at 50 °C, without the need for a structure factor. The presence of multiple objects in the P6 SANS data is likely a result of its high polydispersity (θ = 8.8). Overall, these SANS experiments varying Mn demonstrate reproducibly the presence of oblate ellipsoids at high temperatures amenable to a gel state.



Figure 7. a. Dimensions of BCS ellipsoidal particles with variation of Mn and temperature, derived from SANS fitting and b. degree of hydration of BCS particles estimated from SLD. Please note P6 is omitted due to the different approach to fitting used and presence of multiple species in solution.

Effect of chain-transfer agent (CTA)

The effect of the polymer chain end hydrophobicity on thermoresponsive behaviour of the emulsions was studied. P1, P7 and P8 were synthesised with mercaptoalkanes comprising of C12, C14 and C16 carbon chain lengths leading to hydrophobic tails on the BCS. BCS P9 was synthesised with mercaptoethanol as a CTA to generate a "zero-length" chain end with 2-hydroxyethyl- functionality which is expected to be hydrophilic. Emulsions prepared with hydrophilic and hydrophobic thiols as CTAs at different polymeric concentration levels provided an insight of thermoresponsive activity as shown in Figure 8.



Figure 8: Effect of hydrophobic alkyl tail length on thermo-gelation activity of emulsions as determined by rheometric temperature ramp. P9 is synthesized with mercaptoethnol, P1 with dodecanethiol, P7 with tetradecanethiol and P8 with hexadecanethiol, leading to alkyl chain lengths of 0, 12, 14, and 16 C atoms, respectively.

The thermoresponsive activity of emulsions at 2.5 wt% polymer concentration P1, P7, P8 and P9 showed very weak response and were comparable. At 5 wt% polymeric concentration, the thermo-thickening activity was enhanced with P7 and P8 as compared to P1. Tgel occurred at 50 °C, however this gel state was only stable over a small temperature range. Similarly, emulsion stabilised with P7 at 10 wt% polymer concentration showed themo-thickening but no gelation. The thermoresponsive behaviour of emulsion stabilised with P8 resembled P1 with gelation occurring ca. 47 °C however, the gelation activity seemed to be unstable beyond 55 °C. The rheology of the emulsion stabilised with P9 (Figure 4a) demonstrated that hydrophobic CTA is necessary for thermoresponsive activity. It was observed that P9 stabilised the emulsion system despite a lack of hydrophobic tails, forming emulsions at various polymeric concentrations of 2.5, 5 and 10 wt% but demonstrated weak thermoresponsive activity. The rheology data suggested that the CTAs exert control over the thermo-thickening behaviour of emulsion systems because these hydrophobic alkyl groups with strong interaction to the oil phase are required for gelation to occur. Dynamic light scattering (Figure 9) revealed further differences in self-assembly between the BCS with hydrophobic alkyl groups (P7) and those terminated with -CH₂CH₂-OH (P9). P9 (C0) showed a single, sharp transition at 29 °C to switch from a low scattering sample to a high scattering sample of large D_H and PDI (270 nm, 0.27 PDI). P7 (C14) scattered light, associated with clusters of polymer (169 nm diameter, 0.09 PDI), below 25 °C before transitioning to smaller nanoparticles (ca 34 nm, 0.12 PDI, at 30 °C) in the region ca 27-35 °C. A second transition then occurs > 35 °C resulting in the nanoparticles transitioning to a high D_{H} (51 nm, 0.07 PDI). In line with SANS, DLS of P7 evidenced larger aggregates at low temperatures, followed by the formation of well-defined nanoparticles above PDEGMA LCST which grew with temperature increase. Without the presence of hydrophobic alkyl groups, P9 (C0) simply underwent transition from polymer in solution to heterogeneous aggregates.



Figure 9. Dynamic light scattering (DLS) of P7 (C14) and P9 (C0) solutions showing normalized scattered count rates with temperature.

The rheology of BCS solutions (20 wt%) in D₂O was also studied to probe whether structuration in SANS is associated with phase changes and if the presence of emulsion droplets is required for network formation (Figure 10). This concentration is equivalent to 10 wt% BCS-stabilised emulsions, in which the mass of the oil phase reduces the overall BCS concentration, but the hydrophilic BCS is only present in the aqueous phase, at approximately 20 wt%. All BCS solutions retained thermal transitions which were in accordance with their emulsion rheology, showing sharp thickening with temperature except for P9 which had no hydrophobic chain end, which showed a weaker response. However, a notable difference is in the overall liquid-like behavior of the systems, in which G' never exceeds G", giving rheology like a thick paste when heated. Thus, the presence of emulsion droplets is crucial for the formation of a gel network. This connectivity could arise from interaction of BCS at the oil-water interface with the nano-aggregates in the bulk or from interface-interface interactions between droplets, as hypothesized by Weaver et al in pH responsive engineered emulsions.¹⁵ The rheology of emulsions does not, however, show thermal gelation at low BCS

concentrations (2.5 wt%) which are sufficient to stabilize the emulsion, thus it is proposed that BCS interaction from the interface to bulk above LCST is the mechanism for gel formation in these systems.



Figure 10. Temperature ramp rheology of 20 wt% BCS solutions in D₂O. Filled and open circles show the heating and cooling cycles, respectively.

Conclusions

This research demonstrated that thermoresponsive engineered emulsions may be generated from branched copolymers of polyDEGMA and PEGMA. The materials generated reliably

exhibit sol-gel transition upon warming across a number of different concentrations and architectures, forming materials up to ten-times stronger than the previously reported thermoresponsive engineered emulsions.¹⁷ Variation of cross-linker density proves for the first time that a branched structure is required for effective transition to the gel state, with the phenomenon "turned off" in the non-branched systems. Increasing molecular weight of the BCS made improvements to the gel strength (G') and pushed thickening of the system into a physiologically relevant temperature range (ca 35 °C). Variation of alkyl length on the BCS chain ends showed that the blueprint reliably exhibited thermoreversible gelation across C12-C16, but that the phenomenon was lost when this hydrophobic character was removed. This demonstrates that effective tethering to the emulsion droplets with this hydrophobic anchor is required to form a gel phase, which is believed to require both BCS-BCS interaction in the bulk and at the interface. Bulk behavior of the BCS was explored by SANS and revealed that the materials form oblate ellipsoids in solution which grew anisotropically with temperature, accompanying the thermothickening/ thermoreversible gelation events. This builds up a hierarchical picture of self-assembly. At low temperatures, polymer exists at the interface, tethered by hydrophobic end groups, and in the bulk as a mixture of ellipsoidal particles and large clusters of polymer. Upon heating the system switches to producing oblate ellipsoids of increasingly greater size, with equatorial growth exceeding growth at the polar radius. It is believed that connectivity of these nanoparticles and polymer at the interface leads to the formation of a gel state. This new class of BCS allows effective generation of thermoresponsive engineered emulsions along with principles for the design of advanced materials for exploitation in a wide range of fields, particularly in pharmaceuticals.

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Data access statement

Data available on reasonable request from the corresponding author

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