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CO₂-responsive polyacrylamide copolymer vesicles with acid-sensitive morpholine moieties and large hydrophobic RAFT end-group

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ABSTRACT: Sequential Reversible Addition Fragmentation Chain Transfer (RAFT) polymerizations using 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) and 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) were used to give amphiphilic polyacrylamide block copolymers containing *N*-(2-morpholin-4-ylethyl) acrylamide (MEA), where the morpholine moieties are CO₂-responsive. The order in which monomers were polymerized determined the placement of the large hydrophobic RAFT end group with more complex ABA' type self-assembly (e.g. patterned and large worm-like vesicles, large compound micelles) obtained when the dodecyl trithiocarbonate end-group was attached to the hydrophilic poly(MEA) block. Cleaving the hydrophobic end group reverts self-assembly to simpler spherical vesicles observed in the triblock of the same chemical composition, but with the RAFT end group attached to the hydrophobic poly(*tert*-butyl acrylamide) block. Ionization of the hydrophilic poly(MEA) block through flushing with CO₂ irreversibly shifts self-assembly towards lower order morphologies with spherical micelles being more favoured than vesicles.

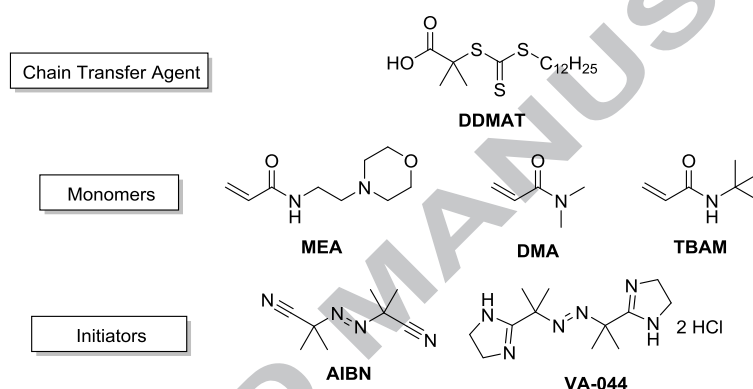
Keywords: Block copolymer, Micelle; Radical polymerization; Self-assembly

1. Introduction

Benign CO₂ gas is an attractive stimulus for smart or switchable polymers due to its easy removal by flushing with an inert gas such as N₂ or Ar.¹⁻³ The main advantage of using CO₂ over solutions of acids and bases is the convenience of repeated switching of cycles without salt accumulation. A large number of factors can affect the self-assembly of amphiphilic block copolymers in aqueous solutions,⁴ including tuning of the morphology by altering the degree of protonation or solubility of the block containing basic sites. The two types of CO₂-sensitive moieties (basic sites) typically utilised are amidine and tertiary amines. For example, self-assembled amphiphilic block copolymers containing hydrophilic poly(ethylene oxide) and hydrophobic poly(*N*-amidino)dodecyl acrylamide blocks form spherical vesicles that expand and contract upon the reversible reaction of the amidine moieties of the polymer core with CO₂.^{5,6} Increasing the size of the polystyrene (St) spacer in the amphiphilic triblock copolymer poly(ethylene oxide, EO)-*b*-(styrene, St)-*b*-(*N*-(2-diethylamino)ethyl) methacrylate) enabled spherical micelles to transform into worm-like nanostructures by applying CO₂.⁷ Sensitivity to more than one stimulus has been established as exemplified by dual CO₂ and temperature response enabling the variation of the polymer lower critical solution temperature (LCST).⁸⁻¹⁰ The copolymer consisting of poly(*N*-(2-dialkylamino)ethyl) methacrylate) and poly(*N*-isopropylacrylamide, NIPAM) blocks in dilute aqueous solutions underwent vesicle to micelle phase transitions with CO₂ and temperature due to the conversion of both blocks into hydrophilic states.⁹ Incorporating a hydrophobic UV-responsive block with poly(*N*-(2-dimethylamino)ethyl) methacrylate) in a diblock copolymer gave micelles that allow controlled drug release using a variety of stimuli.¹¹ Gao *et al.* reported amphiphilic block copolymers of poly(EO) and 2-(cycloalkylamino)ethyl methacrylates (where cycloalkylamino = pyrrolidine, piperidine and azepane), which self-assembled into micelles before a reduction in pH caused complete dissociation, allowing for selective payload release in endosomes/lysosomes.¹² However, nitrogen heterocycles have thus far not been utilised as the CO₂-responsive moieties of the polymer - we here report amphiphilic polymers containing ionizable morpholine moieties.

The conventional radical and nitroxide-mediated polymerization (NMP) of *N*-(2-morpholin-4-ylethyl)acrylamide (MEA, Scheme 1) has been reported,^{13,14} and although NMP gives controlled/living character, extensive chain transfer at the polymerization temperature of 120 °C limited the achievable molecular weights.¹⁴ The advantages of Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization over other types of reversible

deactivation radical polymerizations are well-documented,^{1,15,16} and include its application to a wider range of monomers and lower reaction temperatures. RAFT allows the rapid synthesis of water-soluble multi-block polyacrylamides with high degrees of livingness and narrow molecular weight distributions (MWDs),¹⁷⁻¹⁹ including polyacrylamides containing methylene substituted saturated nitrogen heterocycles.¹⁹ The present article describes the efficient syntheses of unique amphiphilic di- and tri-block copolymers consisting of exclusively polyacrylamide blocks containing ionisable morpholine moieties that self-assemble into a variety of morphologies. The influence of the order of polymerization, block length, end-group, CO₂ and pH on self-assembly in dilute aqueous solutions is examined.



Scheme 1 General structures of reactants used.

2. Experimental

2.1. Materials

N-(2-morpholin-4-ylethyl) acrylamide (MEA) was prepared by the reaction of acryloyl chloride (Sigma-Aldrich, $\geq 97.0\%$) with 4-(2-aminoethyl)morpholine (TCI Chemicals, $>98\%$) and triethylamine (Sigma-Aldrich, 99%) in CH₂Cl₂ and recrystallized from ethyl acetate.²⁰ 2-(Dodecylthiocarbonylthio)-2-methylpropanoic acid (DDMAT, Sigma-Aldrich, 98%) and 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044, Wako) were used as received. 1,4-Dioxane (Sigma-Aldrich, $\geq 99.0\%$) and Milli-Q water were used directly as solvents for polymerization. *N*-*tert*-butylacrylamide (TBAM, TCI Chemicals) was recrystallised from hexanes and *N,N*-dimethylacrylamide (DMA, TCI, 98%) was distilled *in vacuo* to remove radical inhibitor. *N,N*-dimethylformamide (DMF; Sigma-Aldrich, HPLC-grade $\geq 99.9\%$), CH₂Cl₂ (Sigma-Aldrich, $>99\%$), CDCl₃ (Aldrich, 99.8 atom %), D₂O

(Aldrich, 99.9 atom %), LiBr (Aldrich, 99%), CO_{2(g)} (Food Grade, BOC), and N_{2(g)} (O₂ free, BOC) were used as received.

2.2. Measurements

Nuclear Magnetic Resonance (NMR) Spectroscopy. ¹H NMR spectra were recorded using a Joel GXFT 400 MHz instrument equipped with a DEC AXP 300 computer workstation. Conversions for all polymer samples were estimated by deducting the monomeric contribution of an integral, which contains both polymer and monomer contributions. For the polymerization of MEA conversion was estimated using the integral for the polymer contribution at 2.35 – 2.70 ppm (NCH₂, 2-CH₂, 6H) relative to the monomer peak at 5.65 ppm (vinyl, 1H). For the polymerization of TBAM conversion was estimated using the integral for the polymer contribution at 1.05-1.45 ppm (^tBu, 9H) relative to the monomer peak at 5.57 ppm (vinyl, 1H). For the polymerization of DMA conversion was estimated using the integral for the polymer contribution at 2.76–3.22 ppm (Me, 6H) relative to the monomer peak at 5.67 ppm (vinyl, 1H). The relative composition or the degree of polymerization for the purified block copolymers containing both TBAM and MEA is estimated by comparing MEA integral at 3.50 – 4.00 ppm (OCH₂, 4H) with the TBAM integral at 1.05-1.45 ppm (^tBu, 9H).

Theoretical number average molecular weight ($M_{n,th}$) was calculated according to equation 1:

$$M_{n,th} = \left(\frac{[Monomer]_0}{[RAFT]_0} \times MW_{Monomer} \times Conversion \right) + MW_{RAFT} \quad (1)$$

RAFT represents DDMAT or polymeric macroRAFT agent. $MW_{monomer}$ and MW_{RAFT} are the molecular weights of the monomer and (macro)RAFT agent respectively. Conversion was measured by ¹H NMR (as above) and isolated yields for the triblocks are by gravimetry.

Calculating the Theoretical Number of Living Chains (L). In degenerative transfer systems such as RAFT, it is advantageous to use low initiator concentrations in order to improve L , since the number of chains that undergo bimolecular termination directly corresponds to the number of radicals generated from decomposition of the initiator during polymerization:¹⁷

$$L = \frac{[RAFT]_0}{[RAFT]_0 + 2f[I]_0(1 - e^{-k_{at}t}) \left(1 - \frac{f_c}{2}\right)} \quad (2)$$

Equation 2 estimates L , where f is the initiator efficiency (assumed to be 0.5), and the decomposition rate constant k_d is taken as $3.20 \times 10^{-4} \text{ s}^{-1}$ for AIBN at $70 \text{ }^\circ\text{C}$ in dioxane/water (80:20),²¹ and $4.30 \times 10^{-4} \text{ s}^{-1}$ for VA-044 at $70 \text{ }^\circ\text{C}$ in water/dioxane (80:20) (conditions employed in the present work).¹⁸ The quantity $1 - \frac{f_c}{2}$ accounts for the effect of the termination mechanism (combination or disproportionation) on the number of chains.

Gel Permeation Chromatography (GPC). Molar mass distributions were measured using Agilent Technologies 1260 Infinity liquid chromatography system using a Polar Gel-M guard column ($50 \times 7.5 \text{ mm}$) and two Polar Gel-M columns ($300 \times 7.5 \text{ mm}$). DMF containing LiBr (0.01 M) was used as eluent at $1.0 \text{ mL}\cdot\text{min}^{-1}$ at $60 \text{ }^\circ\text{C}$. Twelve narrow polydispersity poly(methyl methacrylate, MMA) standards (EasiVial PM 2 mL, Agilent) were used to calibrate the GPC system. Samples were dissolved in the eluent and filtered through a PTFE membrane with $0.2 \text{ }\mu\text{m}$ pore size before injection ($100 \text{ }\mu\text{L}$). Experimental molar mass (M_n) and dispersity (\mathcal{D}) values were determined by conventional calibration using Agilent GPC/SEC Software for Windows (version 1.2; Build 3182.29519). ($M_n = 550 - 2,136,000 \text{ g}\cdot\text{mol}^{-1}$). Number average molecular weight (M_n) values are not absolute, but relative to linear poly(MMA) standards (as above).

Transmission Electron Microscopy (TEM). TEM images were obtained using a Hitachi H7000 using formvar/carbon 200 mesh Cu grids. One drop ($\sim 0.1 \text{ ml}$) of the micellar solution was placed on the copper grid and allowed to settle. After 10 min excess solution was carefully wicked with filter paper and allowed to dry for a further 5 min prior to TEM analysis.

2.3. General polymerization procedure

All polymerization solutions were added to borosilicate glass tubes sealed with septa and flushed with N_2 for 30 min. The solutions were heated at $70 \text{ }^\circ\text{C}$ in an aluminium heating block for 2 h unless otherwise stated. Polymerizations were stopped by placing glass tubes in an ice-water bath. Unless otherwise stated iterative chain extension reactions were performed directly on the macroRAFT agent reaction solution with the amount of initiator remaining after each cycle taken into account, according to equation 2. Where the block copolymer is isolated, unless otherwise stated, the polymerization mixture was dissolved in DMF (1-2 mL) and precipitated from an excess of petroleum ether, filtered, and dried at room temperature under vacuum for 24 h.

2.4. Preparation of poly(TBAM)_{80-b}-(TBAM)_{80-b}-(MEA)₈-S(C=S)SC₁₂H₂₅ copolymer

A stock solution containing DDMAT (45.7 mg, 0.125 mmol), VA-044 (1.0 mg, 0.0031 mmol) in 10 mL dioxane/water (80:20) was prepared. One aliquot (1 mL) was added to TBAM (0.128 g, 1.00 mmol) to give 1.0 M monomer solution, and heated as described above. TBAM (0.128 g, 1.00 mmol) and VA-044 (2.98×10^{-4} mmol from a stock solution) in 0.5 mL dioxane/water (80:20) were added to the latter poly(TBAM)₈₀-S(C=S)SC₁₂H₂₅ solution, and heated as described above. MEA (23 mg, 0.125 mmol) and VA-044 (0.00124 mmol from a stock solution) in 0.5 mL dioxane/water (80:20) were added to the latter poly(TBAM)_{80-b}-(TBAM)₈₀-S(C=S)SC₁₂H₂₅ solution, and heated as described above. Poly(TBAM)_{80-b}-(TBAM)_{80-b}-(MEA)₈-S(C=S)SC₁₂H₂₅ was isolated as described above, $M_n = 20,700 \text{ g}\cdot\text{mol}^{-1}$, $M_w/M_n = 1.26$, 80% conv., isolated yield = 0.248 g.

2.5. Preparation of poly(TBAM)_{40-b}-(DMA)_{50-b}-(MEA)₄₉-S(C=S)SC₁₂H₂₅ copolymer

A stock solution containing DDMAT (91.4 mg, 0.25 mmol), VA-044 (2.0 mg, 0.0063 mmol) in 10 mL dioxane/water (80:20) was prepared. One aliquot (1 mL) was added to TBAM (0.128 g, 1.00 mmol) to give 1.0 M monomer solution, and heated as described above. DMA (0.125 g, 1.26 mmol) and VA-044 (9.59×10^{-5} mmol from a stock solution) in 0.5 mL dioxane/water (80:20) were added to the latter poly(TBAM)₄₀-S(C=S)SC₁₂H₂₅ solution, and heated as described above. MEA (0.325 g, 1.76 mmol) and VA-044 (0.0025 mmol from a stock solution) in 0.5 mL dioxane/water (80:20) were added to the latter poly(TBAM)_{40-b}-(DMA)₅₀-S(C=S)SC₁₂H₂₅ solution, and heated as described above. Poly(TBAM)_{40-b}-(DMA)_{50-b}-(MEA)₄₉-S(C=S)SC₁₂H₂₅ was isolated as described above, $M_n = 18,000 \text{ g}\cdot\text{mol}^{-1}$, $M_w/M_n = 1.28$, 70% conv., isolated yield = 0.410 g.

2.6. End Group Removal from poly(TBAM)_{40-b}-(DMA)_{50-b}-(MEA)₄₉-S(C=S)SC₁₂H₂₅ copolymer

A solution containing poly(TBAM)_{40-b}-(DMA)_{50-b}-(MEA)₄₉-S(C=S)SC₁₂H₂₅ (0.03 g, 1.55×10^{-3} mmol), AIBN (5.10 mg, 0.031 mmol) in 0.5 mL dioxane was prepared and heated at 80 °C for 2.5 h. The resultant modified polymer was isolated as described above.

2.7. Preparation of poly(MEA)₅₁-S(C=S)SC₁₂H₁₅

A stock solution containing DDMAT (83.0 mg, 0.23 mmol), VA-044 (0.74 mg, 0.0023 mmol) in 10 mL dioxane/water (80:20) was prepared. One aliquot (1 mL) was added to MEA (0.461 g, 2.50 mmol), and heated as described above. The polymer was precipitated by drop

wise addition to an excess of diethyl ether, filtered, and dried at room temperature under vacuum for 24 hours to give poly(MEA)₅₁-S(C=S)SC₁₂H₂₅, $M_n = 9,700 \text{ g}\cdot\text{mol}^{-1}$, $M_w/M_n = 1.28$, 46% conv., isolated yield = 0.205 g.

2.8. Preparation of poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ copolymer

Poly(MEA)₅₁ (0.0155 mmol) and DMA (77 mg, 0.775 mmol) were added to VA-044 (1.55×10^{-4} mmol from a stock solution) in 0.5 mL dioxane/water (80:20), and heated as described above. TBAM (79 mg, 0.622 mmol) and VA-044 (4.04×10^{-4} mmol from a stock solution) in 0.5 mL dioxane/water (80:20) were added to the latter poly(MEA)₅₁-*b*-(DMA)₅₀-S(C=S)SC₁₂H₁₅ solution, and heated as described above. Poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ was isolated as described above, $M_n = 18,350 \text{ g}\cdot\text{mol}^{-1}$, $M_w/M_n = 1.29$, 96% conv., isolated yield = 0.269 g.

2.9. Self-assembly and TEM analysis

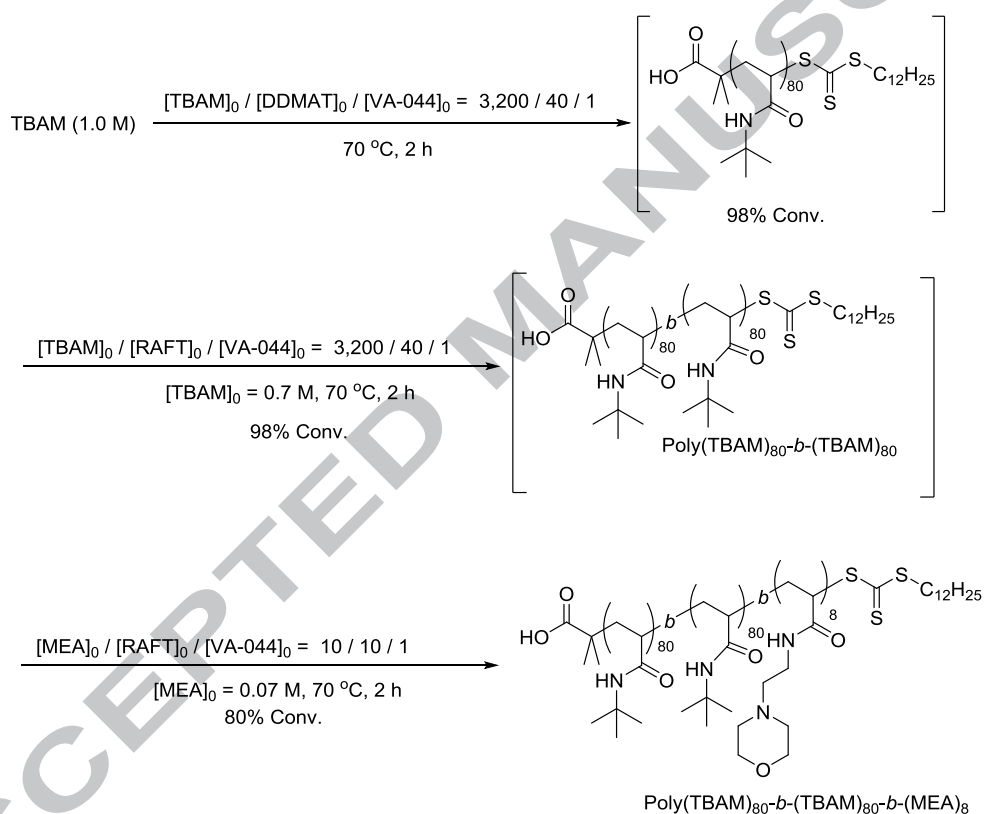
Self-assembly of polymer samples were carried out using the method of Zhang and Eisenberg.²² For example, precipitated poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ (44.0 mg, 0.0024 mmol) was dissolved in DMF (3.87 mL), and distilled water (0.91 mL) added slowly with vigorous stirring at a rate of ~1 drop every 10 s. The resulting solution placed in a dialysis bag (molecular weight cut-off, MWCO = 3500) and exchanged with water (500 ml) for 12 h to exclude the organic solvent. The distilled water was replaced twice and dialyzed for a further 6 h each time. A direct sample and a ~3 mL, CO₂-flushed (30 min.) sample of the micelle solution were analysed *via* TEM. The CO₂-flushed sample was then flushed with N_{2(g)} (30 min.) and analysed *via* TEM.

3. Results and discussion

3.1. Polymer synthesis

Amphiphilic diblock copolymers were prepared starting with polymerizations of the hydrophobic monomer *tert*-butyl acrylamide (TBAM) and extending the resulting macroRAFT agent with hydrophilic *N*-(2-morpholin-4-ylethyl) acrylamide (MEA). NMP and RAFT of TBAM have been reported at 120 and 90 °C, respectively, in alcohols,¹⁴ dioxane,^{23,24} and DMF^{23,25} giving controlled/living character, but accompanied by significant low molecular weight tailing and M_n deviating from theoretical values at higher conversions

due to chain transfer.¹⁴ The reported RAFT polymerizations of TBAM used relatively low $[\text{RAFT}]_0/[\text{2,2'-azobisisobutyronitrile (AIBN)}]_0$ ratios (3.3 and 10),^{23,24} and would thus proceed with a significant theoretical fraction of dead chains (livingness $L = 75\text{-}91\%$ after 3 h according to equation 2). In order to maximize the fraction of living chains¹⁷⁻¹⁹ in the present work, low concentrations of the water soluble initiator VA-044 were used at 70 °C in 2 h polymerizations of TBAM in a mixture of 80:20 dioxane/water. The polymerizations were taken to 98% conversion, for example giving poly(TBAM)₈₀-S(C=S)SC₁₂H₂₅ with $L = 98\%$, $M_n = 12,450 \text{ g}\cdot\text{mol}^{-1}$ and $\bar{D} = 1.12$ (Scheme 2, Table 1).



Scheme 2. Preparation of poly(TBAM)₈₀-*b*-(DMA)₈₀-*b*-(MEA)₈-S(C=S)SC₁₂H₂₅ shown in Figure 1c, where all polymerizations were carried out in dioxane /water (80:20).

Table 1 - Characterization of the polymers prepared.

Figure	Polymer ^a	$M_{n,th}$ ^b	% Conv. ^c	M_n ^d	\bar{D} ^d
1a	Poly(TBAM) ₈₀	10350	98	12450	1.12
	Poly(TBAM) ₈₀ - <i>b</i> -(MEA) ₁₁₃	31150	65	30600	1.44
1b	Poly(TBAM) ₈₀	10350	98	11150	1.10
	Poly(TBAM) ₈₀ - <i>b</i> -(MEA) ₄₈	19200	80	20550	1.29
1c	Poly(TBAM) ₈₀ - <i>b</i> -(TBAM) ₈₀	20300	98	19200	1.18
	Poly(TBAM) ₈₀ - <i>b</i> -(TBAM) ₈₀ - <i>b</i> -(MEA) ₈	21800	80	20700	1.26
2a	Poly(TBAM) ₄₀	5350	98	6100	1.09
	Poly(TBAM) ₄₀ - <i>b</i> -(DMA) ₅₀	10250	99	9850	1.12
	Poly(TBAM) ₄₀ - <i>b</i> -(DMA) ₅₀ - <i>b</i> -(MEA) ₄₉	19300	70	18000	1.28
2b	Poly(MEA) ₅₁	9750	46	9700	1.28
	Poly(MEA) ₅₁ - <i>b</i> -(DMA) ₅₀	14600	99	13550	1.28
	Poly(MEA) ₅₁ - <i>b</i> -(DMA) ₅₀ - <i>b</i> -(TBAM) ₄₀	19600	98	18350	1.29

RAFT end group omitted from polymer structure drawings. ^aThe degree of polymerization for poly(MEA)₅₁ is estimated using M_n from GPC, and for all other polymers degree of polymerization is estimated from conversion by ¹H NMR. ^b $M_{n,th}$ is calculated according to equation 1. ^cDetermined by ¹H NMR. ^dDetermined by GPC/RI in DMF (0.01 M LiBr) using commercial linear poly(MMA) as molecular weight standards.

The effect of block size on self-assembly was investigated using diblocks of the structure poly(TBAM)-*b*-(MEA)-S(C=S)SC₁₂H₂₅. The near complete conversion of TBAM during synthesis of the first block allowed chain extensions without purification of the first hydrophobic block. Poly(TBAM)₈₀-*b*-(MEA)₁₁₃-S(C=S)SC₁₂H₂₅ and poly(TBAM)₈₀-*b*-(MEA)₄₈-S(C=S)SC₁₂H₂₅ and were prepared in 65 and 80% conversion with M_n values close to $M_{n,th}$ with $M_n = 30,600 \text{ g}\cdot\text{mol}^{-1}$ and $\bar{D} = 1.44$ and $M_n = 20,550 \text{ g}\cdot\text{mol}^{-1}$ and $\bar{D} = 1.29$, respectively (Figure 1, Table 1). In order to increase the hydrophobicity further, chain extension of poly(TBAM)₈₀-S(C=S)SC₁₂H₂₅ to poly(TBAM)₁₆₀-S(C=S)SC₁₂H₂₅ was

conducted ($M_n = 19,200$; $\mathcal{D} = 1.18$), followed by chain extension with MEA resulting in poly(TBAM)₁₆₀-*b*-(MEA)₈-S(C=S)SC₁₂H₂₅ (Scheme 2; $M_n = 20,700$ g.mol⁻¹; $\mathcal{D} = 1.26$ at 80% conversion (Table 1)).

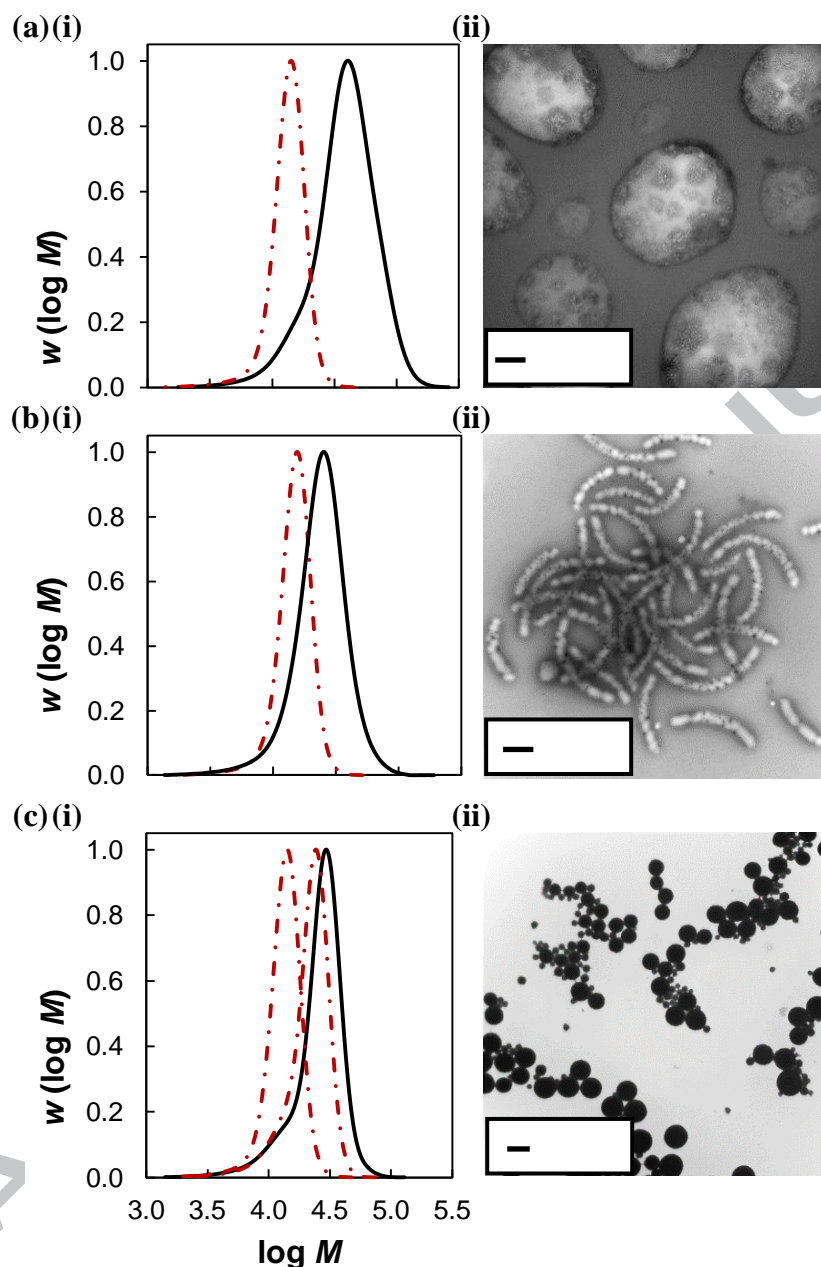
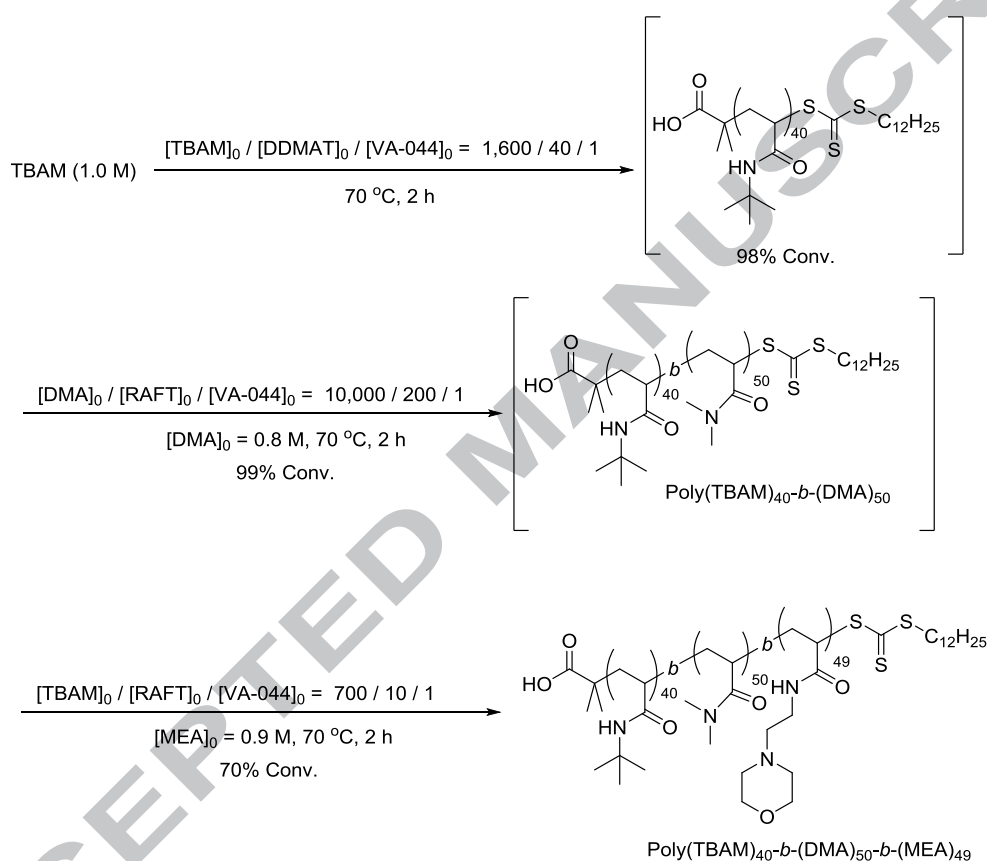


Figure 1. Copolymers ending with -S(C=S)SC₁₂H₂₅ (continuous lines) prepared starting from poly(TBAM) macroRAFT agent (dashed-dotted red lines). (a) Poly(TBAM)₈₀-*b*-(MEA)₁₁₃, (b) poly(TBAM)₈₀-*b*-(MEA)₄₈, and (c) poly(TBAM)₈₀-(TBAM)₈₀-*b*-(MEA)₈, with (i) MWDs of polymer prior to precipitation and (ii) TEM of corresponding precipitated and dialysed block copolymer. See Table 1.

For the synthesis of the triblock copolymer poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅ purification was only carried out after the final chain extension since only the MEA polymerization reached less than near complete conversion of 70% (Scheme 3). The triblock was isolated with $M_n = 18,000$, close to the theoretical value ($M_{n,th} = 19,300 \text{ g}\cdot\text{mol}^{-1}$), and $\mathcal{D} = 1.28$ (Figure 2a, Table 1).



Scheme 3. Preparation of poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅ shown in Figure 2a, where all polymerizations were carried out in dioxane /water (80:20).

The large hydrophobic dodecyl group of the trithiocarbonate derived from DDMAT has been reported to greatly influence self-assembly,^{26,27} which led us to prepare the amphiphilic triblock with reversal of the order of blocks (Scheme 4). Purified poly(MEA)₅₁-S(C=S)SC₁₂H₂₅ was converted into the amphiphilic triblock copolymer poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅. The triblock derived from poly(MEA)₅₁ macroRAFT agent was isolated with $M_n = 18,350 \text{ g}\cdot\text{mol}^{-1}$, close to the theoretical value ($M_{n,th} = 19,600 \text{ g}\cdot\text{mol}^{-1}$) and $\mathcal{D} = 1.29$ (Figure 2b, Table 1).

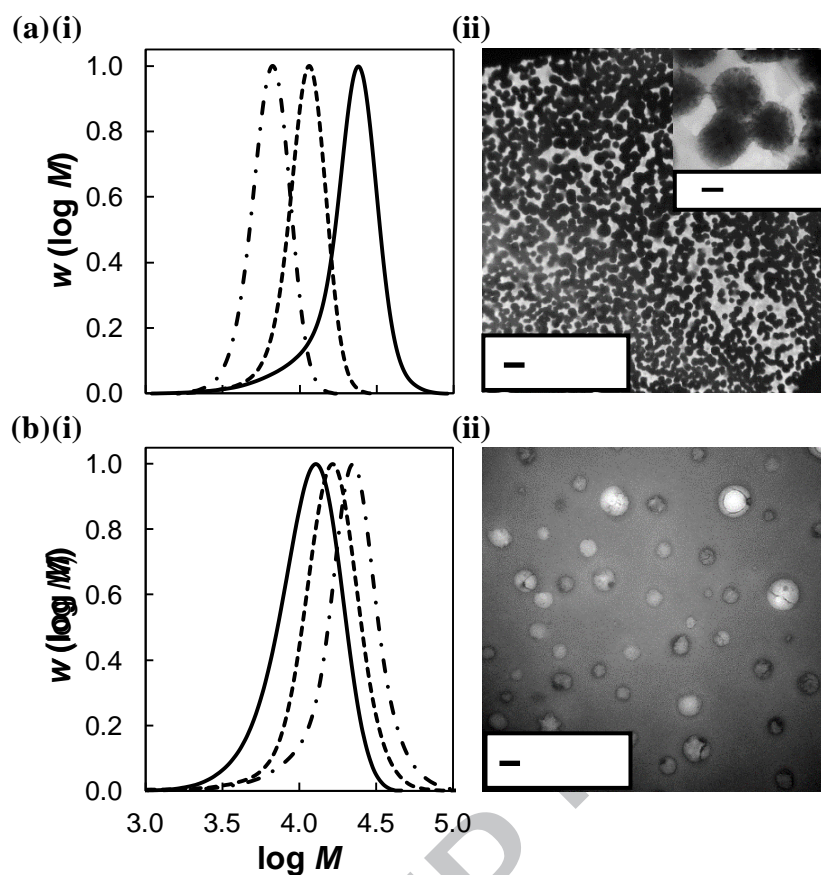
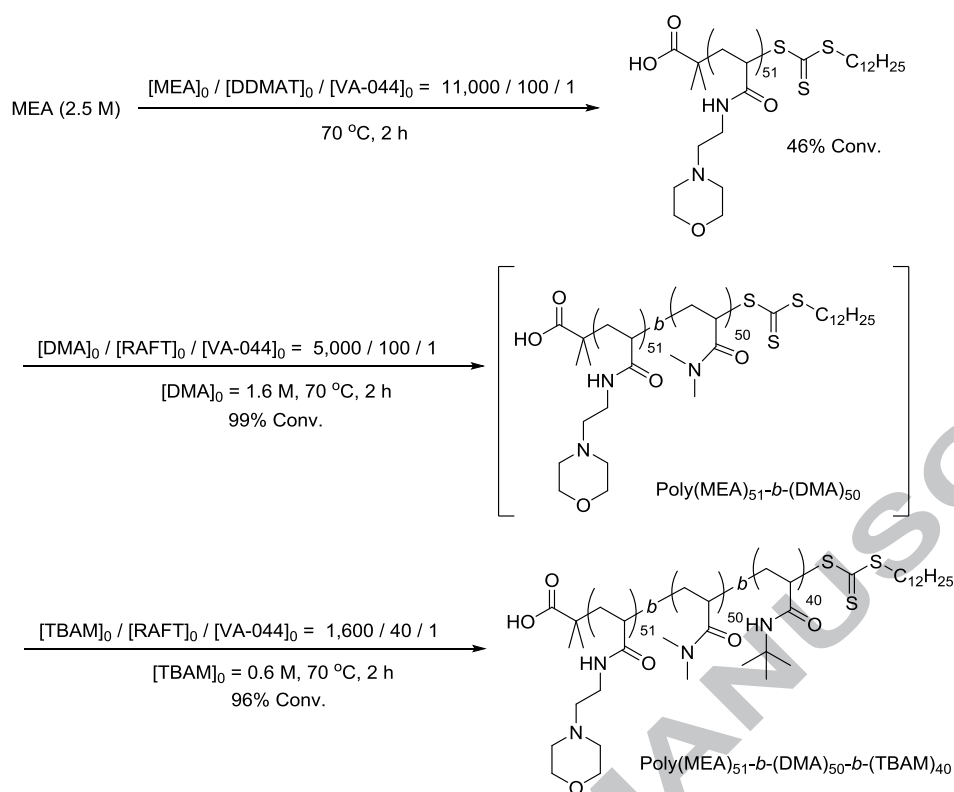


Figure 2. (a) Poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅ prepared starting from poly(TBAM)₄₀ as macroRAFT agent (dashed dotted line) extended with DMA (dashed line) and MEA (continuous line) with (b) Poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ prepared starting from poly(MEA)₅₁ as macroRAFT agent (continuous line) extended with DMA (dashed line) and TBAM (dashed-dotted line) and (i) MWDs of polymer prior to precipitation and (ii) TEM of corresponding precipitated and dialysed triblock copolymer. See Table 1.



Scheme 4. Preparation of poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ shown in Figure 2b, where all polymerizations were carried out in dioxane /water (80:20).

3.2. Self-assembly

The post-polymerization block copolymer self-assembly procedure of Eisenberg was employed,²² whereby the polymer is dissolved in DMF with subsequent water dialysis reducing the solvency of the hydrophobic block to give a translucent colloidal suspension at low polymer concentrations (~1 wt%). For the diblock copolymers comprised of hydrophobic poly(TBAM) and hydrophilic poly(MEA) blocks, various morphologies were found depending on the block lengths (Figures 1 and S1). As a consequence of the large hydrophobic RAFT moiety attached to the hydrophilic block, the diblock copolymer exhibited self-assembly behaviour reminiscent of a more complex ABA' type-structure. The polymer with the largest hydrophilic block, poly(TBAM)₈₀-*b*-(MEA)₁₁₃-S(C=S)SC₁₂H₂₅, self-assembled into near spherical entities, most likely vesicles, with patchy-patterning on the surface (Fig. 1a). The patterning could arise from regions of different thickness in the bilayer due to the hydrophobic end-group with some of the polymer chains in the vesicle bilayer bending to sequester the end group into the hydrophobic core. Other polymer chains may remain straight with the hydrophobic end group exposed to the water with chains aggregating

together to form this patchy structure through phase separation. From the polymer chain length it is expected that the bending of chains will be slow resulting in the patchy surface being kinetically trapped. As the hydrophilic content decreases in poly(TBAM)₈₀-*b*-(MEA)₄₈-S(C=S)SC₁₂H₂₅, large worm-like vesicles with a diameter of approximately 500 nm appear (Fig. 1b). These worm-like vesicles are much larger than the worm-like micelles typically attained from polymerization induced self-assembly RAFT dispersion polymerizations (diblock copolymers).^{28,29} Further reduction in the length of the hydrophilic block as well as an increase in the length of the hydrophobic block (poly(TBAM)₁₆₀-*b*-(MEA)₈-S(C=S)SC₁₂H₂₅) resulted in formation of polydisperse large aggregates where the hydrophilic component is very short in comparison to the large hydrophobic contribution (Fig. 1c).

In regards to the triblock copolymer poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅, hydrophobic interactions between the large end groups (TBAM block and the RAFT end group) leads to large compound micelles (Figures 2a(ii) and S2(a)). Such a large contribution of the RAFT end group in self-assembly has been observed previously for hydrophilic homopolymers.^{26,30,31} In particular alkynyl-terminated poly(NIPAM) were found to form similar compound micelles,³⁰ where the generation of compound micelles (and other structures) was driven by intermolecular hydrogen bonding involving the terminal alkynyl group, suggesting that a specific interaction between the end groups can lead to self-assembly. Such a specific interaction involving the end groups is also responsible for the formation of flower-like micelles and gels in telechelic polymers.³¹

Reversing the order of the blocks to instead have poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ places the hydrophobic RAFT-end group on the hydrophobic poly(TBAM) block. This results in self-assembly following simple diblock-like behavior (both poly(MEA) and poly(DMA) are hydrophilic so can be considered as a single hydrophilic block) leading to what appears to be spherical vesicles (Figure 2b(ii) and S2(b)). In this case, ¹H NMR spectroscopy was used to demonstrate self-assembly in D₂O (1 wt%, Figure 3a).³² The *tert*-butyl signal of TBAM was greatly reduced in size compared to the spectrum in CDCl₃ with the self-assembly leading to suppression of the hydrophobic block (poly(TBAM)) concealed at the core. In CDCl₃ all three blocks were soluble, providing integrations in accordance with copolymer composition.

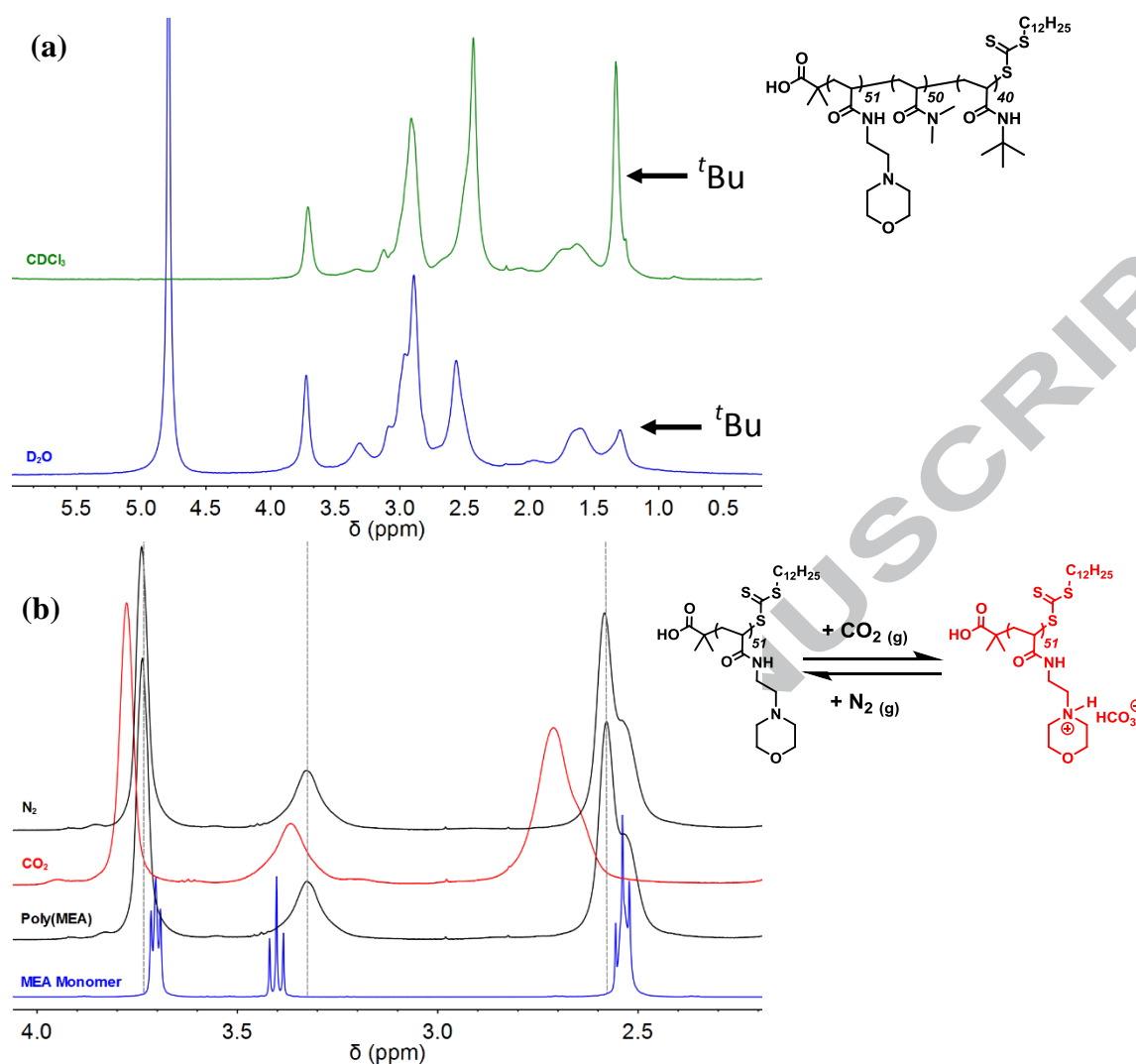


Figure 3. ^1H NMR spectra of (a) poly(TBAM) $_{51}$ -*b*-(DMA) $_{50}$ -*b*-(MEA) $_{40}$ -S(C=S)SC $_{12}$ H $_{25}$ in CDCl $_3$ and D $_2$ O with reduction of the t Bu peak in D $_2$ O indicating self-assembly with poly(TBAM) core and (b) poly(MEA) $_{51}$ -S(C=S)SC $_{12}$ H $_{25}$ in D $_2$ O indicating switchability after flushing the solution with CO $_{2(g)}$ and N $_{2(g)}$.

To further explore the effect of the large hydrophobic dodecyl moiety derived from the RAFT agent on poly(TBAM) $_{40}$ -*b*-(DMA) $_{50}$ -*b*-(MEA) $_{49}$ -S(C=S)SC $_{12}$ H $_{25}$, this moiety was removed by reaction with excess AIBN (20 eq.) in dioxane solution,³³ with the disappearance of the trithiocarbonate evidenced by a reduction in UV-absorbance of the polymer (Figure 4).³⁴ As a consequence, the large compound micelle structure due to ABA' self-assembly reverted to smaller spherical entities, most likely vesicles, indicative of the diblock-like structure observed with poly(MEA) $_{51}$ -*b*-(DMA) $_{50}$ -*b*-(TBAM) $_{40}$ -S(C=S)SC $_{12}$ H $_{25}$, where the large hydrophobic end-group is attached to the hydrophobic poly(TBAM) block.

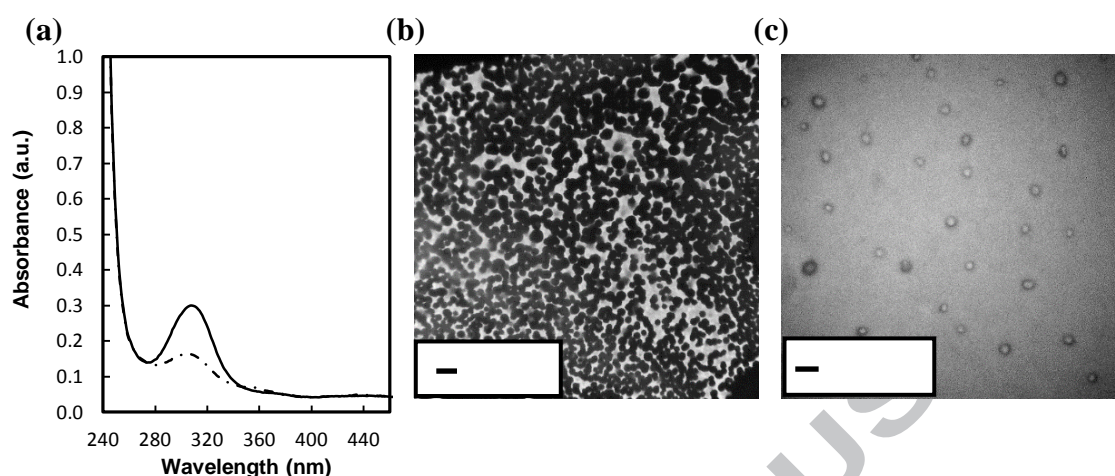


Figure 4. (a) UV-Vis spectra showing poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅ before (solid line) and after cleavage (dashed line) of the RAFT moiety and TEM images of the aqueous polymer (5% w/w) solutions before (b) and after (c) cleavage.

3.3. CO₂-response

Dilute D₂O solutions of poly(MEA)₅₁-S(C=S)SC₁₂H₂₅ were used to confirm CO₂-responsivity by ¹H NMR spectroscopy (Figure 3b).³⁵ After bubbling CO₂ through the solution for 30 min, the morpholine moieties reacted with the acidic solution, and downfield shifts in polymer signals were observed. The most striking change was for the signal at $\delta_{\text{H}} = 2.40\text{-}2.72$ shifting to $\delta_{\text{H}} = 2.55\text{-}2.88$ for the bicarbonate salt. The CO₂-response was found to be switchable - passing N₂ through the CO₂-enriched polymer solution resulted in the chemical shifts reverting to the original values.

Reducing the pH, either through the addition of CO₂ or hydrochloric acid solution, causes the MEA block to become (partially) protonated, both increasing the hydrophilicity of the polymer and introducing electrostatic repulsion between the chains. When such a hydrophilic block forms the corona of a self-assembled entity, an increase in charge density of the corona segment results in electrostatic repulsion, which in turn tends to drive the system towards lower order morphologies (which have lower curvature, *i.e.* spherical micelles are thus energetically favored relative to vesicles).^{4,29,36} For poly(TBAM)₈₀-*b*-(MEA)₁₁₃-S(C=S)SC₁₂H₂₅, the presence of CO₂ causes a change from vesicles (pH 7) to spherical micelles at pH 5 (Figure 5(i) and S5(i)) with flushing with N₂ back to pH 7 returning

the vesicles but to a non-spherical form. In the case of poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅, the presence of CO₂ causes the large compound micelles to break up into much smaller entities (due to increased hydrophilicity of the MEA block, equivalent to making it larger, thus moving away from large compound micelles: Figure 5(ii)). Subsequent flushing with N₂ does not however reverse morphology to the pre-CO₂ morphology. The addition of hydrochloric acid (instead of CO₂) to lower the pH to 1 almost solubilizes the block copolymer, which reassembles into spherical particles under basic conditions (pH ~8) upon the addition of sodium carbonate solution.

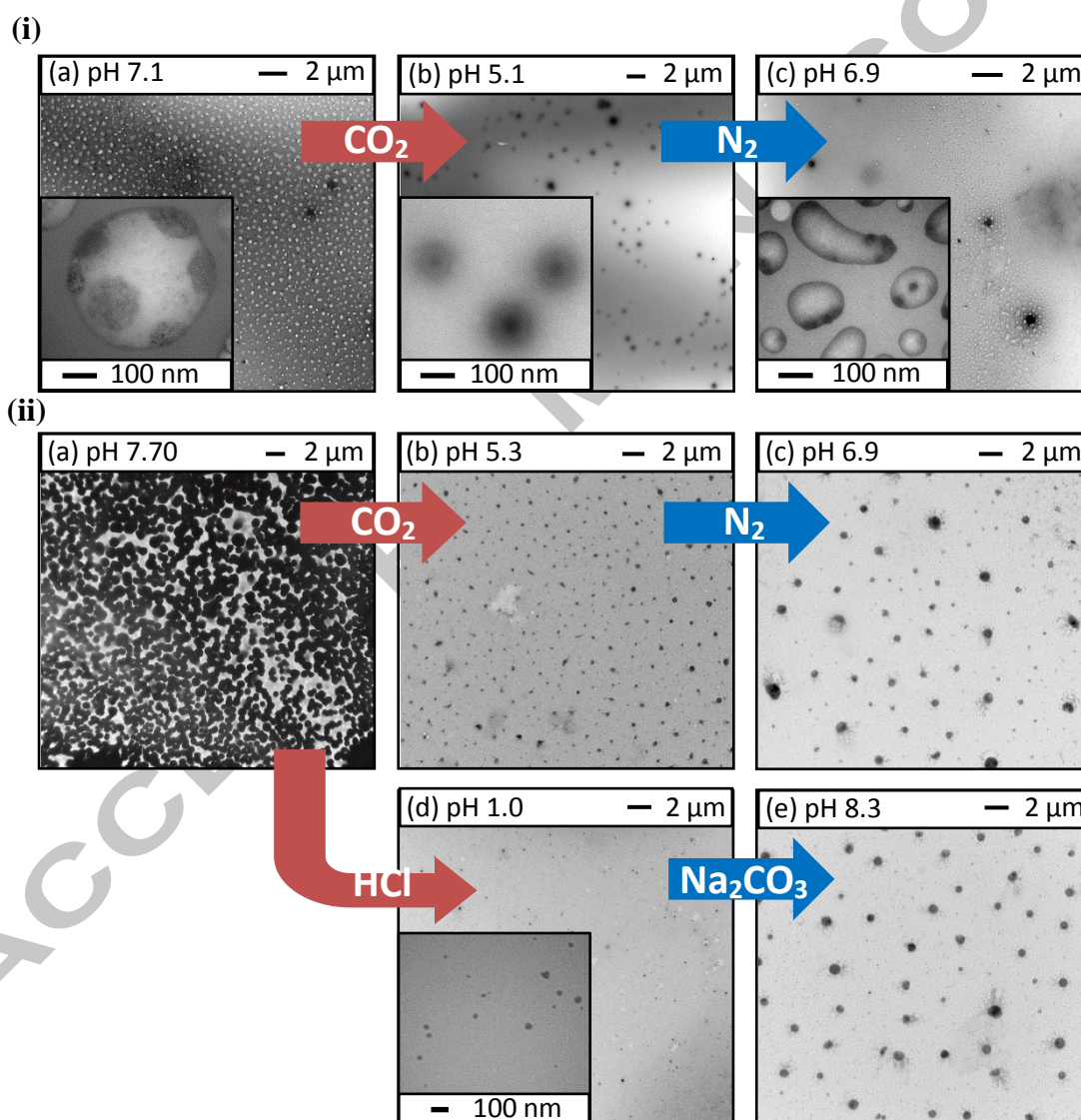


Figure 5. TEM of the aqueous polymer (5% w/w) solutions showing pH-response of (i) poly(TBAM)₈₀-*b*-(MEA)₁₁₃-S(C=S)SC₁₂H₂₅ after flushing with CO_{2(g)} and N_{2(g)} and (ii) poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅ after flushing with CO_{2(g)} and N_{2(g)}, and after the addition of 5% HCl and saturated Na₂CO₃ solutions.

4. Conclusions

RAFT polymerizations involving the monomer *N*-(2-morpholin-4-ylethyl) acrylamide (MEA) have been used to prepare amphiphilic block copolymers containing CO₂-responsive morpholine moieties. It is demonstrated how diblock and triblock copolymers are able to self-assemble in water into a wide variety of morphologies. The triblock copolymer poly(MEA)₅₁-*b*-(DMA)₅₀-*b*-(TBAM)₄₀-S(C=S)SC₁₂H₂₅ gave spherical vesicles. Self-assembly was more complicated when the large hydrophobic RAFT dodecyl moiety was attached to the hydrophilic poly(MEA) block, leading to an ABA' type-structure for poly(TBAM)-*b*-(MEA)-S(C=S)SC₁₂H₂₅ diblock copolymers. Depending on the size of the MEA fraction, patterned vesicles and large worm like vesicles were obtained. When the MEA content was small, the vesicular morphology was replaced by large aggregates of solid spherical hydrophobic particles. Poly(TBAM)₄₀-*b*-(DMA)₅₀-*b*-(MEA)₄₉-S(C=S)SC₁₂H₂₅ resulted in large compound micelles owing to interactions of the large hydrophobic RAFT end group attached to the large hydrophilic portion of the triblock copolymer. These large compound structures due to ABA' self-assembly reverted to simpler spherical vesicles upon cleavage of the solvophobicity/solvophilicity balance distorting RAFT end-group. Decreasing the pH through CO₂ or HCl addition irreversibly shifts the system towards lower order morphologies with spherical micelles being more favoured than vesicles. Overall, the present results show that a wide range of CO₂-tunable morphologies are accessible using well-defined polyacrylamide block copolymers containing heterocyclic moieties.

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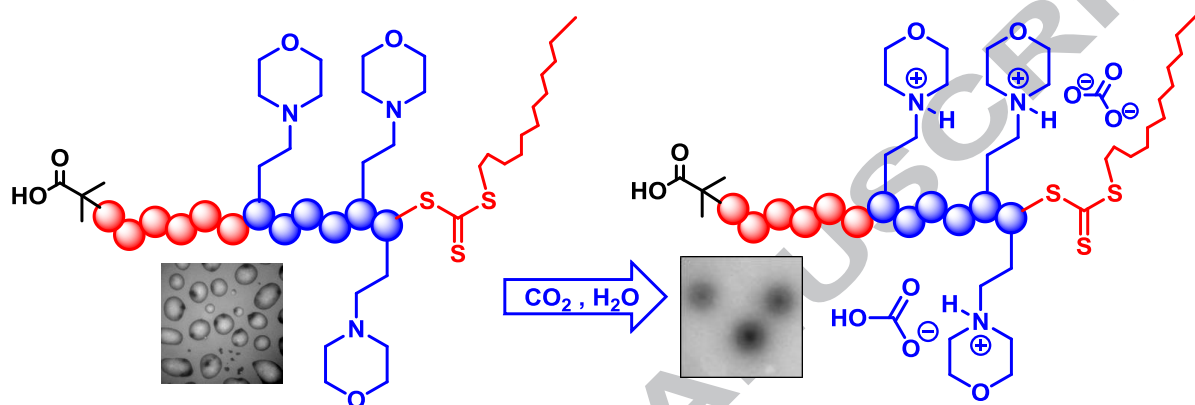
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GRAPHICAL ABSTRACT

CO₂-responsive polyacrylamide copolymer vesicles with acid-sensitive morpholine moieties and large hydrophobic RAFT end-group

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Highlights

- Morpholine is used as the CO₂-responsive part of amphiphilic copolymers.
- The order of RAFT polymerization greatly effects polyacrylamide self-assembly.
- Cleavage of the RAFT-end group revert ABA' morphologies to simpler spherical vesicles.
- CO₂-irreversibly shifts self-assembly to lower order morphologies.