Title

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Journal

Macromolecules, 48(16)

ISSN

0024-9297

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Publication Date

2015-08-25

DOI

10.1021/acs.macromol.5b01453

Peer reviewed

Macromolecules

Article

pubs.acs.org/Macromolecules

Tunable pH- and CO_2-Responsive Sulfonamide-Containing Polymers by RAFT Polymerization

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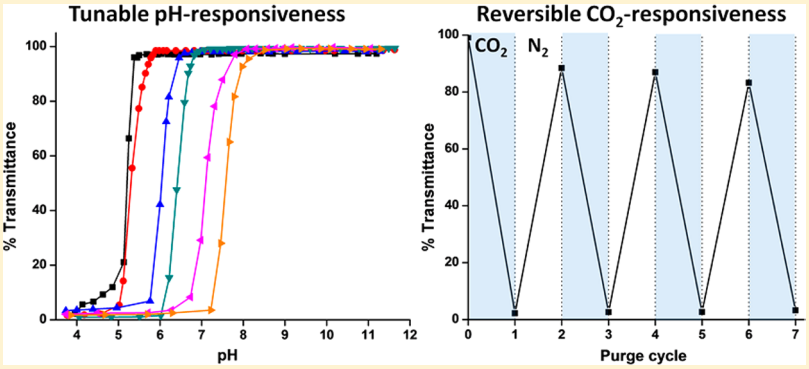


ABSTRACT: The controlled RAFT polymerization of a library of pH- and CO_2-responsive methacryloyl sulfonamides (MSAs) that possess pK_a values in the biologically relevant regime (pH = 4.5−7.4) is reported. Initial polymerizations were conducted at 70 °C in DMF with 4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (CEP) or 4-cyanopentanoic acid dithiobenzoate (CTP), resulting in polymers of broad molecular weight distributions (M_w/M_n > 1.20). As well, chain extension of a poly(methacryloyl sulfacetamide) (pSAC) macro-CTA at 70 °C was unsuccessful, indicating a loss of "living" chain ends during polymerization. However, by conducting the RAFT polymerization of MSAs at 30 °C with 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile), polymers with narrow molecular weight distributions (M_w/M_n < 1.15) and improved chain end retention were obtained. Homopolymers of each MSA derivative were synthesized, and the influence of the sulfonamide R group on monomer pK_a and pH-dependent polymer solubility was determined during these studies. The facility by which these controlled poly(MSAs) can be prepared via low-temperature RAFT without the need for functional group protection and the resulting pK_a-dependent pH- and CO_2-responsive properties point to significant potential in areas including drug and gene delivery and environmental remediation.

INTRODUCTION

Recently, extensive research efforts have been directed toward the synthesis of well-defined (co)polymers capable of rapid and reversible changes in solubility and/or conformation in response to external stimuli including pH,[1−3] temperature,[4,5] or ionic strength,[6] among others.[7−9] Of particular interest are "smart" nanocarriers for drug and gene delivery that exploit discrete changes in physiological pH to elicit the desired therapeutic effect.[10−14] Designing such polymeric systems requires that the morphological transitions occur over a very narrow designated pH range. Commonly, this specificity is achieved by the selection of a monomer with a pK_a at or near the target transition pH; however, polymer design is accordingly restricted by the limited choice in monomers and their respective pK_a values. Consequently, a facile method of specifically tuning polymer pH-responsiveness while maintaining a narrow transition range is needed.

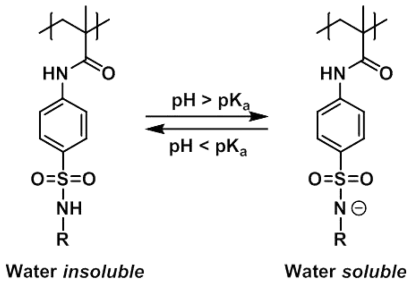
A number of attempts have been made to systematically vary the pH-responsiveness of polymers.[15,16] One versatile approach toward modification of polymer pK_a was reported by Ringsdorf in seminal work in which a library of sulfonamide-containing polymers derived from sulfa drugs was synthesized by classical free radical or Michael-addition techniques.[17] Variation of the sulfonamide R group afforded facile, tunable control over polymer pK_a and subsequent pH-dependent solubility (Scheme 1). Recently, Bae and co-workers further demonstrated this versatility in pK_a selection for a variety of polymer-based therapeutic applications.[11,18−20] However, until now the uncontrolled nature of the polymerization methods used to prepare such polymers has limited the ability to attain well-defined polymer architectures with the specific molecular weights and narrow molecular weight distributions required for responsive nanotherapeutics.

Reversible-deactivation radical polymerization (RDRP) techniques such as nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP), and reversible addition−fragmentation chain transfer (RAFT) polymerization have made possible the synthesis of (co)-

Received: July 2, 2015
Revised: July 29, 2015
Published: August 7, 2015

DOI: 10.1021/acs.macromol.5b01453
Macromolecules 2015, 48, 5487−5495

Scheme 1. pH-Dependent Solubility of pMSAs



polymers of a wide variety of architectures with predictable molecular weights and narrow molecular weight distributions.[21−23] In particular, RAFT has been used to directly polymerize a variety of cationic, anionic, and other functional monomers in organic or aqueous media without the necessity of protecting group chemistries or postpolymerization modification.[23,24] The facility of polymerization and excellent functional group tolerance of RAFT polymerization have driven our current objectives of synthesizing sulfonamide-containing polymers in a controlled fashion.

In this article we report, to our knowledge, the first controlled RAFT polymerization of a library of methacryloyl sulfonamide (MSA) monomers possessing pK_a values in the biologically relevant regime (pH = 4.5−7.4). In this work we show that temperature has a significant influence on the polymerization of MSAs, with lower reaction temperatures affording improved molecular weight control and functional chain end retention. Varying the sulfonamide R group is shown to be an effective means of adjusting monomer pK_a and subsequently the pH-dependent solubility of the resulting polymethacryloyl sulfonamides (pMSAs). During our study of the weakly acidic/basic nature of the MSA derivatives chosen, we found a remarkably facile and reversible CO_2-induced solubility transition in aqueous solutions. The demonstrated control over RAFT polymerization of MSAs now allows new routes for the synthesis of advanced polymer architectures with tunable pH- and CO_2-responsive properties for ultimate use in biological and therapeutic applications.

EXPERIMENTAL SECTION

Materials. 4-Cyanopentanoic acid dithiobenzoate[25] (CTP) and 4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid[12] (CEP) were synthesized according to literature procedures. Methacryloyl chloride (Aldrich, 97%) was distilled under vacuum and stored under N_2 at −10 °C prior to use. 4,4-Azobis(4-cyanovaleric acid) was recrystallized from methanol and stored at −10 °C. *N*,*N*′-Dimethylformamide (Acros, extra dry with sieves) was stirred under vacuum at room temperature for 60 min prior to use in order to remove traces of dimethylamine. 2,2′-Azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70) (Wako, 96%) sulfacetamide (Aldrich, >98%), sulfamethazine (Aldrich, >99%), sulfamethizole (Aldrich, >99%), sulfadimethoxine (Aldrich, >98.5%), sulfadoxine (Aldrich, >95%), sulfabenzamide (TCI, >98%), trimesic acid, (Aldrich, 95%), 0.1 N NaOH (Alfa Aesar, standardized), and 0.05 N HCl (Alfa Aesar, standardized) were used as received.

Characterization. NMR spectra and monomer conversions were obtained using a Varian INOVA 300 MHz NMR spectrometer in DMSO-d_6. Polymer molecular weights and molecular weight distributions (M_w/M_n) were determined by size exclusion chromatography (SEC) using 95:5 (v:v) DMF:CH_3COOH 20 mM LiBr as the eluent at a flow rate of 1.0 mL/min in combination with two Agilent PolarGel-M columns heated to 50 °C and connected in series with a Wyatt Optilab DSP interferometric refractometer and Wyatt DAWN

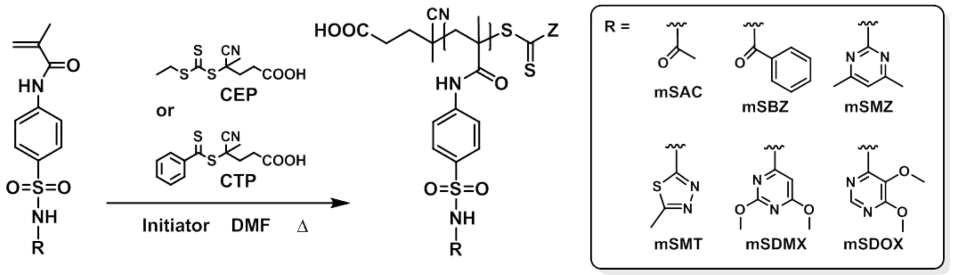
Table 1. Conversion, Molar Mass, and Molecular Weight Distribution Data for the RAFT Polymerization of MSAs in DMF at 70 °C[a]

entry	monomer deriv	CTA	time (min)	conv[b] (%)	$[M]_0$ (mol/L)	$M_{ntheory}$[c] (g/mol)	M_{nexp}[d] (g/mol)	M_w/M_n[d]
1a	mSAC	CTP	120	7	1.0	3200	4400	1.19
1b	mSAC	CTP	360	10		4500	5800	1.18
1c	mSAC	CTP	600	12		5400	6200	1.27
2a	mSAC	CEP	120	22	1.0	9400	14600	1.27
2b	mSAC	CEP	360	67		28500	26400	1.41
2c	mSAC	CEP	600	81		34700	29700	1.44
3a	mSBZ	CEP	120	13	1.0	7000	7400	1.27
3b	mSBZ	CEP	360	48		25000	22000	1.24
3c	mSBZ	CEP	600	66		34200	28100	1.26
4a	mSMZ	CEP	120	16	0.83	8800	13900	1.22
4b	mSMZ	CEP	360	51		26800	29800	1.27
4c	mSMZ	CEP	600	69		36300	35500	1.29
5a	mSMT	CEP	120	35	1.0	18000	22000	1.55
5b	mSMT	CEP	420	79		40600	34900	1.78
5c	mSMT	CEP	600	85		43300	35200	1.81
6a	mSDMX	CEP	120	12	0.83	6900	15100	1.23
6b	mSDMX	CEP	360	47		26800	34600	1.20
6c	mSDMX	CEP	600	73		41900	44400	1.28
7a	mSDOX	CEP	120	15	0.83	8800	11100	1.10
7b	mSDOX	CEP	420	62		35500	25900	1.45
7c	mSDOX	CEP	600	67		38500	27100	1.47

[a]Sulfonamide monomers were polymerized at 70 °C in DMF ($[M]_0$:$[CTA]_0$:$[I]_0$ = 150:1.0:0.2) using V-501 as the initiator. [b]Conversions were determined by ^{1}H NMR (DMSO-d_6) by comparing the relative integral areas of trimesic acid (internal standard) aromatic protons (8.64 ppm, 3H) to the vinyl proton of the sulfonamide monomer (5.84 ppm, 1H). [c]Theoretical number-average molecular weights were calculated according to the equation $M_n = (\rho MW_{mon}[M]/[CTA]) + MW_{CTA}$, where ρ is the fractional monomer conversion, MW_{mon} is the molecular weight of the monomer, and MW_{CTA} is the molecular weight of the CTA. [d]As determined by SEC-MALLS (95:5 (v:v) DMF:CH_3COOH 20 mM LiBr).

DOI: 10.1021/acs.macromol.5b01453
Macromolecules 2015, 48, 5487−5495

Scheme 2. Synthetic Pathway for the CEP- or CTP-Mediated RAFT Polymerization of MSAs in DMF



EOS multiangle laser light scattering (MALLS) detector (λ = 633 nm). Absolute molecular weights and M_w/M_n were calculated using a Wyatt ASTRA SEC/LS software package. The dn/dc values for each polymer derivative in the above eluent at 35 °C were determined offline using a Wyatt Optilab DSP interferometric refractometer and Wyatt ASTRA dn/dc software.

General Procedure for Methacryloyl Sulfonamide Synthesis. Using a modified procedure,[11] sulfa drug (40.0 mmol) was dissolved in 160 mL of a 1:1 (v:v) mixture of acetone and 0.5 N aqueous NaOH and stirred while cooling in an ice bath. Methacryloyl chloride (4.10 mL, 42.0 mmol) was then added dropwise over 30 min followed by removing the ice bath and stirring the reaction at room temperature for an additional 60 min. The acetone was removed by rotary evaporation, followed by adjusting the solution to pH = 2 with 6 N HCl. The resulting solids were isolated using vacuum filtration and washed with 100 mL of dilute HCl (0.01 N) prior to drying *in vacuo* for 48 h, yielding the desired monomers as colorless to off-white solids. The synthesis of methacryloyl sulfadoxine (mSDOX) required the use of 240 mL of a 1:2 (v:v) mixture of acetone and 0.5 N aqueous NaOH.

Methacryloyl Sulfacetamide (mSAC). Yield: 10.29 g, 91%; mp 203−205 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.99 (s, 1H), 10.20 (s, 1H), 8.11−7.65 (m, 4H), 5.84 (s, 1H), 5.58 (s, 1H), 1.93 (s, 3H), 1.89 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 168.69, 167.34, 143.72, 139.99, 133.13, 128.67, 121.03, 119.50, 23.22, 18.63.

Methacryloyl Sulfabenzamide (mSBZ). Yield: 12.89 g, 94%; mp 228−229 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.46 (s, 1H), 10.22 (s, 1H), 8.02−7.87 (m, 4H), 7.83 (d, J = 7.2 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.47 (t, J = 7.6 Hz, 2H), 5.84 (s, 1H), 5.58 (s, 1H), 1.93 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.36, 165.38, 143.79, 139.99, 133.24, 133.15, 131.54, 128.93, 128.61, 128.40, 121.03, 119.50, 18.62.

Methacryloyl Sulfadimethoxine (mSDMX). Yield: 14.72 g, 97%; mp 216−218 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.50 (s, 1H), 10.17 (s, 1H), 7.86 (m, 4H), 5.92 (s, 1H), 5.82 (s, 1H), 5.57 (s, 1H), 3.77 (s, 3H), 3.73 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 171.67, 167.30, 164.26, 159.90, 143.41, 139.98, 133.68, 128.30, 120.96, 119.64, 84.57, 54.54, 53.81, 18.59.

Methacryloyl Sulfadoxine (mSDOX). Yield: 14.10 g, 93%; mp 198−199 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.09 (s, 1H), 10.17 (s, 1H), 8.12 (s, 1H), 5.84 (s, 1H), 5.59 (s, 1H), 3.90 (s, 3H), 3.70 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.32, 161.63, 150.43, 143.14, 140.06, 134.61, 129.88, 128.61, 127.21, 120.92, 119.40, 60.28, 54.08, 18.64.

Methacryloyl Sulfamethazine (mSMZ). Yield: 13.39 g, 97%; mp 234−235 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.47 (s, 1H), 10.10 (s, 1H), 7.86 (dd, J = 28.3, 8.6 Hz, 4H), 6.74 (s, 1H), 5.81 (s, 1H), 5.55 (s, 1H), 2.23 (s, 6H), 1.92 (s, 3H). ^{13}C NMR (75 MHz, DMSO): δ 167.61, 156.65, 143.19, 140.43, 135.08, 129.49, 121.24, 119.42, 113.97, 23.37, 19.04.

Methacryloyl Sulfamethizole (mSMT). Yield: 12.42 g, 91%; mp 215−217 °C dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.90 (s, 1H), 10.12 (s, 1H), 7.77 (dd, J = 37.4, 8.4 Hz, 4H), 5.82 (s, 1H), 5.56 (s, 1H), 2.44 (s, 3H), 1.92 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.80, 167.20, 154.46, 142.65, 140.03, 136.05, 126.71, 120.83, 119.70, 18.65, 16.10.

General Procedure for RAFT Polymerization of Methacryloyl Sulfonamides. Briefly, MSA (5.0×10^{-3} mol, 150 equiv), CTA (CTP or CEP) (3.3×10^{-5} mol, 1 equiv), initiator (V-70 or V-501) (6.7×10^{-7} mol, 0.2 equiv), and trimesic acid (50 mg, ^{1}H NMR internal standard) were combined in a 10 mL graduated cylinder, and DMF was added to bring the final solution volume to 5.0 mL ($[M]_0$ = 1 M) or 6.0 mL (0.83 M) depending upon monomer solubility as indicated in Table 1. The solution was then transferred to a 10 mL test tube equipped with a magnetic stir bar and rubber septum followed by purging with N_2 for 40 min. An initial aliquot (200 μL) was taken prior to heating the reaction vessel at the indicated temperature with subsequent aliquots taken at timed intervals and analyzed by ^{1}H NMR (DMSO-d_6) to determine monomer conversion by comparing the relative integral areas of the trimesic acid aromatic protons (8.64 ppm, 3H) to the monomer vinyl proton (5.84 ppm, 1H). SEC-MALLS (95% DMF/5% CH_3COOH, 20 mM LiBr) was used to monitor the progression of molecular weight and molecular weight distribution (M_w/M_n) throughout each polymerization. Polymers isolated for solubility studies were purified by precipitating the reaction mixture into a 10-fold excess of MeOH followed by isolating the resulting solids by ultracentrifugation. The isolated polymers were precipitated a total of three times from DMF into MeOH before drying overnight *in vacuo*.

Monomer Titrations. Monomer stock solutions (1 mM) were prepared by weighing each MSA (0.1 mmol) into separate 100 mL volumetric flasks, followed by the addition of 2.00 mL of 0.1 N NaOH (0.2 mmol) to each flask. Once the monomers were completely dissolved, DI H_2O (18.2 MΩ resistance) was added to each volumetric flask to achieve a final volume of 100 mL. 25 mL of each stock solution was transferred to a 100 mL beaker containing a stir bar and titrated against 0.05 N HCl in volume increments of 5 μL at 25 °C using a Metrohm 848 Titrino Plus autotitrator. All titrations were performed in triplicate.

pH-Dependent Polymer Solubility. Polymer solutions were first prepared by dissolving each pMSA derivative (1 equiv of sulfonamide, 2.5×10^{-5} mol of sulfonamide functional groups) in 1.00 mL of 0.05 N NaOH (2 equiv, 5×10^{-5} mol) followed by dilution with DI H_2O (18.2 MΩ resistance) to a final volume of 2.50 mL ($[SO_2NH]$ = 10 mM). The polymer solution was transferred into a quartz cuvette, and the solution pH adjusted incrementally by adding 1−10 μL of 0.2 N HCl followed by measuring the % transmittance at λ = 500 nm using a UV−vis spectrophotometer.

CO_2-Dependent Polymer Solubility. In a 20 mL vial equipped with magnetic stir bar and pierceable cap, pMSA (1 equiv sulfonamide, 2.0×10^{-5} mol sulfonamide functional groups) was dissolved in 400 μL of 0.05 N NaOH (1.25 equiv, 2.5×10^{-5} mol) and subsequently diluted to a final volume of 3.00 mL ($[SO_2NH]$ = 6.7 mM) with DI H_2O (18.2 MΩ resistance). CO_2-dependent polymer solubility was examined between purge cycles by transferring the solutions to a quartz cuvette and measuring the percent transmittance at 500 nm. Purge cycles consisted of purging the solution with CO_2 for 10 s or N_2 for 25 min.

RESULTS AND DISCUSSION

RAFT Polymerization of Methacryloyl Sulfonamides (MSAs) at 70 °C. The MSA monomers (R groups shown in

DOI: 10.1021/acs.macromol.5b01453
Macromolecules 2015, 48, 5487−5495

Scheme 2) were targeted for this work based upon their respective pK_a values (Table 3) that reside within the biologically relevant pH range of 4.5−7.4. Utilizing a modified literature procedure,[11] high monomer yields (>90%) were obtained from the reaction of methacryloyl chloride and the appropriate sulfa drug precursor, as outlined in the Experimental Section.

Achieving controlled RAFT polymerization of a given monomer requires appropriate choice of CTA and polymerization conditions. Previously, our group successfully utilized the trithiocarbonate 4-cyano-4-(ethylsulfanylthiocarbonylsulfanyl)pentanoic acid (CEP) and the dithioester 4-cyanopentanoic acid dithiobenzoate (CTP) to polymerize a wide variety of (meth)acrylamide monomers in aqueous or organic media in a controlled fashion.[25,26] On the basis of that work, we have investigated the RAFT polymerization of MSAs using CEP and CTP as outlined in Scheme 2. It is worth noting that although these monomers are water-soluble, polymerizations were conducted in DMF in order to avoid CTA hydrolysis or aminolysis.[27]

Initially, CEP- and CTP-mediated RAFT polymerizations of methacryloyl sulfacetamide (mSAC) were carried out at 70 °C in DMF using V-501 as the initiator at molar ratios of $[M]_0$:$[CTA]_0$:$[I]_0$ = 150:1:0.2. As illustrated in Figure 1, a near-linear

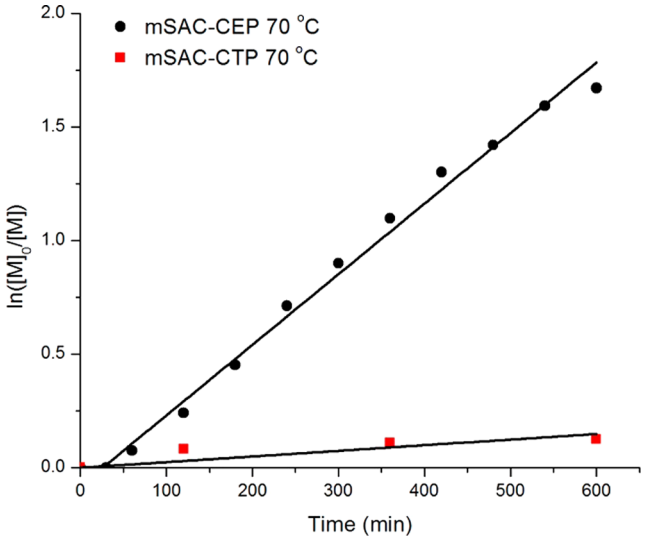


Figure 1. Kinetic plots for the CTP- and CEP-mediated RAFT polymerization of mSAC at 70 °C in DMF ($[M]_0$:$[CTA]_0$:$[I]_0$ = 150:1:0.2).

pseudo-first-order kinetic plot is observed for the polymerization of mSAC with CEP at 70 °C. After an initialization period of approximately 30 min, monomer conversion reached 81% after 600 min. The CTP-mediated polymerization of mSAC at 70 °C under analogous conditions was significantly slower, reaching only 12% monomer conversion after 600 min. Retardation in rate of dithiobenzoate-mediated polymerizations as compared to analogous reactions mediated by trithiocarbonates has been observed previously for styrenics, acrylates, and acrylamides with some monomers failing to polymerize in the presence of a dithiobenzoate RAFT agent.[28,29]

Despite near-ideal linear pseudo-first-order kinetic behavior, the CEP-mediated polymerization of mSAC at 70 °C produced polymers with M_w/M_n of 1.27 or higher (Table 1). Similarly, the polymerization of mSAC with CTP yielded polymers with M_w/M_n > 1.20. The increased conversions achieved during the CEP-mediated polymerization of mSAC prompted our use of this CTA to polymerize each monomer derivative in order to ascertain what influences the sulfonamide R group might have on conversion, molar mass, and molecular weight distribution (Table 1). As with the CEP-mediated polymerization of mSAC at 70 °C, each substituted monomer derivative also yielded moderately broad molecular weight distributions, typically increasing with conversion, and indicative of limited polymerization control.

Chain Extension of pSAC-CEP Macro-CTA at 70 °C. The degree of "living" chain end retention was investigated by synthesizing and isolating a macro-CTA (pSAC-CEP) (M_n = 7300 g/mol, M_w/M_n = 1.35), followed by chain extension with mSAC to yield the corresponding chain extended polymer (pSAC-*b*-pSAC-CEP). Figure 2 shows the SEC traces of both

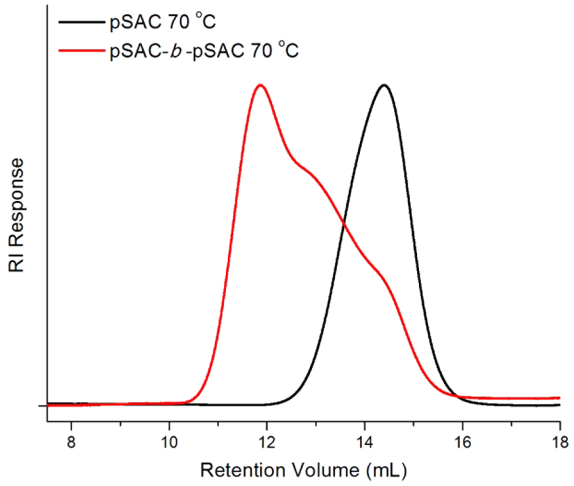


Figure 2. SEC traces of pSAC macro-CTA (M_n = 7300 g/mol, M_w/M_n = 1.35) and pSAC-*b*-pSAC after chain extension at 70 °C in DMF.

the initial monomodal pSAC-CEP macro-CTA and the corresponding pSAC-*b*-pSAC-CEP polymer after chain extension with mSAC. The latter exhibits multimodality and broad molecular weight distribution, indicating extensive loss of "living" polymer chain ends during the initial polymerization of the pSAC-CEP macro-CTA. Loss of "living" polymer chains is most often attributed to irreversible radical termination, undesirable chain transfer events, or degradation of the thiocarbonylthio chain ends. During the CEP- and CTP-mediated polymerizations of MSAs at 70 °C, we observed a loss of the characteristic color of CEP (yellow) and CTP (pink) after extended polymerization times, qualitatively indicating degradation of the trithiocarbonate and dithioester moieties, respectively. A quantitative study of the extent of this degradation, as well as the precise mechanism by which it occurs, is currently underway in our laboratories and is the subject of a manuscript to be submitted.

RAFT Polymerization of Methacryloyl Sulfonamides at 30 °C. Hypothesizing that a deleterious side reaction was competing with chain extension during the CTA-mediated polymerization, we lowered the reaction temperature. Such approaches have been previously successful in RAFT polymerizations, yielding well-defined copolymers that maintained a high degree of chain-end functionality.[30−32] Figure 3 shows the comparative SEC chromatograms of the CEP-mediated polymerizations of mSAC at 70 and 30 °C under the

DOI: 10.1021/acs.macromol.5b01453
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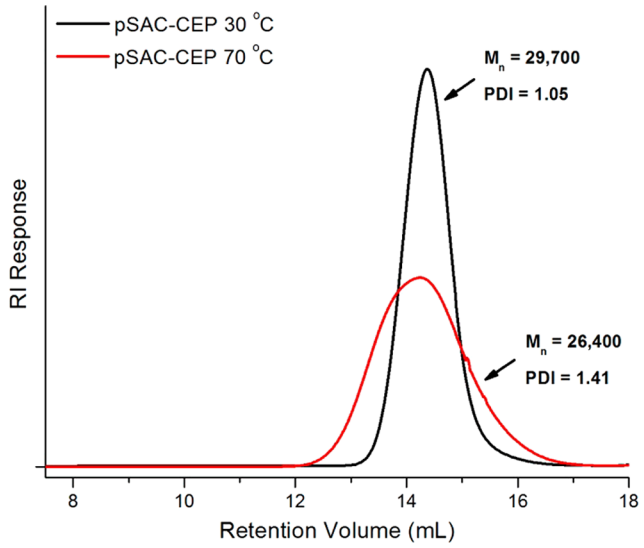

Figure 3. DMF SEC RI traces of pSAC-CEP polymerized at 30 and 70 °C using V-70 and V-501, respectively.

polymerization conditions outlined in Table 1. It should be noted that the 30 °C reaction utilized the low decomposition temperature initiator 2,2′-azobis(4-methoxy-2,4-dimethyl-valeronitrile) (V-70). While both reactions produced polymers with similar number-average molecular weights, the resulting molecular weight distribution of the polymer synthesized at 30 °C (58% conversion, M_n = 29 700 g/mol, M_w/M_n = 1.05) was substantially lower than the polymer prepared at 70 °C (67% conversion, M_n = 26 400 g/mol, M_w/M_n = 1.41).

Figure 4a shows the kinetic plots for the respective CEP- and CTP-mediated polymerizations of mSAC at 30 °C. The former exhibited a longer pre-equilibrium (initialization) period (~60 min) as compared to polymerization at 70 °C; however, linear pseudo-first-order kinetic behavior was observed up to 600 min. Deviation from linearity at longer times in this particular case is possibly due to the reduced radical flux observed as the initiator concentration decreases substantially at prolonged reaction times, as we have previously reported.[33] Figure 4b shows the SEC chromatogram overlay at specified times during the 30 °C polymerization of mSAC with CEP. The progression of the polymer traces to lower elution volumes with corresponding increases in RI intensity, without high molecular weight shouldering, is indicative of controlled polymerization behavior and thus maintenance of thiocarbonylthio functionality. This is further indicated by the narrow molecular weight distributions (Figure 4c) and linear progression of M_n vs monomer conversion (Figure 4d) observed for the 30 °C polymerization of mSAC. While M_n increases in a linear fashion during the RAFT polymerization of mSAC at 30 °C, experimentally determined molecular weights (M_{nexp}) are marginally higher than those theoretically predicted ($M_{ntheory}$) based upon monomer conversion. The higher than expected molecular weights determined by MALLS directly of aliquots taken from the polymerization could be indicative of irreversible coupling of CTA intermediate radicals during the initialization stage.[34−36]

Table 2 summarizes the conversion, molar mass, and molecular weight distribution data for the RAFT polymerization of each MSA derivative in DMF at 30 °C using either CTP or CEP as the RAFT agent and V-70 as the initiator. Reducing the polymerization temperature to 30 °C results in

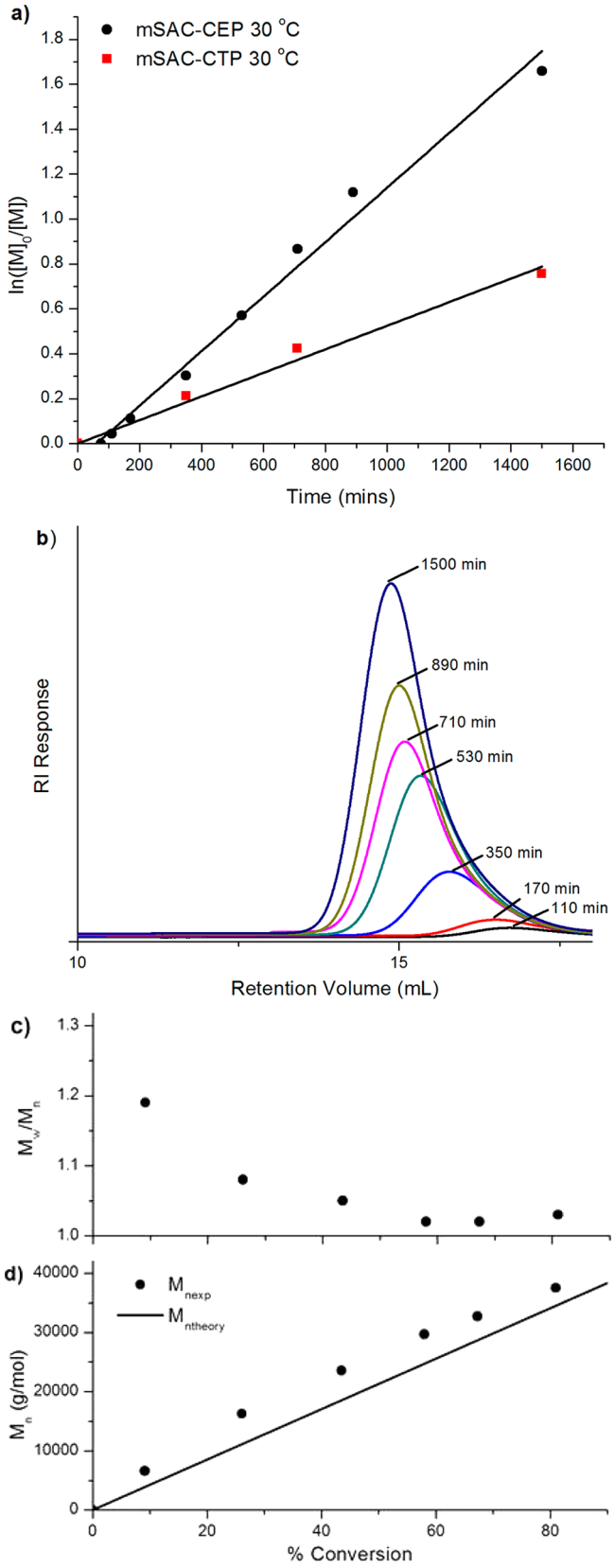

Figure 4. (a) Pseudo-first-order kinetic plots for the CTP- and CEP-mediated RAFT polymerization of mSAC at 30 °C in DMF ($[M]_0$:$[CTA]_0$:$[I]_0$ = 150:1:0.2). (b) SEC overlay for CEP-mediated polymerization of mSAC at 30 °C in DMF. (c) M_w/M_n versus conversion. (d) M_n versus conversion.

DOI: 10.1021/acs.macromol.5b01453
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Table 2. Conversion, Molar Mass, and Molecular Weight Distribution Data for the RAFT Polymerization of MSAs in DMF at 30 °C[a]

entry	monomer deriv	CTA	time (min)	conv[b] (%)	$[M]_0$ (mol/L)	$M_{ntheory}$[c] (g/mol)	M_{nexp}[d] (g/mol)	M_w/M_n[d]
1a	mSAC	CTP	350	19	1.0	8300	9500	1.02
1b	mSAC	CTP	710	34		14700	16700	1.01
1c	mSAC	CTP	1500	53		22700	20500	1.03
2a	mSAC	CEP	350	26	1.0	11300	16300	1.08
2b	mSAC	CEP	710	58		24800	29700	1.05
2c	mSAC	CEP	1500	81		34600	37500	1.03
3a	mSBZ	CEP	350	10	1.0	5600	8100	1.19
3b	mSBZ	CEP	710	30		15900	14500	1.12
3c	mSBZ	CEP	1500	69		36000	28600	1.02
4a	mSMZ	CEP	350	11	0.83	5700	10300	1.12
4b	mSMZ	CEP	710	35		18200	22100	1.06
4c	mSMZ	CEP	1500	61		32200	35400	1.06
5a	mSMT	CEP	240	8	1.0	4200	8100	1.16
5b	mSMT	CEP	360	14		7500	12200	1.06
5c	mSMT	CEP	780	54		28200	33200	1.05
6a	mSDMX	CEP	240	7	0.83	6300	10800	1.11
6b	mSDMX	CEP	360	13		14800	16700	1.05
6c	mSDMX	CEP	780	44		35500	43800	1.04
7a	mSDOX	CEP	240	11	0.83	4200	8700	1.10
7b	mSDOX	CEP	360	26		7500	11800	1.06
7c	mSDOX	CEP	780	62		25000	30100	1.07

[a]Sulfonamide monomers were polymerized at 30 °C in DMF ($[M]_0$:$[CTA]_0$:$[I]_0$ = 150:1.0:0.2) using V-70 as the initiator. [b]Conversions were determined by 1H NMR (DMSO-d_6) by comparing the relative integral areas of trimesic acid (internal standard) aromatic protons (8.64 ppm, 3H) to the vinyl proton of the sulfonamide monomer (5.84 ppm, 1H). [c]Theoretical number-average molecular weights were calculated according to the equation $M_n = (\rho MW_{mon}[M]/[CTA]) + MW_{CTA}$, where ρ is the fractional monomer conversion, MW_{mon} is the molecular weight of the monomer, and MW_{CTA} is the molecular weight of the CTA. [d]As determined by SEC-MALLS (95:5 (v:v) DMF:CH_3COOH 20 mM LiBr).

M_w/M_n values typically below 1.10 for all monomer derivatives. M_n values determined by DMF SEC-MALLS are in reasonable agreement with theoretical values calculated from monomer conversion; however, M_{nexp} exceeds $M_{ntheory}$ in a similar manner to that discussed earlier. Furthermore, all polymerizations conducted at 30 °C maintained the characteristic color of the parent CTA, indicating limited degradation as compared to that at 70 °C.

The CTP-mediated polymerization of mSAC conducted at 30 °C resulted in 34% monomer conversion after 710 min and narrow molecular weight distributions even after 1500 min of polymerization (53% conversion, M_n = 20 500 g/mol, M_w/M_n = 1.03) (Table 2) with the M_n values determined by DMF SEC-MALLS agreeing well with the theoretical values. The analogous reaction conducted at 70 °C yielded 12% monomer conversion after 600 min and relatively broad molecular weight distributions (M_n = 6200 g/mol, M_w/M_n = 1.27) (Table 1). The strikingly higher rate of polymerization observed for the CTP-mediated polymerization of mSAC performed at 30 °C as compared to 70 °C is consistent with effectively minimizing (though not completely eliminating) competing dithioester degradation and limiting the accumulation of potentially rate-retarding degradation byproducts.

Chain Extension of PSAC-CEP Macro-CTA at 30 °C. To further demonstrate the controlled RAFT polymerization of MSAs at low temperatures, a pSAC-CEP macro-CTA was prepared at 30 °C using V-70 as the initiator and isolated before chain extending with additional mSAC at 30 °C. Figure 5 shows the SEC chromatogram of the pSAC-CEP macro-CTA (M_n = 25 100 g/mol, M_w/M_n = 1.09) and a distinct decrease in elution volume of the chain-extended polymer (pSAC-*b*-pSAC-CEP) (M_n = 49 600 g/mol, M_w/M_n = 1.07). The monomodal SEC chromatogram and absence of low molecular weight tailing at higher elution volumes of the chain extended polymer are additional evidence of improved chain-end retention during the polymerization of MSAs at 30 °C as compared to the analogous chain extension conducted at 70 °C (Figure 2).

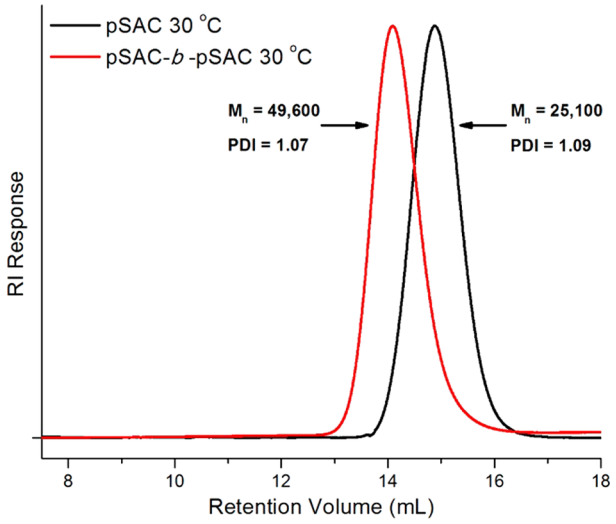

Figure 5. SEC traces of pSAC-CEP macro-CTA (M_n= 25 100 g/mol, M_w/M_n = 1.09) and pSAC-*b*-pSAC-CEP (M_n = 49 600 g/mol, M_w/M_n = 1.07) after chain extension in DMF. Both polymerizations were conducted at 30 °C.

Methacryloyl Sulfonamide Monomer pK_a Studies. MSA monomer titrations were performed to determine the pK_a of each monomer derivative after converting the respective sulfa drug precursors into the corresponding methacrylamides.

DOI: 10.1021/acs.macromol.5b01453
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The pK_a of the sulfonamide (SO_2NH) group of each monomer derivative was determined by eq 1, where $pH_{EP1/2}$ is the pH corresponding to the half equivalence point ($EP_{1/2}$) of the titration curve. The volume of HCl titrant required to reach the $EP_{1/2}$ ($Vol_{EP_{1/2}}$) was determined by eq 2, where Vol_{EP} is the volume of HCl titrant required to reach the equivalence point of the titration curve, [SO_2NH] is the sulfonamide concentration, [HCl] is the concentration of HCl titrant used, and Vol_{sol} is the initial volume of the monomer solution being titrated. Figure 6 shows the positions of the EP and $EP_{1/2}$ on the titration curve for mSAC.

$$pK_a = pH_{EP_{1/2}} \quad (1)$$

$$Vol_{EP_{1/2}} = Vol_{EP} + \frac{1}{2}\frac{[SO_2NH]}{[HCl]}Vol_{sol} \quad (2)$$

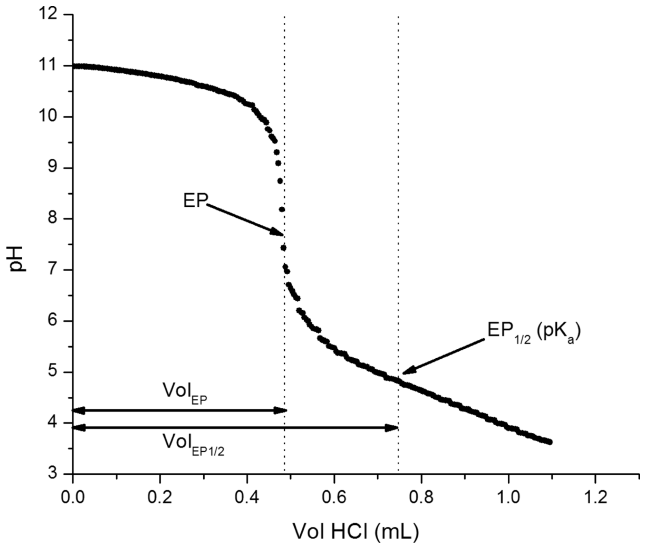

Figure 6. EP and $EP_{1/2}$ locations on the titration curve of mSAC (1 mM) titrated against HCl (0.05 N) at 25 °C using a Metrohm 848 Titrino Plus autotitrator.

Table 3 contains the pK_a values for each monomer calculated using eq 1 along with the literature reported pK_a values for the corresponding sulfa drug precursors. A general trend is observed whereby the pK_a of the MSA is lower than that of the sulfa drug precursor which is consistent with the decrease in pK_a observed upon acetylation of the *p*-amino group of sulfa drugs.[37]

Table 3. MSA Monomer and Polymer Titration Data

polymer	M_{nexp} (g/mol)	M_w/M_n	sulfadrug pK_a	monomer pK_a	pH*
pSAC	31400	1.04	5.38	4.88 ± 0.01	5.3
pSBZ	27500	1.03	4.57	4.51 ± 0.01	5.7
pSMT	32000	1.05	5.29	5.19 ± 0.03	6.3
pSDOX	24400	1.03	6.16	5.44 ± 0.01	6.7
pSDMX	43800	1.04	6.70	5.75 ± 0.01	7.5
pSMZ	34400	1.08	7.49	7.33 ± 0.02	7.9

pH-Dependent Solubility of Poly(methacryloyl sulfonamides). The titration curves (Figure 7) demonstrate the facility by which the pH-dependent solubility of pMSAs can be "tuned" by simply varying the sulfonamide R-group of the monomer. The changes in polymer solubility occur over a very narrow range of typically 0.5 pH units. Table 3 summarizes the pH-dependent solubility of each MSA derivative. The critical onset of precipitation (pH*) is defined as the pH corresponding to 90% light transmittance. For each of the MSA derivatives, pH* of the polymer is greater than the pK_a of the corresponding monomer. The pH* of a particular pMSA is dependent upon the monomer pK_a and the relative hydrophobicity of the monomer derivative, both influenced by the sulfonamide R group. The mutual influence of these two parameters is readily apparent by comparing the pH* and pK_a values for pSAC and pSBZ (Table 3). While the pK_a of mSBZ (4.51) is lower than that of mSAC (4.88), the pH* for pSBZ (5.3) is higher than that of pSAC (5.1) due to the greater hydrophobicity of the benzoyl R group.

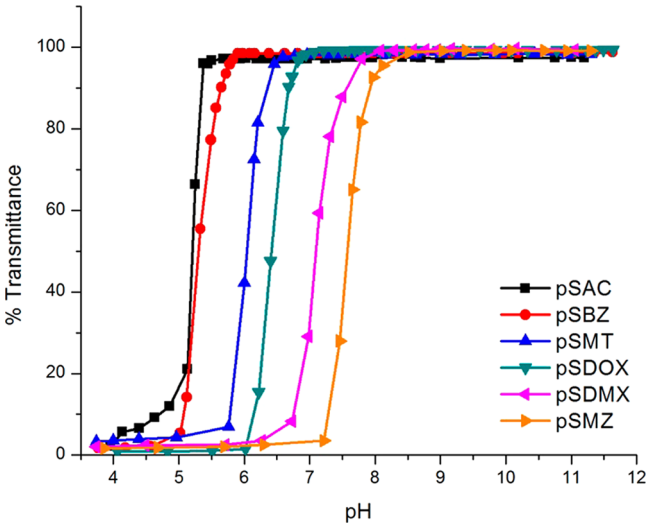

Figure 7. Substituent effects on pH-dependent solubility transitions of sulfonamide-containing polymers. Percent transmittance was measured using a UV−vis spectrophotometer (λ = 500 nm).

CO_2-Dependent Solubility of Poly(methacryloyl sulfonamides). To date, CO_2-responsive polymers rely almost exclusively upon protonation of amine or amidine functional groups by carbonic acid (produced upon dissolution of CO_2 in water) that alters polymer solubility and conformation in solution.[9,38] However, there are very few examples of CO_2-responsive polymers based upon acidic functional groups.[39] In order for acid-functional polymers to exhibit CO_2-induced changes in phase or conformation, the pK_a of the acidic functional group and more importantly the pH* of the corresponding polymer must be greater than the pH of the solution upon production of carbonic acid via dissolution of CO_2. Therefore, weakly acidic polyacids that exhibit pH-responsive behaviors above pH = 4 (the pH of an aqueous solution in equilibrium with 1 atm of CO_2 at 25 °C) should also exhibit similar changes in properties upon CO_2-induced solution acidification.

The weakly acidic pMSA derivatives we report here exhibit pH* values above pH = 5.0, making these ideal candidates as CO_2-responsive polymers. To demonstrate the reversible CO_2-responsiveness of pMSAs, polymethacryloyl sulfamethazine (pSMZ) (M_n = 34 400 g/mol, M_w/M_n = 1.08) (1 equiv of sulfonamide functional group) was dissolved in 0.05 N NaOH (1.25 equiv) and diluted with DI H_2O to yield a final [SO_2NH] = 6.7 mM and [NaOH] = 8.4 mM. The solution was purged with CO_2 (10 s) and then N_2 (25 min) and the % transmittance

DOI: 10.1021/acs.macromol.5b01453
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(λ = 500 nm) of the polymer solution measured before and after each purge cycle using a UV−vis spectrophotometer. Figure 8 shows % transmittance as a function of purge cycle and illustrates the reversible CO_2-triggered change in aqueous solubility of pSMZ.

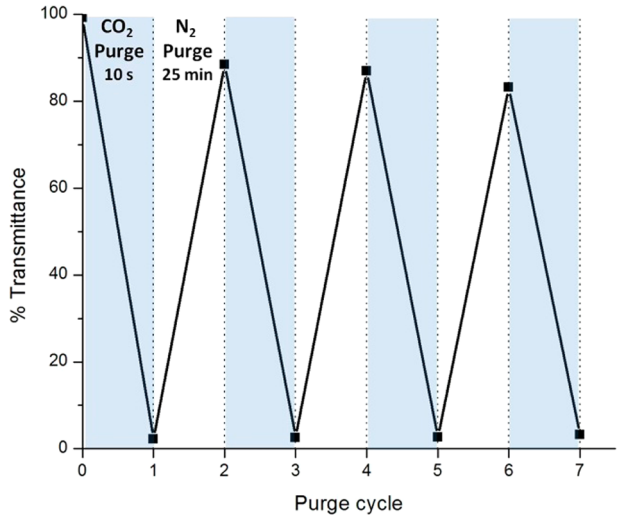

Figure 8. Reversible solubility of pSMZ in response to presence or absence of CO_2. Solutions were purged with either CO_2 for 10 s (shaded regions) or N_2 for 25 min (unshaded regions) and % transmittance measured using a UV−vis spectrophotometer (λ = 500 nm).

CONCLUSIONS

A series of pMSA polymers with tunable, pH-dependent solubility in aqueous media have been synthesized by RAFT polymerization. Initially, polymerizations conducted in DMF at 70 °C gave polymers with broad molecular weight distributions, but upon reducing the polymerization temperature to 30 °C and employing the low decomposition temperature initiator V-70, polymers of narrow molecular weight distribution and increased thiocarbonylthio chain-end functionality were obtained. Selection of the sulfonamide R group of MSA monomers is a facile means of adjusting pK_a and ultimately the critical onset of precipitation pH (pH*) of the corresponding pMSA. Thus, it is possible to "fine tune" pH-dependent polymer solubility in the biologically relevant regime (pH = 4.5−7.4). Additionally, we demonstrated the reversible CO_2-responsiveness of pMSAs in aqueous media, further indicating the potential of pMSAs in biological and nanotherapeutic applications.

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Notes

The authors declare no competing financial interest.

This is paper number 157 in a series entitled "Water-Soluble Polymers".

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided in party by the National Science Foundation Graduate Research Fellowship Program under Grant GM004636/GR04355 and the National Science Foundation under Grant IIA-1430364.

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