Design of Thermoresponse Polymers with Aqueous LCST, UCST, or Both: Modification of a Reactive Poly(2-vinyl-4,4-dimethylazlactone) Scaffold

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S Supporting Information

ABSTRACT: The synthesis and aqueous solution properties of a family of zwitterionic homo-, co-, and terpolymers derived from poly(2-vinyl-4,4-dimethylazlactone) (pVDMA) with tunable lower and upper critical solution temperatures (LCST and UCST) are presented. A RAFT-made pVDMA precursor was reacted with mixtures of zwitterionic sulfopropylbetaine (SPB) amine or sulfobutylbetaine (SBB) amine, tetrahydrofurfurylamine (THF amine), and benzylamine (Bz amine) in varying molar ratios. Products were characterized by variable temperature (VT) NMR spectroscopy, FT-IR spectroscopy, size exclusion chromatography, turbidity, and VT dynamic light scattering in order to confirm quantitative postpolymerization modification, determine molar compositions, and elucidate structure–property relationships. Polymers comprising large molar fractions of THF groups showed LCST behavior due to a polarity change of the THF-functional segments, while SPB/SBB-rich samples, including the zwitterionic homopolymers, showed UCST behavior in ultrapure water based on electrostatic polymer–polymer attractions. Binary SPB–THF copolymers were water-soluble between 0 and 90 °C for a large compositional range. Terpolymers comprising molar SPB:THF:Bz ratios of approximately 50:25:25 showed a low LCST and a high UCST (LCST < UCST) with a miscibility gap in which the SPB groups and THF groups were not fully hydrated. In the one-phase regions below the LCST and above the UCST, polymer chains were presumed to be unimerically dissolved with partially solvated domains undergoing intrachain associations. Addition of NaCl caused LCST and UCST behavior to disappear, resulting in temperature-independent solubility. Molecular insights presented herein are anticipated to aid in the development of smart materials with double LCST < UCST or UCST < LCST thermoresponsiveness.

INTRODUCTION

Stimulus-responsive polymers, a class of smart materials, have influenced many fundamental and applied research fields as diverse as nanotechnology, sensors, catalysis, coatings, and biomedicine.^{1–4} Polymers that exhibit temperature responsiveness, especially in aqueous solution, are particularly versatile materials.^{5,6} Their ability to change abruptly and reversibly between hydrophobic globular and hydrophilic coil states can be triggered not only by subtle temperature changes but also, in specifically engineered multiresponsive copolymers,⁷ at constant temperature⁸ through a range of other stimuli including pH,⁹ CO₂,⁹ mechanical agitation,¹⁰ light,^{11,12} redox potential,² or metal ions.^{8,13} There has been long-standing interest in the polymer science arena to develop tunable thermoresponsive polymers and to understand structure–property relationships of their sometimes complex phase behavior.

There are two main types of temperature responsiveness in water. Polymers with a lower critical solution temperature (LCST) phase separate upon heating based on a loss of

entropically unfavorable “hydrophobic hydration” of hydrophobic segments at the critical temperature. This behavior can be evoked and tuned by adjusting the hydrophilic–hydrophobic balance within a (macro)molecule, and a large number of (co)polymers have been demonstrated to have measurable LCST transitions under standard conditions (0–100 °C, $p = 1$ atm).^{14–16} The opposite behavior, phase separation upon cooling, characterized by an upper critical solution temperature (UCST), is far less common in water and known only for a few types of (co)polymers.¹⁷ These polymers are typically markedly hydrophilic species that are insoluble below a critical temperature because of strongly attractive polymer–polymer interactions that are broken by water when the temperature-weighted entropy of mixing, $T\Delta S_{\text{mix}}$, outbalances these enthalpic attractions.

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From an application perspective, doubly thermoresponsive polymers that exhibit LCST and UCST behavior are attractive because they offer the potential to engineer smart materials that respond only within a specific range of an environmental variable rather than just beyond a critical value.

Reversible deactivation radical polymerization (RDRP) techniques have been applied to produce block copolymers comprising LCST- and UCST-type blocks in which the different parts of the macromolecule respond separately to temperature changes resulting, in most presented cases, in self-assembly.^{18–23} In contrast, examples of homopolymers or statistical copolymers showing LCST and UCST in aqueous solution under standard conditions are sparse. Such polymers can fall into two categories: Species with LCST < UCST are *insoluble* within a temperature window (circular temperature–concentration phase diagram) while polymers with UCST < LCST are *soluble* only within a temperature window (phase diagram with two U-shaped binodal curves bending away from each other). Several OH-functional (co)polymers, for example, including partially hydrolyzed poly(vinyl acetate/butyrate),^{24,25} copolymers of (protonated) acrylic acid,^{26,27} and poly(2-hydroxyethyl methacrylate) of low molar masses,²⁸ can show LCST < UCST behavior depending on copolymer composition, added salt, and solution pH. Recently, Cai et al. described OH- and tertiary amine-functional homopolymers prepared through a combination of click chemistry and postpolymerization modification to show LCST < UCST behavior.²⁹ Copolymers of a zwitterionic sulfopropylbetaine methacrylate (SPB MA) (known to produce homopolymers with aqueous UCST) with *N*-isopropylacrylamide (NIPAM)³⁰ or (2-dimethylaminoethyl methacrylate (DMAEMA)³¹ (both typical LCST-type monomers), on the other hand, have been shown to exhibit UCST < LCST behavior at precisely adjusted molar compositions.

Several (co)polymers show double thermoresponsiveness under nonstandard conditions. Poly(ethylene glycol), PEG, for example, has an LCST < UCST-type miscibility gap in water above 100 °C which is experimentally assessable at elevated pressures.^{32,33} Several polycations including partially protonated poly(DMAEMA)^{34,35} and quaternized ammonium species^{28,36} have been shown to exhibit LCST and UCST transitions at appropriate ionic strengths and/or in the presence of multivalent or hydrophobic anions. These examples demonstrate the often very specific solutions conditions under which double thermoresponsiveness is observable.

Postpolymerization modification, the functionalization of reactive pendant groups, has emerged as a versatile synthetic tool to study structure–property relationships. A main reason for this is that libraries of homopolymers and statistical copolymers with smart characteristics and identical degrees of polymerization can be produced from a single precursor modified with a very wide range of functional reagents.^{37,38} Poly(pentafluorophenyl acrylate), a common activated ester precursor,^{39,40} for example, can conveniently be converted into thermoresponsive poly(NIPAM)-based (co)polymers through an acyl substitution reaction with isopropylamine and in mixtures with other amines.⁴¹ Our group recently demonstrated the synthesis of sulfobetaine (co)polymers with aqueous UCSTs from this reactive scaffold by modification with the zwitterionic amines 3-((3-aminopropyl)dimethylammonio)propane-1-sulfonate and 4-((3-aminopropyl)dimethylammonio)butane-1-sulfonate.^{42,43} A far less commonly investigated reactive platform is poly(2-vinyl-4,4-dimethylazlactone),

pVDMA, which undergoes rapid, atom-economic ring-opening addition reactions with amines yielding poly(2-acrylamido-isobutyramide)s.^{44,45} We recently presented a first study into producing homo- and copolymers with aqueous LCST through postpolymerization modification of pVDMA with amines.⁴⁶ To the best of our knowledge, polymers with aqueous UCST have not been prepared from pVDMA.

Herein, we describe the synthesis of homo-, co-, and terpolymers with aqueous UCST, LCST, and both from a pVDMA precursor. By systematically varying the molar compositions of zwitterionic sulfobetaine, intermediately polar tetrahydrofurfuryl, and hydrophobic benzyl functionality, the effect of each group on the water solubility and their interplay in achieving tunable LCST < UCST double thermoresponsiveness under standard conditions are presented.

■ EXPERIMENTAL SECTION

Materials. All reagents were purchased from Sigma-Aldrich and were used as received unless stated otherwise. Propylene carbonate (99.7%, anhydrous) was stored in a glovebox. Azobis(isobutyronitrile) (AIBN) was recrystallized from methanol and stored at –24 °C. The syntheses of chain transfer agent (CTA) benzylpropyl trithiocarbonate (BPTC)⁴⁷ and the zwitterionic amines 3-((3-aminopropyl)dimethylammonio)propane-1-sulfonate, (SPB amine)⁴² and 4-((3-aminopropyl)dimethylammonio)butane-1-sulfonate (SBB amine)⁴³ are described elsewhere. Monomer 2-vinyl-4,4-dimethylazlactone (VDMA) was prepared according to a literature procedure.⁴⁸

Methods. NMR spectroscopic measurements in D₂O were performed on Bruker Avance 300 or 500 MHz instruments in 5 mm NMR tubes. Measurements of polymers were done on D₂O solutions containing up to 0.5 M NaCl. The internal solvent signal $\delta(\text{HDO}) = 4.79$ ppm was used as reference. For variable temperature NMR spectroscopic measurements, samples were equilibrated until the measured temperature in the probe was stable within ± 0.2 °C. The internal solvent signal $\delta(\text{HDO}) = 4.79$ ppm was used for calibration at 25 °C, and spectra recorded at other temperatures were calibrated to the chemical shift of a methylene segment of the zwitterionic side groups at $\delta = 3.48$ ppm.

Size exclusion chromatography (SEC) in dimethylacetamide (DMAc) was performed on a Shimadzu system with four 300 $\times$ 7.8 mm² linear phenogel columns (10⁵, 10⁴, 10³, and 500 Å) operating at a flow rate of 1 mL/min. The system was calibrated with a series of narrow molar mass distribution polystyrene (PS) standards. Chromatograms were analyzed by Cirrus SEC software version 3.0.

Fourier transform infrared spectroscopy (FT-IR) was performed on a Bruker IFS 66/S instrument under attenuated total reflectance (ATR), and data were analyzed on OPUS software version 4.0.

Turbidity measurements were performed on a Varian Cary 300 Scan spectrophotometer equipped with a Cary temperature controller and a Peltier heating element in quartz cuvettes of 10 mm path length at a wavelength of 520 nm with heating/cooling rates of 1 °C/min. Polymer concentrations were 10 g/L. For clear solutions the baseline was corrected to zero absorbance, A . Transmittance, $t = 10^{-A}$, was plotted against temperature, and cloud points, T_{CP} , were determined at $t = 50\%$.

Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano ZS at a scattering angle of 173° and were analyzed by Malvern Zetasizer Software version 6.20. Samples had a concentration of 1 g/L and were equilibrated at each temperature for 6 min before measurements.

Poly(2-vinyl-4,4-dimethylazlactone), PVDMA. Monomer VDMA (1.0 g, 7.2 mmol, 100 equiv), BPTC (17.4 mg, 0.072 mmol, 1 equiv), AIBN (1.2 mg, 0.0072 mmol, 0.1 equiv), and acetonitrile (2 mL) were combined in a flask, which was equipped with a stir bar and sealed with a rubber septum. After purging with nitrogen for 20 min, the mixture was stirred in a preheated oil bath at 70 °C for 6 h. The polymerization was stopped by quenching the reaction with liquid nitrogen. A sample (100 μL) was withdrawn, diluted with CDCl₃ (550

Table 1. Homo-, Co-, and Terpolymers Derived from PVDMA with Zwitterionic Amines, Benzylamine, and THF Amine

entry	polymer ^a	amine feed ratio SPB(SBB):Bz:THF ^b (mol %)	$M_n^{\text{theor } c}$ (kg/mol)	CP ^d LCST (°C)	CP ^d UCST (°C)
1	pSPB	100:0:0	28.6		10.3
2	pSBB	100:0:0 (SBB)	29.7		41.4
3	pTHF	0:0:100		31 ^e	
4	p[SPB _{0.33} -co-THF _{0.67}]	50:0:50	22.2	81.7	
5	p[SPB _{0.78} -co-THF _{0.22}]	85:0:15	26.5	S ^f	S
6	p[SPB _{0.80} -co-Bz _{0.20}]	70:30:0	26.8	S	S
7	p[SPB _{0.63} -co-Bz _{0.37}]	50:50:0	25.2		54.0
8	p[SPB _{0.58} -co-Bz _{0.26} -co-THF _{0.16}]	45:45:10	24.7		75.5
9	p[SPB _{0.42} -co-Bz _{0.24} -co-THF _{0.34}]	35:35:30	23.1	9.1	
10	p[SPB _{0.21} -co-Bz _{0.19} -co-THF _{0.60}]	25:25:50	21.1	13.7	
11	p[SPB _{0.43} -co-Bz _{0.19} -co-THF _{0.38}]	40:35:25	23.2	30.9	
12	p[SPB _{0.57} -co-Bz _{0.23} -co-THF _{0.20}]	50:35:15	24.6	0	53.4
13	p[SPB _{0.51} -co-Bz _{0.23} -co-THF _{0.26}]	45:35:20	24.0	37.9	67.8
14	p[SPB _{0.45} -co-Bz _{0.24} -co-THF _{0.31}]	40:40:20	23.4	15.8	76.5
15	p[SPB _{0.48} -co-Bz _{0.15} -co-THF _{0.37}]	50:25:25	23.7	S	S
16	p[SPB _{0.67} -co-Bz _{0.18} -co-THF _{0.15}]	60:30:10	24.7	S	S

^aMolar composition determined by ¹H NMR spectroscopy. ^bRatio of amines in reaction. 100% corresponds to a 1.5 equiv feed of amines. ^cMolar mass calculated from DP of precursor and copolymer composition from ¹H NMR spectroscopy assuming full conversion. ^dCloud point measured in Milli-Q water at a concentration of 10 g/L by heating (LCST type) or cooling (UCST type). ^eValue from our previous study for homopolymers of comparable molar mass. ^fSoluble between 0 and 90 °C at a concentration of 10 g/L.

different separation than for the reactive precursor (see Figure S2). For this reason and limited solubility in the eluent, SEC analysis was not possible for all samples. Given the postpolymerization synthetic strategy, and the absence of observable polymer–polymer coupling reactions in the measured samples, however, all daughter polymers were assumed to have the same average degree of polymerization of 78 as the reactive precursor. ¹H NMR spectra of polymer products conformed to the expected structures and allowed for an approximation of molar compositions through integration (see Figure S3).

In all cases, except for homopolymers, the observed molar compositions deviated from the amine feed ratios as a result of (i) a total feed of 1.5 equiv of amines per pVDMA repeat unit and (ii) different apparent reactivities of the amines toward the azlactone functionality. The incorporation of the THF functionality was consistently higher than the feed ratio, reaching the maximum of 1.5-fold in many cases, while the incorporation of the benzyl functionality was consistently lower than the feed ratio, resulting an observed amine reactivity in the order THF amine > SPB amine > Bz amine presumably reflecting steric effects (THF amine vs SPB amine) and a reduced basicity/nucleophilicity of Bz amine. By considering these reactivity differences, terpolymers with (near) targeted molar compositions were obtained through adjusting the amine feed ratios, making this synthetic strategy a convenient approach for the preparation of a library of functional co- and terpolymers from a single parent homopolymer.

The temperature-dependent aqueous solution behavior of the pVDMA-derived species was assessed through turbidity measurements, variable temperature (VT) NMR spectroscopy, and VT dynamic light scattering. Depending on their molar composition, polymers with UCST, LCST, or both were identified. Turbidity curves are plotted in Figure 1, and cloud points are summarized in Table 1. Transitions were typically fully reversible (see Figure S4).

The zwitterionic homopolymers pSPB and pSBB (Table 1, entries 1 and 2) showed UCST behavior in ultrapure water with cloud points of 10 and 41 °C, respectively. This is, to the

Figure 1. Turbidity curves of aqueous solutions of homo-, co-, and terpolymers at a concentration of 10 g/L found to exhibit (A) UCST, (B) LCST, and (C) both UCST and LCST behaviors.

best of our knowledge, the first report on pVDMA-derived poly(2-acrylamidoisobutyramides) with an aqueous UCST and extends the smart polymer family of the related zwitterionic poly(meth)acrylates and poly(meth)acrylamides.⁵⁷

The higher cloud point of the sulfobutylbetaine species compared to the sulfopropylbetaine-functional sister polymer is in agreement with the trend observed for polymethacrylates and polyacrylamides.⁴³ In a comparison of backbone structures, the pVDMA-derived species appear to be less water-soluble, i.e., have higher UCST cloud points, than the respective SPB- and SBB-functional polymethacrylate and polyacrylamide derivatives of comparable degrees of polymerization.^{43,56} Decreased water solubility of poly(*N*-alkyl 2-acrylamidoisobutyramides) compared to the respective poly(*N*-alkyl acrylamides) has been reported and attributed to the 2-methylalanyl segment in the former species.⁴⁶

For SPB–THF copolymers (Table 1, entries 4 and 5), LCST and UCST behaviors were found to be mutually exclusive. The LCST cloud point of 31 °C for pTHF (entry 3) was found to increase with the incorporation of zwitterionic comonomer units, while the UCST cloud point of pSPB was assumed to decrease with the incorporation of THF-functional comonomer units as copolymer p[SPB_{0.78}-co-THF_{0.22}] showed temperature-independent aqueous solubility (see Figure 2A).

Figure 2. Temperature–molar composition phase diagrams showing the interplay of zwitterionic SPB and intermediately polar THF groups for (A) copolymers of these two functionalities where combination of the two groups mutually increases solubility and (B) terpolymers containing 0.19–0.24 mol % of benzyl pendant groups in dependence of the molar composition of the residual ~80%. Short dashed curves are extrapolated and indicate the assumed trend of the phase separation boundary taking the composition of fully soluble terpolymer samples into account. The dashed curve in part B connects the highest LCST-type cloud points to guide the eye along a presumed phase boundary. The region in which samples showed LCST and UCST behavior is shaded.

Table 2. Overview of Temperature-Dependent Influences of Each Functional Group on Water Solubility with Examples from Table 1

Functional Group	Influence on Water Solubility	Observed Effects (Example entries in Table 1)
SPB/SBB $q = 3 \text{ or } 4$	decreased solubility below a critical temperature increased solubility above a critical temperature	<ul style="list-style-type: none"> UCST transitions of zwitterionic homopolymers (1,2) UCST transitions of zwitterionic co- and terpolymers (7,8,12,13,14) incorporation of SPB groups into THF-functional polymers increases the LCST (3/4/5)
THF	decreased solubility above a critical temperature increased solubility below a critical temperature	<ul style="list-style-type: none"> LCST transitions of homopolymers (3) LCST transitions of co- and terpolymers (4,9,10,11,13,14) incorporation into hydrophobically modified sulfobetaine copolymers increases the UCST (7/8 (comparable SPB content); 12/13/14 (comparable Bz content)) incorporation into zwitterionic SPB-functional polymers (with comparatively low UCSTs) decreases the UCST (1/5) terpolymers that are phase separated below their UCST and contain a sufficient amount of THF groups redissolve upon further cooling (4/12/13/14 (comparable Bz content and UCST CP; LCST only for 20–31% THF, not 16%))
Bz	generally decreased solubility	<ul style="list-style-type: none"> hydrophobic modification generally decreases LCST transitions³⁸ UCST transitions of zwitterionic copolymers are increased above a critical Bz content (1/7). Conversely, incorporation of a small amount of hydrophobic groups can increase solubility⁴² (1/6/7)

With the incorporation of hydrophobic benzyl segments, the terpolymers $p[\text{SPB}_x\text{-co-Bz}_y\text{-co-THF}_z]$ exhibited rich aqueous solution behavior encompassing double LCST and UCST thermoresponsiveness. In order to understand the interplay of the three functional groups, a 3D-phase diagram was constructed (see Figure S5). Observed influences on the aqueous solution behavior of each functional group are summarized in Table 2.

A 2-D temperature–molar composition phase diagram of terpolymers containing a similar (0.19–0.24) molar content of benzyl functionality, shown in Figure 2B, indicated the importance of the benzyl functionality in achieving double thermoresponsiveness. Incorporation of benzyl groups was expected to decrease the LCST transition of THF-functional

copolymers and thereby counteract the solubility increase found for the incorporation of SPB groups, thus shifting the LCST behavior into the 0–100 °C range for terpolymers. Importantly, the presence of benzyl comonomer units apparently inverted the effect of the THF groups on the UCST behavior of SPB-functional species (circles in Figures 2A,B), bringing the UCST phase separation boundary into an intermediate compositional range. As a result, double LCST < UCST thermoresponsiveness was observed for terpolymers containing molar contents of ~0.2 benzyl groups and 0.2–0.4 THF groups (shaded area in Figure 2B). Within this range, both transition temperatures were strongly composition dependent. Sample $p[\text{SPB}_{0.57}\text{-co-Bz}_{0.23}\text{-co-THF}_{0.20}]$ (Table 1, entry 12), for example, was found to be insoluble between 0

and 53 °C, while terpolymer p[SPB_{0.51-co}-Bz_{0.23-co}-THF_{0.26}] (entry 13) with the same measured Bz molar content and only 6 mol % difference in the SPB–THF balance had a miscibility gap from 38 to 68 °C. This compositional dependence suggested difficulties in a precise tuning of the LCST transition and, considering an assumed relative uncertainty of up to $\pm 10\%$ in the determination of molar compositions through ^1H NMR spectroscopy, may be a reason for the two apparent outlying LCST cloud points in Figure 2B.

Temperature-independent solubility within the observed range of 0–90 °C was expected for (ter)polymers with (i) a theoretical LCST > 100 °C, (ii) a theoretical UCST < 0 °C, or (iii) the case LCST > UCST (*vide infra*). Given the narrow miscibility gap between the (extrapolated) phase boundaries in Figure 2B, a crossing of the two phase boundary curves for molar Bz contents other than 0.2 is conceivable and may explain why some terpolymer samples (e.g., Table 1, entries 15 and 16) were found to be fully soluble, in spite of having similar molar compositions as samples that showed thermal transitions (e.g., compare entries 8 and 16).

The UCST transitions of zwitterionic (co)polymers are known to be strongly dependent on the ionic strength showing an antipolyelectrolyte effect and increased solubility (decreased UCSTs) with increasing salt concentration.⁵⁸ LCST transitions are typically also influenced by added salt with effects in agreement with the Hofmeister series typically found at significantly higher salt concentrations.⁵⁹ The addition of small amounts of NaCl to aqueous solutions of doubly thermoresponsive terpolymers may thus be expected to affect only the UCST transition. Cloud point curves of sample p[SPB_{0.51-co}-Bz_{0.23-co}-THF_{0.26}] (Table 1, entry 13) in water containing 0, 1, 2, and 3 mM NaCl obtained during heating and cooling are shown in Figure 3. Indeed, the UCST transition

Figure 3. Influence of added NaCl concentration on the aqueous solution behavior of terpolymer p[SPB_{0.51-co}-Bz_{0.23-co}-THF_{0.26}].

(solid arrow connecting solid lines) shifted to lower temperatures with increasing salt concentration. Simultaneously, however, the LCST transition (dashed arrow indicating trend of dashed heating curves) shifted to higher temperatures with the effect of NaCl concentration being of similar magnitude for the UCST and LCST transitions. Simultaneously, hystereses between the heating and cooling curves increased with NaCl concentration. Most noticeably, the clouding became less pronounced with the minimum transmittances increasing until, above an NaCl concentration of 3 mM, the polymer was found

to be soluble over the entire observed temperature range with both UCST and LCST behavior disappearing. This generally increased solubility (*vide infra*) was assumed to cause also the unexpected shift of the LCST transition temperature.

In order to investigate the molecular basis of temperature-dependent (in)solubility, ^1H NMR measurements were conducted on a solution of p[SPB_{0.51-co}-Bz_{0.23-co}-THF_{0.26}] in D₂O at 10 °C (soluble), 45 °C (cloudy), and 80 °C (soluble). Though isotope effects have been shown to cause UCST transitions of polyzwitterions to be several °C higher in D₂O than in H₂O,⁵¹ the deuterated solvent was still considered a suitable model to elucidate molecular events in H₂O. Spectra are plotted in Figure 4. Two opposing trends were apparent

Figure 4. Sections of ^1H NMR spectra of p[SPB_{0.51-co}-Bz_{0.23-co}-THF_{0.26}] (Table 1, entry 13) recorded at 10 °C (blue), 45 °C (green), and 80 °C (red) in D₂O (300 MHz) with arrows indicating the broadening of the THF signals and a sharpening of the SPB-related signals with increasing temperature.

with the THF groups and the SPB groups each behaving as expected in their respective homopolymers. Upon heating, especially from 10 to 45 °C, the signals associated with the $-\text{CH}_2\text{O}-$ segment of the THF groups became broader, suggesting shorter relaxation times, more rigidity, and slower molecular movements caused through the loss of a certain amount of hydration as these groups become hydrophobic above their critical temperature. The opposite effect was found for the zwitterionic segments of the terpolymers. Upon increasing the temperature, the associated signals became markedly sharper, suggesting increased hydration. The sharpness of the signals associated with the benzyl protons did not change strongly with temperature. The chemical shift of the highest peak in this broad multiplet and the intensity of a shoulder toward lower field, however, changed slightly, suggesting slightly different chemical environments of the benzyl groups at different temperatures. These observations indicated that both thermosensitive groups (SPB and THF) behaved largely independent of each other. In line with the observed LCST < UCST behavior, the terpolymers were insoluble when the THF groups and the SPB groups both were not fully hydrated with hydration of one of these groups sufficient to make the entire polymer soluble (see Table 3). When the two critical temperatures became closer, e.g. through variation of the molar composition or through addition of salt, the overlap region in which the SPB and THF groups are not fully hydrated became smaller and disappeared, explaining the occurrence of samples with temperature-independent solubility.

Table 3. Summary of Interplay between SPB and THF Groups with Opposing Effects in Producing LCST < UCST Behavior

temperature	THF groups	SPB groups	terpolymer solubility
$T < \text{LCST}$	solvated, hydrophilic	not fully solvated, presumed intrachain association	soluble
$\text{LCST} < T < \text{UCST}$	not fully solvated, hydrophobic, presumed interchain aggregation	not fully solvated, presumed interchain interlocking	insoluble
$T > \text{UCST}$	not fully solvated, hydrophobic, presumed intrachain association	solvated	soluble

The same set of VT NMR measurements at 10, 45, and 80 °C was performed on terpolymer $p[\text{SPB}_{0.48}\text{-co-Bz}_{0.15}\text{-co-THF}_{0.37}]$ which showed temperature-independent solubility (Table 1, entry 15). The spectra, shown in Figure S6, offered two observations. First, the behavior of the SPB side groups was identical to that observed for the doubly responsive sample (Figure 4)—increasing solvation and peak sharpness with increasing temperature. This observation may appear surprising, since this sample did not undergo any observable UCST transition. In this context it is noteworthy that unlike LCST transitions, UCST transitions can be the result of very gradual microscopic changes, as demonstrated recently on PEG methacrylate-containing polymers in isopropanol.⁶⁰ The thermoresponsive side chains (PEG in alcohol or zwitterionic groups in water) gradually lose solvation with decreasing temperature and can be assumed to “crumple” onto the backbone. This behavior is independent of polymer concentration⁶⁰ and, as indicated in this study, independent of the functional group molar content in a copolymer. On the other hand, whether or not a (partially desolvated) polymer phase separates macroscopically depends on interpolymer forces during polymer–polymer collisions and therefore depends on polymer concentration and functional group molar content.⁶¹ For such cases, UCST transitions cannot be observed by means of VT NMR measurements. Second, the signals associated with the THF side groups became only very slightly weaker and broader with increasing temperature. In an LCST transition, thermoresponsive side groups suddenly switch from hydrophilic to hydrophobic (though they typically still retain some bound water molecules). The resulting hydrophobic effect then causes the polymer to phase separate. Commonly, VT NMR measurements present an ideal tool to monitor the LCST transition because of shortened relaxation times in the phase separating material. The observed slight weakening and broadening of the signals associated with the THF group may thus suggest slight changes in the hydration of these thermoresponsive groups in the absence of a macroscopic phase separation.

Notably, the LCST < UCST behavior observed for double responsive samples is opposite to UCST < LCST behavior documented for copolymers of SPB methacrylate with NIPAM (prepared by free radical polymerization)³⁰ and SPB methacrylate with DMAEMA (prepared by surface-initiated atom transfer radical polymerization).³¹ In these literature examples, the copolymers appear to dissolve only when both respective comonomer units are solvated and are insoluble when one (or no) comonomer type is solvated. In order to further characterize the behavior of the terpolymers derived by postpolymerization modification of pVDMA, an aqueous solution of sample $p[\text{SPB}_{0.51}\text{-co-Bz}_{0.23}\text{-co-THF}_{0.26}]$ was analyzed by variable temperature dynamic light scattering (see Figure 5). At temperatures $T \leq 30$ °C and $T \geq 75$ °C the volume-average diameter of scatterers was around 10 nm, averaging 9.50 nm at 10 °C and 11.42 nm at 80 °C (Figure 5A and blue and red curves in Figure 5B). Between these temperatures and

Figure 5. (A) Average hydrodynamic diameter of scatterers by volume (averaged from four runs with standard deviation shown as error bars) for sample $p[\text{SPB}_{0.51}\text{-co-Bz}_{0.23}\text{-co-THF}_{0.26}]$ in water in dependence of temperature. (B) Four size distribution plots each measured on a sample at 10, 80, and 10 °C in the presence of 5 mM NaCl.

coinciding with the region of reduced transmittance (Figure 3, black curve) the average diameter increased drastically with large calculated standard deviations, suggesting the formation of large, poorly defined aggregates in the two-phase domain. The smaller observed sizes above and below this temperature range were interpreted as hydrodynamic diameters of unimerically dissolved terpolymer chains, which suggested water solubility when only one type of comonomer unit was assumed to be fully hydrated (see Table 3).⁶⁰ Taking the incomplete hydration observed by VT NMR measurements into account, in these one-phase regions, the not fully hydrated comonomer units were assumed to undergo intrachain associations, with partially collapsed terpolymer domains presumably forming intrachain “knots”.⁶² Such “pearl necklace” conformations (see Scheme 2) featuring intrachain associated domains connected through extended, solvated sections have been described theoretically for amphiphilic copolymers consisting of a hydrophobic backbone carrying hydrophobic and charged side groups^{63–67} and have been experimentally verified for model copolymers in aqueous solution through small-angle neutron/X-ray scattering^{68–71} and AFM.^{68,72} Pearl necklace configurations have also been associated with the early stages of homopolymer collapse where this configuration has decreased overall chain dimensions compared to fully hydrated chains.^{61,73} Partial chain “knotting” may be a reason for the slightly different hydrodynamic diameters measured at 10 °C (9.50 nm) and 80 °C (11.42 nm) at which temperatures VT NMR measurements suggested different domains of the terpolymer chains to be fully solvated.⁶² Additionally, DLS analysis of the same sample at 10 °C in the presence of 5 mM NaCl (assumed to break electrostatic association of zwitterionic groups and make the polymer fully hydrophilic) gave a hydrodynamic diameter of 13.05 nm (yellow curves in Figure 5B), which was interpreted to reflect fully hydrated, “untied” chains, underpinning the

Scheme 2. Proposed Conformations of a Zwitterionic P[SPB_x-co-Bz_y-co-THF_z] Terpolymer Showing LCST < UCST Behavior at Different Temperatures Including Single Chain “Pearl Necklace” Conformations at Low and High Temperatures and Interchain Entanglement and Aggregation between the Critical Temperatures^a

^aThe electrostatic interactions of the zwitterionic groups dominate below the UCST, while the THF-functional segments are hydrophobic above the LCST.

formation of smaller conformations of single chains in the one-phase regions without NaCl.

Ultimately, whether suitably functional (co)polymers show solubility, LCST < UCST, UCST < LCST, or insolubility in water will depend on what percentage of repeat unit hydration is required to render entire chains soluble. Conceivably, for a given set of functional comonomer units, this percentage may depend strongly on the copolymer microstructure, i.e., the precise comonomer sequence, backbone tacticity, and whether “knotted” conformations are possible in which poorly solvated domains are stabilized by sufficiently long, adjacent, solvated chain sections. It can thus be envisaged that the design of advanced doubly thermoresponsive polymers and their engineering into smart materials will benefit from emerging techniques to determine and control comonomer sequence.⁷⁴

CONCLUSION

Only very few types of polymers are known to show LCST and UCST behavior in water under standard conditions. This study takes advantage of the postpolymerization modification concept to generate a library of statistical copolymers with the same degree of polymerization and elucidate structure–property relationships in a novel class of copolymers containing zwitterionic SPB, hydrophobic Bz, and intermediately hydrophilic THF functional groups derived from pVDMA. The combination of SPB and THF functional groups, known to impart UCST and LCST thermoresponsiveness, respectively, resulted in mutually exclusive LCST or UCST transitions and samples with temperature-independent water solubility. The incorporation of Bz groups and the associated reduced water solubility proved crucial to achieve double LCST and UCST thermoresponsiveness under standard conditions. Importantly, terpolymers dissolved unimerically in water at temperatures where only one type of comonomer units was fully solvated (full hydration of THF groups causing solubility at low temperatures and full hydration of SPB groups causing solubility at high temperatures), enabling LCST < UCST double thermoresponsiveness featuring a miscibility gap or, in the case of a

theoretical “LCST > UCST” scenario, temperature-independent solubility. A comparison with literature examples of zwitterionic methacrylate-based zwitterionic copolymers indicated that copolymers comprising very similar building blocks can show UCST < LCST behavior or, in the case of a theoretical “UCST > LCST” scenario for such species, insolubility over the entire observable temperature range. An entirely independent tuning of the LCST and UCST transitions in doubly thermoresponsive (co)polymers over the entire temperature range is thus not possible in either case but results in either solubility or insolubility. The high solubility of pVDMA-derived terpolymers and the associated LCST < UCST behavior was attributed to intrachain associations of poorly hydrated chain segments, causing a “pearl necklace” configuration. It is concluded that the (typically unknown) microstructure and comonomer sequence in statistical copolymers (whether prepared through direct copolymerization or postpolymerization modification) may have a profound impact on the solubility and thermal solution behavior of species containing groups with opposing thermal responses.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.5b02056.

FT-IR spectra, SEC curves, and exemplary ¹H NMR spectrum supporting successful terpolymers synthesis, turbidity curves showing reversibility and hysteresis, and 3-D phase diagram of cloud points vs terpolymer molar composition (PDF)

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Notes

The authors declare no competing financial interest.

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