Synthesis and Rheological Characterization of Poly(vinyl acetate-b-vinyl alcohol-b-vinyl acetate) Triblock Copolymer Hydrogels

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Supporting Information

ABSTRACT: We report the synthesis and shear rheology of a series of hydrogels derived from poly(vinyl acetate-b-vinyl alcohol-b-vinyl acetate) (PVAc-b-PVOH-b-PVAc) triblock copolymers. Bidirectional, reversible-addition-fragmentation chain transfer free radical polymerizations of vinyl acetate (VAc) and vinyl chloroacetate (VClAc) are optimized to produce a series of relatively narrow dispersity PVAc-b-P(VAc-ran-VClAc)-b-PVAc triblock copolymers with controlled molecular weights and compositions. Rapid and selective hydrolysis of P(VAc-ran-VClAc) blocks with K2CO3/CH3OH furnishes access to a series of PVAc-b-PVOH-b-PVAc amphiphiles. Hydration of solvent-cast films of these copolymers, comprising PVAc blocks of comparable degrees of polymerization with variable PVOH segment lengths, yields soft hydrogels ($G'(\omega) \sim 1-10$ kPa). Synchrotron small-angle and wide-angle X-ray scattering reveals that these hydrogels microphase separate into spherical hydrophobic PVAc domains that are interconnected by solvated PVOH network strands. The strain-dependent rheology of these hydrogels depends sensitively on the length of the center PVOH segment. This gel rheology is shown to stem from the hydrogen bond donor–acceptor capabilities of PVOH, which leads to the formation of weak, dynamic noncovalent crosslinks in the aqueous domains of these gels. Thus, the observed gel rheology may be rationalized in terms of the shear-induced reorganization of these H-bonding crosslinks as a function of the applied strain.

INTRODUCTION

Block copolymer gel networks have attracted substantial interest by virtue of their wide-ranging applications in personal care products, therapeutic delivery, tissue scaffolding, and as ion-conducting media.1–4 In contrast to solvent-swollen gels comprising a chemically crosslinked single or double network,5,6 introduction of a selective solvent to a segmented copolymer drives associations of polymer chains to yield a physically crosslinked network.7–9 Hydrogels derived from water-swollen amphiphilic ABA- or ABC-type triblocks, in which hydrophobic end blocks flank a hydrophilic B segment, are well-studied materials.10–12 Water induces the hydrophobic association of the end blocks to yield micelles that physically crosslink the hydrophilic B segments into a gel network.13–15 These materials typically exhibit solid-like rheological properties at low strains, with weakly frequency-dependent dynamic elastic storage and loss moduli $G'(\omega)$ and $G''(\omega)$, respectively.12

Many reports have described the materials properties and applications of ABA-type triblock hydrogels with various hydrophilic and hydrophobic block combinations, with an emphasis on economical and biocompatible polymer segments.2,4,18 Poly(ethylene oxide) (PEO) is one of the most widely incorporated hydrophilic blocks in physical hydrogels by virtue of its known biocompatibility and resistance to nonspecific protein adsorption in biomedical applications.19 Detailed studies of poly(ether)/PEO7,14,15,17,20 and poly(acrylate)-based triblock hydrogels2,17 reveal the systematic tunability of their properties with copolymer structure. In their studies of block copolymer hydrogels derived from admixtures of AB diblocks with ABBA triblocks, Guo and Bailey further demonstrated that they could finely tailor the distensibility and consequent mechanical properties of these water-laden materials.17 More recently, the groups of Tew and Bhatia described the morphology-dependent rheology of biocompatible PLA-b-PEO-b-PLA triblocks (PLA = poly(lactide)).12,16 Armes and co-workers also reported biocompatible, amphiphilic methacrylate block copolymers such as PHPMA-b-PMPC-b-PHPMA (PHPMA = poly(2-hydroxypropylmethacrylamide); PMPC = poly(2-(methacryloyloxy)ethylphosphorylcholine)), which are promising potential tissue scaffolds.5,6 Unfortunately, the limited degradabilities of the hydrophilic segments in these copolymers restrict their applications.

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Block copolymer hydrogels based on water-soluble and fully degradable poly(vinyl alcohol) (PVOH) segments, have not been previously studied due to synthetic difficulties that curtail access to well-defined PVOH-based block copolymers. Controlled freezing of aqueous solutions of PVOH homopolymers is known to form physical “cryogels” with ill-defined structures, comprising irregular yet interconnected semicrystalline (water-poor) and amorphous (water-solvent) polymer domains. However, the sensitivity of PVOH cryogel morphology and attendant properties to the exact preparation method renders their reproducible fabrication problematic. On the other hand, well-defined amphiphilic PVOH copolymers constitute a previously unexploited chemical platform for rationally designing useful hydrogels with tunable physical properties. Incorporation of chemically degradable, hydrophobic end blocks into these copolymers would render these PVOH-based gels completely degradable for wider potential applications. Recent advances in reversible addition-fragmentation chain transfer (RAFT) and cobalt-mediated free radical polymerizations (CMRP) permit access to narrow dispersity PVOH-based diblock copolymers, especially, those bearing chemically degradable and biocompatible poly(vinyl acetate) hydrophobic segments.

Herein, we report the synthesis and characterization of a series of well-defined poly(vinyl acetate-b-vinyl alcohol-b-vinyl acetate) (AcVOHAc) triblock copolymer hydrogels. Upon establishing an optimized RAFT synthesis of copolymers with well-defined molecular weights, dispersities, and compositions, the rheological properties of three homologous triblock gels with constant PVAc end blocks and variable length PVOH center blocks are examined. Shear rheology measurements reveal that the gel moduli exhibit an unexpected dependence on the magnitude of the applied strain and that this rheological response depends on the PVOH center segment molecular weight. By studying the properties of these gels in the presence and absence of acetic acid, a hydrogen-bonding inhibitor that solubilizes PVOH, the unusual rheology of these hydrogels is shown to originate from a convolution of the microphase-separated block copolymer morphology and dynamic non-covalent crosslinking afforded by hydrogen bonding in the water-solvent PVOH domains.

■ EXPERIMENTAL SECTION

Materials. All reagents were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and used as received unless otherwise noted. 2,2′-Azobis(isobutyronitrile) (AIBN) and 2,2′-azoisocyclohexane-1-carboxonitrile (V-40) (Wako Chemicals, USA) were both recrystallized from CH3OH prior to use. Vinyl acetate (VAc) was distilled from NaBH4 at ambient pressure, while vinyl chloroacetate (VClAc) (TCI America, Inc.) was distilled from NaBH4 under reduced pressure (~1 mmHg) prior to use. RAFT agents 1 and 2 were synthesized according to previously reported procedures.

NMR Spectroscopy. 1H NMR spectra were acquired on a 300 MHz Bruker AC-300 spectrometer or a Varian UI-500 spectrometer operating at 500 MHz. All spectra were referenced to the residual protiated solvent peak in the samples. 13C NMR spectra were obtained at 125 MHz using the Varian UI-500 spectrometer. Copolymer compositions were determined by integration of quantitative 1H NMR spectra. The degree of polymerization (N_{PVOH}) for the end segments of the triblock copolymers derived from bidirectional chain extension of P(VClAc-ran-VAc) RAFT macroinitiators were calculated from the random copolymer molecular weight (M_r) derived from SEC analyses (vide infra).

Size Exclusion Chromatography (SEC). Polymer molecular weights were determined by SEC analyses in a THF mobile phase at 40 °C with flow rate of 0.8 mL/min using a Viscotek GPCMax System employing two Polymer Laboratories Polypropylene columns (250 mm × 4.6 mm), with in-line differential refractometer, 7° and 90° light scattering module, and four-capillary differential viscometer. M_r and M_w values for PVAc, PVClAc, and P(VClAc-ran-VAc) materials are reported against a Mark–Houwink corrected poly(vinyl acetate) calibration curve, which was constructed using 10 narrow molecular weight distribution poly(styrene) standards with M_w = 580–377400 g/mol (Polymer Laboratories, Amherst, MA).

Dynamic Mechanical Spectroscopy. Dynamic mechanical spectroscopy experiments employed a TA Instruments ARES-L2 strain-controlled parallel-plate rheometer fitted with a 25 mm diameter upper plate and a 50 mm diameter lower, temperature-controlled Peltier plate. Upon sample loading, hydrogels were covered with plastic cap lined with water-saturated sponges to mitigate sample dehydration during analyses. Dynamic strain sweep measurements were conducted at 25 °C and ω = 1 rad/s with |γ| = 0.01–10%, whereas dynamic frequency sweep measurements employed |γ| = 1% with 0.10 ≤ ω ≤ 10 Hz.

X-ray Scattering. Synchrotron small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) measurements were performed at the Sector 12-ID-B beamline of the Advanced Photon Source at Argonne National Laboratory (Argonne, IL). Experiments employed 12 keV (λ = 1.03 Å) incident photons with SAXS/WAXS detectors sample-to-detector distances of 2.215 and 0.455 m, respectively. 2D SAXS patterns were recorded on a Pilatus 2M detector (25.4 cm × 28.9 cm active area) with 1475 × 1679 pixel resolution, and 2D WAXS patterns were recorded on a Pilatus 300 K (8.4 cm × 10.7 cm active area) with 487 × 619 pixel resolution. Hydrogel samples were hermetically sealed in aluminum DSC pans for data acquisition, with typical exposure times of ~1 s. Data reduction was performed using Igor Pro Procedures files developed by Schmitt and Mahanthappa to produce azimuthally integrated one-dimensional intensity (I(q)) versus q plots. Form factor scattering was modeled using the Scattering (Version 0.4) software package.

Representative Synthesis of Poly(vinyl acetate-ran-vinyl chloroacetate) (P(VAc-r-VClAc)). Monofunctional RAFT agent 1 (1.150 g, 5.47 mmol) and V-40 (173 mg, 0.71 mmol) were dissolved in a mixture of CICH2CH2Cl (30 mL) and freshly distilled VAc (30.0 mL, 325 mmol) and VClAc (30.0 mL, 297 mmol) to yield a final solution with [monomer] = 5.2 M, [1] = 45.6 mM, and [1]:[V-40] = 7.7:1. 30 mL of this solution was then sealed in a 100 mL Schlenk tube, degassed by three freeze–pump–thaw cycles, and placed in an oil bath at 80 °C. After 4.5 h, the tube was removed from the oil bath and placed in 0 °C bath to terminate the reaction. The resulting poly(VAc-r-VClAc) was precipitated into hexane and then subsequently redissolved in THF and again precipitated into hexanes. The isolated solids were then freeze-dried from C2H6. Quantitative 1H NMR analyses show that the isolated polymer contains F_{VAc} = 0.45. M_w/sec = 5.8 kg/mol, and D = 1.26.

Representative Chain Extension of P(VAc-r-VClAc) with VAc. A monofunctional P(VAc-r-VClAc) macro-chain transfer agent (1.77 g, 0.303 mmol with M_r = 5.8 kg/mol, D = 1.26, F_{VAc} = 0.45) and V-40 (7.6 mg, 0.031 mmol) was dissolved in freshly distilled VAc (6.0 mL, 0.065 mol) and 14 mL of CICH2CH2Cl (14 mL) to produce a solution with [VAc] = 3.25 M, [macro-CTA] = 15.2 mM, and a [macro-CTA]:[V-40] = 9.76:1. The solution was sealed in a 100 mL Schlenk tube, degassed by three freeze–pump–thaw cycles, and placed in a stirred oil bath at 80 °C. After 5 h, the reaction was removed from the oil bath and placed in a 0 °C bath to stop the reaction. The resulting poly(P(VAc-r-VClAc)-b-VAc) was first precipitated into hexanes and then subsequently redissolved in THF and again precipitated into hexanes. The isolated solids were then freeze-dried from C2H6. Quantitative 1H NMR analyses show that the isolated polymer contains F_{VAc} = 0.80. M_w/sec = 12.6 kg/mol and D = 1.48.

Representative Hydrolysis of P((VAc-r-VClAc)-b-VAc) to P(VAc-b-VOH). P((VAc-r-VClAc)-b-VAc) (0.9053 g, 0.072 mmol...
with $M_{	ext{SEC}} = 12.6$ kg/mol, $D = 1.48$, $F_{\text{VAc}} = 0.80$) was dissolved in CH$_2$OH (65 mL) with K$_2$CO$_3$ (29.4 mg, 0.213 mmol) and stirred at 22 °C. Reaction aliquots were periodically removed, concentrated by rotary evaporation, and treated with trifluoroacetic anhydride, prior to quantitative $^1$H NMR analyses in acetone-$d_6$ that enabled monitoring of the hydrolysis reaction extent per a previous report. NMR analyses reveal complete random copolymer segment hydrolysis after 6.5 h to yield poly(vinyl acetate-b-vinyl alcohol) (P(VAc-b-VOH). The product was triturated with distilled water (3 × 15 mL), centrifuged to isolate the solids, and dried in vacuo. The resulting VOH$_2$Ac$_{58}$ polymer exhibits $F_{\text{VAc}} = 0.61$.

**Representative Hydrogel Preparation.** P(VAc-b-VOH-b-VAc) (0.75 g) film was cast from a 15:5:1 (v/v/v) CH$_3$OH:H$_2$O:DMSO (20 mL) solution into a Teflon-lined Petri dish at 30 °C by repeatedly aliquoting 0.5 mL portions of the solution and allowing the solvent to evaporate until the solution was consumed. This solvent-cast film was swollen in excess Milli-Q water at 22 °C overnight. After 2 h, THF (5 mL) was added, and the reaction was warmed to 40 °C and stirred overnight. The resulting PVAc was isolated by removal of the volatiles via rotary evaporation, and precipitation from CH$_2$Cl$_2$ into hexanes twice, prior to freeze-drying from C$_6$H$_6$. Complete acetylation was confirmed via $^1$H NMR. For the resulting PVAc, $M_{\text{SEC}} = 21.6$ kg/mol and $D = 1.30$.

**Acetic Acid Treatment of Hydrogels.** Water-swollen P(VAc-b-VOH-b-VAc) hydrogels were placed in either 1 or 10% (v/v) CH$_3$COOH(aq) for 4 h prior to SAXS and rheological analyses. Upon completion of these analyses, the hydrogels were placed in a fresh Milli-Q water bath that was changed every 10–15 h for a total of 7 days to drive acetic acid removal.

## RESULTS

### Optimized Synthesis of PVAc/PVOH Block Copolymers

Repollet-Pedrosa et al. previously reported the synthesis of PVAc-b-PVOH diblock copolymers, hereafter denoted VOH$_x$Ac$_y$, wherein $x$ and $y$ are the degrees of polymerization for each segment. Their approach relied on sequential RAFT block copolymerization of vinyl acetate (VAc) and vinyl chloroacetate (VClAc) to produce a PVAc-b-PVClAc diblock copolymer, followed by selective hydrolysis of the PVClAc block with K$_2$CO$_3$/CH$_2$OH to unmask a poly(vinyl alcohol) (PVOH) segment. The success of this strategy relies on the known 750:1 differential hydrolytic selectivity for chloroacetate esters over the acetate esters under basic conditions. Trifluoroacetylation of the resulting VOH$_x$Ac$_y$ diblock copolymer amphiphile and $^{13}$C NMR analysis of the resulting polymer demonstrated the efficiency and selectivity of this synthetic protocol. While this methodology offers straightforward access to a new class of degradable block copolymer amphiphiles, the molar mass dispersions of the PVClAc segments were broad ($D = 1.35$–1.50). Segmental dispersity in the polymer precursor translates into a broad dispersity PVOH block in the amphiphile, and segmental dispersity is known to complicate studies of the morphological behavior of these new materials.

Destarac and co-workers recently reported a related route to PVAc-b-PVOH diblock copolymers by sequential RAFT block copolymerization of VAc and vinyl trifluoroacetate (VTFA), followed by selective saponification of the PVTF block. In their syntheses, the PVTF blocks in the amphiphile precursors exhibit lower molar mass dispersions ($D = 1.07$–1.43). However, their approach is limited by the fact that the VTFA monomer must be sequentially added after VAc to maintain polymerization control, which precludes synthesis of well-defined Ac$_x$VOH$_x$Ac$_y$ triblock copolymers for hydrogel studies.

To access narrower dispersity VOH$_x$Ac$_y$ diblock and Ac$_x$VOH$_x$Ac$_y$ triblock copolymers, a method for better controlling the xanthate-mediated RAFT polymerization of VClAc was sought. Kaneyoshi and Matyjaszewski demonstrated improved control over the cobalt-mediated radical polymerization (CMRP) of VClAc by the addition of VAc as a comonomer. Hence, we hypothesized that an analogous VClAc/VAc copolymerization strategy would enable better control over the molecular weights and dispersities of the hydrolytically cleavable segments in our RAFT route to PVAc/PVOH block copolymer amphiphiles.

We initially studied RAFT copolymerizations of VClAc/VAc in CICH$_2$CH$_2$Cl as a reaction solvent (Scheme 1). Atanase et al. previously reported that VClAc/VAc RAFT copolymerizations conducted in ethyl acetate yield only low molecular weight materials with broad dispersities ($D ≥ 1.69$) at high conversions. The broad dispersities and low molecular weights likely originate from a combination of the high monomer conversions used therein and the well-documented chain transfer activity of ethyl acetate. Low conversion RAFT VClAc/VAc copolymerizations in CICH$_2$CH$_2$Cl mediated by xanthate 1 (45 mM) at 80 °C, with a monomer feed $f_{\text{VAc}} = 0.52$ and total [monomer] = 5.2 M, yielded relatively narrow dispersity random copolymers with $M_x = 5.8$–7.0 kg/mol and $D = 1.26$ with composition $F_{\text{VAc}} \sim 0.45$ (Table 1, entries 2 and 3). However, bulk polymerization of VClAc under comparable conditions yielded PVC1Ac with $D = 1.69$ (Table 1, entry 1). Copolymer compositions grossly reflected the monomer feed composition at monomer conversions <50%, albeit with some composition drift consistent with the reported VClAc and VAc reactivity ratios. Since the ultimate goal was to selectively hydrolyze the copolymer to yield PVOH, we sought to maximize the number of VClAc units in the copolymer while retaining control over the xanthate-mediated RAFT polymerizations. Decreasing the copolymerization feed composition to $f_{\text{VAc}} = 0.26$ also resulted in good polymerization control, yielding poly(VAc-x-VClAc) with $M_x = 4.8$–6.3 kg/mol with

![Image](https://example.com/scheme1.png)
In order to demonstrate the feasibility of the proposed PVAc/PVOH block copolymer synthesis, a monofunctional poly(VAc-r-VClAc) macromolecular RAFT agent with $M_n = 5.8$ kg/mol and $Đ_f = 1.26$ (Table 1, entry 2) was chain extended with VAc as shown in Scheme 2 under the reaction conditions given in Table S1. SEC analysis of the resulting block copolymer indicates the success of this PVAc chain extension reaction, albeit with some dispersity broadening. The broader molar mass dispersity of the diblock copolymer is ascribed to the low concentration of RAFT end groups (15 mM) and the high reaction viscosity, which reduces the rate of xanthate interchange.39 Saponification of this block copolymer with methanolic $K_2CO_3$ at 22°C yielded VOH56Ac97 after 6.5 h, as confirmed by exhaustive trifluoroacetylation and subsequent quantitative $^1H$ NMR analysis in acetone-$d_6$. Triﬂuoroacetylation of the free –OH groups of the PVOH segment improves polymer solubility in acetone-$d_6$ and mitigates polymer aggregation, while facilitating wider peak dispersion of the backbone methine peaks associated with the distinct homopolymer segments. Thus, this analysis revealed the formation of VTFA56Ac97, as expected from the hydrolysis precursor composition (Figure 1). $^{13C}$ NMR analyses of the VTFA56Ac97 demonstrate that the hydrolysis reaction is block selective: within the limit of detection, no resonances in the range $δ = 69−72$ ppm associated with VTFA/VAc hetero-ads and hetero-triads are observed (Figure S1). The lack of any observed VTFA/VAc heterosequences implies the presence of minimal VAc/VOH heterosequences in the hydrolyzed polymer per our previous report.50 Thus, we conclude that both the VClAc and VAc units in the poly(VAc-r-VClAc) block are completely cleaved without any detectable reaction of the PVAc segment. While this hydrolytic selectivity may initially seem surprising, neighboring group effects are known to drive the autocatalytic hydrolysis of poly(vinyl esters) under basic conditions, and polymer hydrolysis kinetics are especially complex for samples with chloroacetate moieties.30 These effects likely hasten the

### Table 1. Molecular Characteristics of Poly(VClAc-ran-VAc) Random Copolymers

<table>
<thead>
<tr>
<th>sample</th>
<th>[vinyl ester] (M)</th>
<th>$f_{VAc}$</th>
<th>CTA</th>
<th>[CTA] (mM)</th>
<th>[CTA]:[I]$^d$</th>
<th>T (°C)</th>
<th>$t_{rxn}$ (h)</th>
<th>$F_{VAc}$</th>
<th>$M_n$ (kg/mol)</th>
<th>$Đ_f$</th>
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<tr>
<td>1</td>
<td>9.9$^a$</td>
<td>0</td>
<td>1</td>
<td>55</td>
<td>10:1</td>
<td>80</td>
<td>0.75</td>
<td>8.6</td>
<td>1.69</td>
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</tr>
<tr>
<td>2</td>
<td>5.2$^b$</td>
<td>0.52</td>
<td>1</td>
<td>45</td>
<td>8:1</td>
<td>80</td>
<td>4.5</td>
<td>5.8</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.2$^b$</td>
<td>0.52</td>
<td>1</td>
<td>45</td>
<td>8:1</td>
<td>80</td>
<td>6</td>
<td>7.0</td>
<td>1.26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.1$^b$</td>
<td>0.26</td>
<td>2</td>
<td>23</td>
<td>10:1</td>
<td>80</td>
<td>5.17</td>
<td>4.8</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.1$^b$</td>
<td>0.26</td>
<td>2</td>
<td>12</td>
<td>11:1</td>
<td>70</td>
<td>6.5</td>
<td>6.3</td>
<td>1.31</td>
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<tr>
<td>6</td>
<td>10.1$^a$</td>
<td>0.26</td>
<td>2</td>
<td>12</td>
<td>11:1</td>
<td>70</td>
<td>7</td>
<td>14.3</td>
<td>1.23</td>
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</tr>
<tr>
<td>7</td>
<td>10.1$^a$</td>
<td>0.26</td>
<td>2</td>
<td>12</td>
<td>11:1</td>
<td>70</td>
<td>3.75</td>
<td>23.4</td>
<td>1.38</td>
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<tr>
<td>8</td>
<td>10.1$^a$</td>
<td>0.26</td>
<td>2</td>
<td>12</td>
<td>11:1</td>
<td>70</td>
<td>7</td>
<td>14.3</td>
<td>1.23</td>
<td></td>
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</table>

$^a$Bulk vinyl ester monomers. $^b$50 vol % monomer in CICH2CH2Cl. $^c$Initial VAc mole fraction in the feed. $^d$I = 1,1’-azobis(cyclohexane-1-carbonitrile) (V-40). $^e$Mole fraction of VAc in the copolymer determined by quantitative $^1H$ NMR in CDCl3. $^f$Determined by SEC in THF at 40°C using a Mark–Houwink corrected calibration curve for PVAc (see Experimental Section).
selective hydrolysis of the poly(VAc-r-VClAc) segment, leaving the PVAc segment untouched at reaction times <6.5 h.

The above synthetic approach also furnished access to a series of Ac$_x$VOH$_y$Ac$_x$ triblock copolymers (Table 2 and Scheme 3). Bidirectional copolymerizations of VClAc/VAc with difunctional RAFT agent 2 initiated by V-40 were well controlled at low monomer conversions (e.g., Table 1, entry 6, with 38% conversion). However, high reaction viscosities led to loss of copolymer dispersity control at conversions >55%. Higher molecular weight poly(VClAc-ran-VAc) samples with $M_n = 13.1$−$23.4$ kg/mol and $Đ = 1.32$−$1.39$ were more readily synthesized using RAFT polymerizations with [2] = 12 mM (Table 1, entries 7−9) in neat monomer mixtures. Chain extension of these difunctional macro-RAFT agents with VAc furnished unimodal triblock copolymers with modest dispersity broadening, according to SEC analyses (Table 2). The center poly(VClAc-ran-VAc) segments of these triblocks were saponified using methanolic K$_2$CO$_3$ over 6−8 h. The copolymer hydrolysis reaction was monitored by periodic removal of reaction aliquots and subsequent $^1$H NMR analysis.

Trifluoroacetylation of the reaction product (vide supra) again enabled confirmation of both the triblock composition and the selectivity of the center segment hydrolysis reaction. However, the above analysis does not establish the integrity of the ester linkage in the center of the putative triblock originating from the “R group” of xanthate 2. We confirmed that the latter linkage is not affected by the hydrolysis conditions, by peracetylating Ac$_{100}$VOH$_{135}$Ac$_{100}$ with acetic anhydride and catalytic DMAP to obtain a PVAc homopolymer. SEC analysis of this PVAc indicates that it is unimodal and that its hydrodynamic volume is larger than that of the poly(VClAc-ran-VAc) precursor (Figure 2). The unimodality of the molar mass distribution coupled with the relative increase in the size of the polymer suggests the integrity of the ester unit at the center of the copolymer chain under the block-selective saponification conditions. Thus, the triblock architecture is apparently preserved.

**Scheme 3. RAFT Synthesis of Poly(vinyl acetate-block-vinyl alcohol-block-vinyl acetate) Triblock Copolymers**

**Table 2. Molecular Parameters of PVOH/PVAc Block Copolymers**

<table>
<thead>
<tr>
<th>sample</th>
<th>$F_{VAc}$</th>
<th>$M_r$ (kg/mol)</th>
<th>$Đ$</th>
<th>$F_{VAc}$</th>
<th>$N_{n,end\ block}$</th>
<th>$Đ$</th>
<th>$F_{VAc,hydro}$</th>
<th>$F_{VAc,snap}$</th>
<th>SR</th>
</tr>
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<tr>
<td>VOH$<em>{10}$Ac$</em>{10}$</td>
<td>0.45</td>
<td>5.8</td>
<td>1.26</td>
<td>0.80</td>
<td>97</td>
<td>1.48</td>
<td>0.64</td>
<td>0.61</td>
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<tr>
<td>Ac$<em>{100}$VOH$</em>{135}$Ac$_{100}$</td>
<td>0.45</td>
<td>14.3</td>
<td>1.23</td>
<td>0.78</td>
<td>200</td>
<td>1.40</td>
<td>0.60</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Ac$<em>{120}$VOH$</em>{117}$Ac$_{120}$</td>
<td>0.25</td>
<td>13.1</td>
<td>1.32</td>
<td>0.74</td>
<td>241</td>
<td>1.42</td>
<td>0.65</td>
<td>0.67</td>
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<tr>
<td>Ac$<em>{127}$VOH$</em>{164}$Ac$_{127}$</td>
<td>0.22</td>
<td>18.6</td>
<td>1.39</td>
<td>0.68</td>
<td>254</td>
<td>1.52</td>
<td>0.59</td>
<td>0.59</td>
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</tr>
<tr>
<td>Ac$<em>{113}$VOH$</em>{208}$Ac$_{113}$</td>
<td>0.24</td>
<td>23.4</td>
<td>1.38</td>
<td>0.64</td>
<td>225</td>
<td>1.60</td>
<td>0.52</td>
<td>0.47</td>
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</table>

Subscripts denote the degree of polymerization for each segment. $^a$Mole fraction of VAc in polymer determined by quantitative $^1$H NMR in CDCl$_3$. $^b$Determined by SEC in THF at 40 °C using a Mark−Houwink corrected calibration curve for PVAc (see Experimental Section). $^c$Expected mole fraction of VAc posthydrolysis from complete midblock hydrolysis. $^d$Observed mole fraction of PVAc determined via $^1$H NMR of the hydrolyzed block copolymer after functionalization with trifluoroacetic anhydride. $^e$SR = swelling ratio = (g H$_2$O)/(g polymer) upon swelling in Milli-Q water at 22 °C for 24 h.

**Block Copolymer Hydrogel Preparation.** The optimized block copolymer synthesis allowed construction a series of Ac$_{10}$VOH$_{12}$Ac$_{10}$ with nearly constant length PVAc segments...
with longer PVOH segments take up water to a greater extent, as quantified by swelling ratios SR = (g water taken up)/(g polymer) given in Table 2. Qualitatively, the opacity of the gels diminishes with increasing PVOH center segment length, such that Ac120VOH117Ac120 is more opaque than the translucent Ac113VOH208Ac113 (Figure 3). While the opacity of these gels suggests possible crystallinity in the water-solvated PVOH domains, synchrotron wide-angle X-ray scattering (WAXS) analyses of these samples do not reveal any quantifiable crystallinity (vide infra). Azimuthally integrated synchrotron small-angle X-ray scattering (SAXS) patterns for these block copolymer hydrogels exhibit broad, form factor scattering peaks consistent with microphase separation of the hydrophobic PVAc segments into micellar aggregates in a PVOH/H2O matrix phase (Figure S2). However, the breadth of these scattering peaks suggests a disorganized micellar microphase separated structure in these as-prepared gels.

**AcxVOHyAc, Triblock Gel Rheology.** The rheological properties of the homologous series of AcxVOHyAc gels were investigated as a function of the PVOH center segment degree of polymerization (y = 117, 164, and 208) with nearly constant length PVAc end segments (113 ≤ x ≤ 127). Representative dynamic elasticity data for each of the three block copolymer hydrogels are shown in Figure 4. For the Ac113VOH208Ac113 hydrogel with the longest PVOH segment, the shear rheological behavior at ω = 1 rad/s may be classified into four strain-dependent regimes (Figure 4a): (I) soft gel-like behavior implied by G′(γ) > G″(γ) when |γ| ≤ 0.08%, (II) strain-softening and viscoelastic behavior over the range |γ| = 0.08–0.3% with a G′ = G″(γ) crossover at |γ| = 0.1%, (III) strain stiffening when |γ| = 0.3–0.8% with another crossover to a solid-like gel at |γ| = 0.4%, and (IV) final strain softening with a crossover to a liquid-like behavior at |γ| ∼ 0.8%. Isothermal frequency sweep tests show that G′(ω) and G″(ω) depend modestly on the frequency of the applied strain when γ = 0.1% and 0.1 ≤ ω ≤ 10 Hz (Figures S3 and S4), similar to other known physically crosslinked, microphase-separated triblock gels at low strains.12

Reducing the center segment degree of polymerization from y = 208 to 164 or 117 results in different shear rheology profiles with a reduced number of G′/G″ crossovers (Figure 4b,c). At strains ∼0.01–0.1%, the x = 164 material behaves as a soft viscoelastic solid with G′(γ) ≤ G″(γ) that are weakly strain-dependent (II). This sample strain hardens when |γ| ≤ 0.7%, including a viscoelastic crossover at |γ| = 0.2% to soft gel-like behavior (III). At strains |γ| > 1%, the gel softens with a crossover into a liquid-like regime (IV). Further reduction of the center PVOH segment degree of polymerization to y = 117 furnishes a gel with a linear viscoelastic response in the range 0.01 ≤ |γ| ≤ 0.20% (I), followed by strain softening at strains exceeding 1% (IV). The fact that the final strain softening event in all three hydrogels occurs when |γ| > 1% suggests a common high strain failure mechanism, whereby the physically crosslinked network is disrupted. Similar high-strain behavior has been observed in other hydrogel systems23,12 and is thought to occur by hydrophobic end block pull out from the microphase-separated domains.24 The low strains at which the AcxVOHyAc gels are disrupted starkly contrast the typically higher strains (~100%) required for failure in triblock hydrogels with hydrophobic and glassy poly(styrene), poly(methyl methacrylate) (PMMA), or PLA domains.12,22 Spontak and co-workers have recently shown that the critical strain required for gel network failure significantly decreases at temperatures near the Tg of the end blocks.25 We thus attribute the low failure strain onset in the AcxVOHyAc gels to the low Tg ∼ 20–22 °C for PVAc plasticized with water, which enables facile copolymer chain pull out from the soft and liquid-like end block domains.

**Effect of Acetic Acid on Hydrogel Rheology.** Since PEO-based block copolymer hydrogels do not exhibit the strain hardening observed in the AcxVOHyAc (y = 164 and 208) gels, these phenomena were studied in more detail. We hypothesized that the unusual low-strain rheological responses in these hydrogels stemmed from hydrogen-bonding donor/
acceptor interactions in the center PVOH segment. Given the apparently enhanced solubility of PVOH in acetic/water mixtures, the effects of treating the Ac\_VOH\_Ac gels with dilute AcOH(aq) solutions of varying concentrations were probed.

Immersion of water-swollen and initially opaque Ac\_113\_VOH\_208\_Ac\_113 and Ac\_127\_VOH\_164\_Ac\_127 hydrogels in 10 vol % AcOH(aq) solutions for 4 h rendered the gels transparent and qualitatively softer. Sample fragility upon handling precluded their loading into a rheometer for in-depth dynamic mechanical analyses. These qualitative changes in gel properties could arise from either AcOH-induced disruption of the low levels of crystallinity in the water-solvated PVOH or further plasticization of the PVAc domains. However, attempts to detect changes in gel crystallinity using synchrotron WAXS failed, since we did not detect any baseline crystallinity in the pristine Ac\_113\_VOH\_208\_Ac\_113 sample prior to acetic acid treatment (Figure 5a). Complementary SAXS analyses of AcOH-treated Ac\_113\_VOH\_208\_Ac\_113 reveal qualitatively similar form factor scattering to that of the pristine gel, indicating retention of a micellar, microphase-separated gel morphology lacking long-range lattice order (Figure 5b). The fact that the first form factor scattering peak shifts to a slightly higher q-value in the presence of AcOH, coupled with the better defined form factor scattering, suggests that AcOH anneals the network to produce a more uniform structure. Attempts to analyze the SAXS form factor scattering with a cylindrical micelle model failed to yield high quality data fits. However, the form factor scattering for Ac\_113\_VOH\_208\_Ac\_113 does fit a disorganized spherical micelle model (Figure S5), from which one finds that the PVAc domains have diameters $d = 16.5 \pm 2.1$ nm.\cite{41,51}

In order to obtain AcOH-treated samples amenable to rheological analyses, we soaked the Ac\_113\_VOH\_208\_Ac\_113 and Ac\_127\_VOH\_164\_Ac\_127 hydrogels in a 1 vol % AcOH(aq) solution for 4 h. The resulting gels were qualitatively less opaque than the native materials, yet they retained enough mechanical integrity for loading into a rheometer. As shown in Figures 6b and 6e, dynamic strain sweeps on both of these AcOH-treated gels do not exhibit the strain hardening behaviors of the pristine samples. For example, Ac\_113\_VOH\_208\_Ac\_113 exhibits gel-like behavior with $G'(\gamma) > G''(\gamma)$ with a slightly reduced $G'(\gamma)$ value when $\gamma < 1\%$, a strain softening onset at $\gamma = 1\%$, and a crossover to liquid-like behavior at high strains (Figure 6b). Additionally, isothermal frequency sweep tests conducted at $\gamma = 0.1\%$ of AcOH-treated Ac\_113\_VOH\_208\_Ac\_113 are consistent with previously reported physically crosslinked, block copolymer hydrogels over the range $0.1 \leq \omega \leq 10$ Hz (Figure S3).\cite{52} While AcOH treatment of Ac\_127\_VOH\_164\_Ac\_127 drives qualitatively similar rheological behaviors, we find that the storage and loss moduli in this sample increase substantially as compared to the pristine sample (Figure 6e). The onset of strain softening for the Ac\_127\_VOH\_164\_Ac\_127 also shifts to lower strains. We note that the rheological properties of these AcOH-treated samples similar are those expected for well-studied ABA block copolymer hydrogels with hydrophobic A end blocks a hydrophilic center B segment, albeit with lower failure strains due to ease of gel network failure by chain pullout from the hydrophilic center B segment. In summary, the above data suggest that PVOH hydrogen bonding plays a key role in the low strain rheological response of the AcVOAc triblock hydrogels.

We assessed the reversibility of the AcOH-induced changes in hydrogel rheology by soaking these gels in Milli-Q water for 7 days to remove the acetic acid. Rheological analyses of these “washed” gels are shown in Figures 6c and 6f for Ac\_113\_VOH\_208\_Ac\_113 and Ac\_127\_VOH\_164\_Ac\_127, respectively. While the absolute magnitudes of $G'(\omega)$ and $G''(\omega)$ for the “washed” gels are lower than those of the pristine gels, the “washed” materials do exhibit a similar strain stiffening signature to that of the pristine materials. We attribute the observed order of magnitude decrease in $G'(\gamma)$ and $G''(\gamma)$ to the fact that prolonged soaking of these gels in acidic water likely induced degradation of the PVOH block or cleavage of the ester linkage in the center of the PVOH segment. Either of these chemical events would lead to formation of a hydrogel comprising a mixture of both AB diblock and AB-BA triblock copolymer chains, which are challenging to analyze by SEC. However, the storage and loss shear moduli of these hydrogels derived from diblock/triblock mixtures decrease dramatically as demonstrated by the groups of Bhatia\cite{52} and Bailey.\cite{57}

**DISCUSSION**

The low strain deformation mechanics of microphase-separated AcVOHAc triblock copolymer hydrogels are distinct from

![Figure 4. Dynamic strain sweep curves for hydrogels (a) Ac\_113\_VOH\_208\_Ac\_113, (b) Ac\_127\_VOH\_164\_Ac\_127, and (c) Ac\_120\_VOH\_117\_Ac\_120 acquired at $\omega = 1$ rad/s at $T = 25^\circ$C demonstrate the dependence of $G'(\omega)$ (filled symbols) and $G''(\omega)$ (open symbols) on the PVOH segment degree of polymerization. Roman numerals indicate (I) soft gel-like behavior, (II) viscoelastic behavior after a crossover, (III) strain stiffening after a crossover, and (IV) high strain network failure after another crossover.](image-url)
those of traditional ABA triblock hydrogel analogues with hydrophilic B segments that lack hydrogen-bonding functionalities (e.g., poly(ethylene oxide)). While the latter ABA gels manifest strain-independent mechanics prior to network failure by A end block chain pull out, the AcVOHAc gels exhibit a more complex viscoelastic response at low strains. Since AcOH treatment of the AcVOHAc gels eliminates these low strain softening effects, we ascribe these new properties to hydrogen bonding in the solvated PVOH blocks. Furthermore, the specific number and nature of the strain softening and strain hardening events observed in AcVOHAc gels depend on the microphase-separated morphology of the hydrogel Ac$_{113}$VOH$_{208}$Ac$_{113}$ in Milli-Q water. The widely variable rheology of associative polymer networks containing dynamic noncovalent crosslinks has motivated numerous experimental and theoretical studies, that provide insight into how crosslink strength and lifetime affect nonlinear network rheology. Craig and co-workers have extensively studied the properties of well-defined metallo-supramolecular polymer networks wherein poly(4-vinylpyridine) strands are noncovalently crosslinked with bis-Pd(II) pincer complexes, for which the kinetics and thermodynamics of crosslink formation are known. They attribute the observed shear thickening in these systems to a shear-induced increase in the number of elastically effective network strands, which stems from the conversion of intrachain crosslinks to interchain crosslinks at high strains. In collagen-based materials, Rajagopalan and co-workers ascribed a similar strain stiffening to deformation-induced reorganization of the dynamic noncovalent crosslinks to increase the number of elastically effective strands. In the latter study, the dynamic crosslinks originate from H-bonding interactions between the collagen fibrils. To the best of our knowledge, only one report studied the effects of incorporating dynamic noncovalent crosslinks into ABA triblock copolymer gels. Shull and co-workers showed network strength PMMA−PMMA−PMMA (PMMA = poly(methyl methacrylate); PMMA = poly-(methacrylic acid)) hydrogels crosslinked with divalent metal counterions depends on the strength of ionic crosslinks. While these systems are conceptually related to our AcVOHAc gels, low strain oscillatory rheological measurements were not reported by Shull and co-workers. Nonetheless, similarities in the low strain rheology of AcVOHAc gels and that of dynamic noncovalent polymer networks suggest a conceptual framework for explaining our observations. All of the AcVOHAc triblock gels appear to form a network of interconnected spherical micelles lacking long-range translational order, in which the copolymer chains adopt a distribution of conformations that either "bridge" adjacent hydrophobic domains or "loop" back into the same hydrophobic domain. Under quiescent conditions, the hydrated PVOH center segments form an equilibrium number of intrachain and interchain H-bonds (Figure 7). The relative number of intra- and interchain H-bonds depends upon the preferred conformations of the PVOH segments, which are a function of PVOH block length and the extent of chain stretching in the microphase-separated morphology. In our series of Ac$_{x}$VOH$_{y}$Ac$_{z}$ hydrogels with nearly constant $x \sim 125$, increasing the PVOH block length leads to decreased chain stretching and thus increased conformational freedom in the backbone. Hence, one would expect that number of intrachain hydrogen bonds is the largest for the block copolymer with the longest PVOH segment. The strain-dependent rheology of AcVOHAc gels reflects rearrangements of the H-bonding networks present in the quiescent gels. Application of an oscillatory deformation to the microphase-separated Ac$_{113}$VOH$_{208}$Ac$_{113}$ results in a soft gel-like response at low strains, consistent with an unperturbed H-bond network (Figure 7). Applying greater strain causes the dissociation of the intrachain H-bonds and drives local alignment of the network strands, which manifests in gel strain softening. Beyond a critical strain, the alignment of the network strands facilitates cooperative formation of new interchain H-bond crosslinks, thereby increasing the density of mechanically active crosslinks in the system. This increase in dynamic crosslink density gives rise to a strain stiffening response. Further increasing the applied strain magnitude results in complete network failure, by end block chain pull out from the microphase separated domains. For the Ac$_{127}$VOH$_{164}$Ac$_{127}$

![Figure 5. (a) Azimuthally integrated WAXS data associated with (1) bulk PVOH homopolymer, (2) a Milli-Q water background, and (3) Ac$_{113}$VOH$_{208}$Ac$_{113}$ in Milli-Q water and (4) in 10% (v/v) AcOH(aq); small, sharp peaks in curves 2−4 are X-ray sample holder artifacts. (b) Azimuthally integrated synchrotron SAXS intensity profiles of the hydrogel Ac$_{113}$VOH$_{208}$Ac$_{113}$ in (5) Milli-Q water, (6) after soaking in 10% (v/v) AcOH(aq), and (7) after AcOH removal, which shows that AcOH does not disrupt the microphase-separated morphology.](image-url)
hydrogel, we expect that the decrease in the PVOH segment length leads to greater chain stretching and better chain alignment, which facilitates interchain hydrogen bonding to yield a more tightly crosslinked network. Thus, application of strain drives immediate disruption of the H-bond network (viscoelastic response), enhanced network strand alignment, and interchain H-bond rearrangement (strain hardening), followed by network failure (strain softening), consistent with experimental observations. This increased interchain H-bonding accounts for the more pronounced strain hardening response as compared to Ac113VOH208Ac113. Decreasing the PVOH chain length further as in Ac120VOH117Ac120 leads to the greatest amount of PVOH chain stretching, thus aligning the hydrophilic segments to form many strong interchain H-bonds. Since atactic PVOH crystallizes through H-bonding, we suspect the large number of interchain associations may result in low levels of crystallinity consistent with the opacity of this gel. We surmise that the strength of these H-bonds coupled with the relative hydrophobicity of this triblock copolymer minimizes its experimentally observed water uptake. These changes in the PVOH domain H-bond network result in solid-like behavior that is proceeded only by network failure, consistent with the lack of experimentally observed strain hardening in this sample.

The impact of H-bonding on the deformation characteristics of the AcVOHAc hydrogels was directly tested by studying their rheology after treatment with AcOH(aq). AcOH reduces the number of intra- and interchain H-bonds in the polymer by more effectively solvating the PVOH segments. Treating the Ac113VOH208Ac113 and Ac127VOH164Ac127 hydrogels with AcOH causes the rheological responses ascribed to rearrangements of the PVOH H-bond networks to vanish, leading only to viscoelastic gel-like behavior with network failure at large strains. The latter behavior is typical in PEO-based hydrogels, in which the backbone ether linkages cannot directly associate to form dynamic crosslinks. Thus, AcOH "turns off" the effects of the dynamic noncovalent H-bond crosslinks in the PVOH gels. We note that acetic acid treatment of Ac113VOH208Ac113 leads to a drop in the gel modulus, which is consistent with softening of the network through a combination of decreased H-bonding and AcOH plasticization of the PVAc domains (vide supra). In the Ac127VOH164Ac127 gels, acetic acid treatment leads to an apparent increase in the observed modulus. However, we desist from any direct comparison of these results given that the pristine gel does not exhibit a low strain gel modulus plateau. Note that the higher modulus of the acetic acid-treated Ac127VOH164Ac127 as compared to Ac113VOH208Ac113 likely arises from the decreased PVOH strand length.

**CONCLUSION**

An optimized RAFT synthesis of chemically degradable PVAc-b-PVOH-b-PVAc amphiphilic block copolymers was developed in order to investigate the physical and rheological properties of
their microphase-separated physical hydrogels formed by swelling in excess water. Studies of the low strain shear rheology of a series of soft hydrogels comprising disorganized spherical PVAc domains of nearly fixed sizes linked by variable length PVOH network strands reveal that these gels exhibit unexpected low strain rheological responses, which are not seen in PEO-based block copolymer hydrogels. Treatment of these gels with aqueous acetic acid, a strong hydrogen-bonding agent and better solvent for PVOH, leads to the disappearance of these low strain rheological features. Hence, the nonlinear viscoelastic properties of these PVAc-b-PVOH-b-PVAc hydrogels are attributed to the unique ability of PVOH to cooperatively form a strain-dependent distribution of intramolecular hydrogen bonds. Deformation of these gels initially leads to strain softening due to dissociation of intramolecular H-bonds in the PVOH, followed by strain hardening stemming from interchain H-bond reassociation. Thus, these materials act as doubly physically crosslinked hydrogels whose properties may be tuned by strategically varying the copolymer composition. Given the known degradability and biocompatibility of the constituent homopolymer segments, these PVAc-b-PVOH-b-PVAc materials may potentially fill an unmet need in the design and development of new hydrogel materials for future biomedical applications.

### Associated Content

#### Supporting Information

Molecular characteristics of P(VClAc-ran-VAc)/PVAc block copolymers, $^{13}$C NMR characterization of VOH$_{56}$Ac$_{97}$, SAXS characterization of Ac$_{113}$VOH$_{208}$Ac$_{113}$ hydrogel, isothermal frequency sweeps for all hydrogel samples, and SAXS analysis of acetic-acid treated Ac$_{113}$VOH$_{208}$Ac$_{113}$ hydrogel. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.5b00410.

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**Notes**

The authors declare no competing financial interest.

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### References


