Impact of Synthetic Technique on PLA−PEO−PLA Physical Hydrogel Properties

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ABSTRACT: Previous work has shown that the stiffness of poly(lactide)-*b*-poly(ethylene oxide)-*b*-poly(lactide) [PLA−PEO−PLA] hydrogels can be influenced by crystallinity. Those hydrogels with crystalline PLLA end blocks had a higher storage modulus (up to 1 order of magnitude) than the amorphous equivalents. All the previous work was done with polymers synthesized in the bulk. This paper reports the difference in mechanical properties when two different synthetic techniques are used—bulk and solution synthesis. Solution-synthesized polymers consistently formed stiffer hydrogels than bulk-synthesized polymers. Further investigation determined the following: crystalline polymers from solution synthesis still form stiffer gels than the amorphous analogues, but not to the extent previously reported; the solution synthesized polymers have narrower distributions, but this alone does not account for the mechanical differences. However, the presence of asymmetric triblock copolymers, which act like an effective diblock copolymer, within the bulk-synthesized materials appears to lower the overall stiffness of the gel. The impact on modulus is much larger for amorphous PLA than for crystalline PLLA end block materials. These findings suggest bulk-synthesized polymers likely have more asymmetric triblock copolymers, that decrease the relaxation time of the system, possibly by lowering the junction lifetime, or lead to dangling ends in the network, which cause a loss in mechanical properties when compared to solution-synthesized polymers.

Introduction

Hydrogel systems are well-known for their potential in the area of biomaterials. These systems are highly aqueous, porous, and can have a certain degree of mechanical tunability[1] making hydrogels very useful for both tissue engineering and drug-delivery applications. However, hydrogels used as biomaterials are limited to biocompatible and/or biodegradable polymers and this restriction leads to a limited range of stiffness.[2−8] Some of the most commonly used polymer biomaterials include: poly(ethylene oxide) [PEO] (known for its biocompatibility), poly(lactide) [PLA], poly(glycolide) [PGA], poly(caprolactone) [PCL], and other biodegradable polyesters. These polymers alone do not assemble into ordered structures that are needed for successful use as a tissue-engineering or drug-delivery construct, so they have often been synthesized as copolymers to take advantage of both the hydrophilic and hydrophobic nature of the above-mentioned polymers.[9] Amphiphilic block copolymers, homopolymers, and graft copolymers can be chemically cross-linked to form hydrogels,[10−13] and adhesion peptides or proteins are often added to aid in cell attachment to the gel.[14−16] Physical hydrogels, such as those formed by poly(lactide)-*b*-poly(ethylene oxide)-*b*-poly(lactide) [PLA−PEO−PLA] triblock copolymers, are self-assembling systems and do not require a covalent cross-linking agent. In this case, assembly occurs through the hydrophobic interactions of the PLA end blocks, which serve as physical cross-links. PEO midblocks, at a critical concentration, can bridge between the PLA hydrophobic micelle cores to form a connected network structure.

The mechanical properties, or specifically the stiffness, of hydrogels will have a great impact on their performance as tissue engineering systems because cells not only receive chemical signals from their environment but mechanical signals as well.[17,18] Cells will not grow properly in a mechanical environment that is not similar to their native tissue. More specifically, if the environment, or gel in this case, is too soft or too stiff, the cells will not proliferate properly.[19,20] For this reason, it is imperative to match the hydrogel's properties to the target tissue. Keeping this in mind, we aim to broaden the range of attainable stiffness in PLA−PEO−PLA physical hydrogels.[7,21]

We have previously reported that the mechanical properties of physical hydrogels formed from PLA−PEO−PLA triblock copolymers can be affected by the crystallization of the hydrophobic (PLA) component.[22] Those gels that were made with crystalline hydrophobic segments (PLLA−PEO−PLLA, synthesized from the L-lactide monomer) were stiffer than the amorphous equivalent (racemic PRLA−PEO−PRLA, synthesized from the D,L-lactide monomer). The latter showed a viscoelastic liquid-like response with a measurable relaxation time within the frequency range tested where this was observed as a crossover between the storage and loss modulus, $G' > G''$. The differences in stiffness were attributed to stabilization of the hydrophobic domains within the physical network structure through crystallization in the PLLA materials, whereas the amorphous polymer forms a more loosely connected network junction with shorter-lived hydrophobic domains. We modified the synthetic procedure used in previous publications to yield a polymer with a narrower molecular weight distribution. Analysis of gels formed with these polymers showed that the synthetic conditions, either bulk polymerization or solution polymerization, also affected the final mechanical properties. A number of experiments point to the presence of asymmetric triblock "contaminants" as the most significant contributor to the bulk

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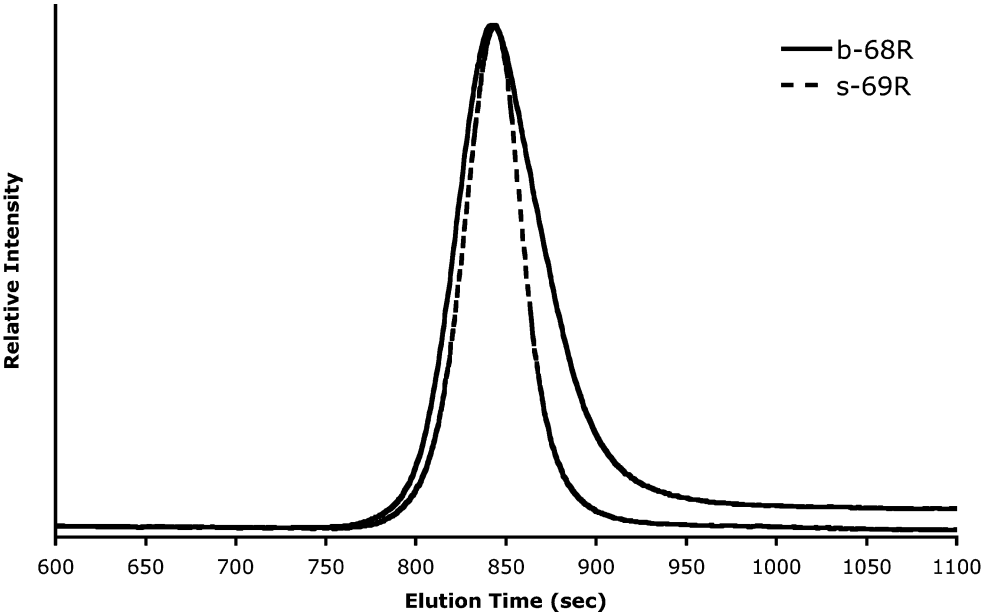

Figure 1. GPC of bulk-synthesized and solution-synthesized triblock copolymers.

Table 1. Sample Polymers Tested

sample	DP_{PLA}[a]	PDI[b]	synthetic method	monomer	stereochemistry	crystallinity
b-72L	72	1.19	bulk	L−lactide	stereoregular	semicrystalline
s-70L	70	1.06	solution	L−lactide	stereoregular	semicrystalline
b-68R	68	1.16	bulk	D,L−lactide	stereorandom	amorphous
s-69R	69	1.07	solution	D,L−lactide	stereorandom	amorphous

[a] On the basis of ^{1}H NMR integrations. [b] Measured from DMF (0.01 M LiCl) GPC.

elastic modulus as opposed to crystallinity and polydispersity. The impact of this asymmetric triblock “contaminant” reduces the modulus of the hydrogels made from amorphous PRLA materials more than those made of crystalline PLLA.

Experimental Section

Materials. (3*S*)-*cis*-3,6-Dimethyl-1,4-dioxane-2,5-dione (L-lactide) and 3,6-dimethyl-1,4-dioxane-2,5-dione (DL-lactide) (Sigma Aldrich) were recrystallized from ethyl acetate and sublimated prior to use. Tin(II) 2-ethylhexanoate catalyst (Alfa Aesar), PEO (M_p = 8 kDa, Sigma Aldrich, previous MALDI−ToF analysis showed the actual molecular weight to be 8.8 kDa), and poly(ethylene glycol)−monomethyl ether (M_n = 9 kDa, Polymer Source) were used without further purification.

General Method for Bulk Synthesis. Bulk-synthesized triblock copolymers were prepared as previously reported.[22] Telechelic PEO macroinitiator was stirred and heated at 150 °C while purged with nitrogen. Tin(II) 2-ethylhexanoate was added to the mixture, followed by immediate addition of lactide. The reaction was capped and reacted at 150 °C for 24 h. The reaction mixture was quenched with methanol, dissolved in tetrahydrofuran (THF), and precipitated using hexanes. Dissolution and precipitation was repeated three more times. The recovered white powder was then dried under vacuum at room temperature.

General Method for Solution Synthesis. Triblock Copolymers. Telechelic PEO macroinitiator (2.50 g, 0.284 mmol, 1 equiv) was weighed into a dry three-neck round-bottom flask with a stir bar and attached to a condenser. The PEO was stirred and heated at 130 °C under nitrogen flow. Tin(II) 2-ethylhexanoate (46 μL, 0.142 mmol, 0.5 equiv) was added to the PEO, followed by immediate addition of lactide (1.43 g, 9.94 mmol, 35 equiv). The condenser was turned on and toluene was added to the reaction mixture (approximate [PEO] = 28 mM). The mixture was refluxed for 24 h under nitrogen flow, then diluted with THF, and precipitated using hexanes. The recovered white powder was separated with a filter funnel, collected, and dried under vacuum at room temperature. Yield = 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.12−5.19 (quartet when polymerizing with L-lactide, multiplet when polymerizing with DL-lactide), δ 3.64 (s), δ 1.48−1.59 (d). M_n = 13 858. GPC (DMF) PDI = 1.06.

Diblock Copolymers. Poly(ethylene glycol) monomethyl ether macroinitiator (1.00 g, 0.111 mmol, 1 equiv, PDI = 1.18) was weighed into a dry 3-neck round-bottom flask with a stir bar and attached to a condenser. The PEO was stirred and heated at 130 °C under nitrogen flow. Tin(II) 2-ethylhexanoate (9 μL, 0.028 mmol, 0.25 equiv) was added to the PEO, followed by immediate addition of lactide (0.281 g, 1.95 mmol, 17.5 equiv). The condenser was turned on and toluene was added to the reaction mixture (approximately [PEO] = 28 mM). The mixture reacted at 130 °C for 24 h under nitrogen flow, then diluted with THF, and precipitated using hexanes. The recovered white powder was separated with a filter funnel, collected, and dried under vacuum at room temperature. Yield = 61%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.15−5.20 (quartet when polymerizing with L-lactide, multiplet when polymerizing with DL-lactide), δ 3.64 (s), δ 1.48−1.60 (d). M_n = 11 826. GPC (DMF) PDI = 1.27 (broadness due to starting material).

Characterization of Polymer (^{1}H NMR and GPC). ^{1}H NMR spectra were recorded with a 300 MHz Bruker Spectrospin 300. Chemical shifts were expressed in parts per million using deuterated chloroform solvent protons as the standard. The average degrees of polymerization (DP) were calculated by comparing the integration of the methyne peak of PLA to the integration of the methylene peak of the PEO block. Gel permeation chromatography (GPC) was performed with a Polymer Laboratories PL-GPC50 with 2 PLGel 5 μm Mixed-D columns, a 5 μm guard column, and a Knauer RI detector vs poly(styrene) standards. The eluent was *N*,*N*-dimethylformamide with 0.01 M LiCl at 50 °C.

Hydrogel Preparation. Dried polymer sample was gradually added to a fixed amount of water while stirring. Once the polymer was added, the dispersion was heated to 80 °C (or 40 °C) for 20 h while stirring. The dispersion gelled upon cooling and was allowed to equilibrate for at least 1 day before testing.

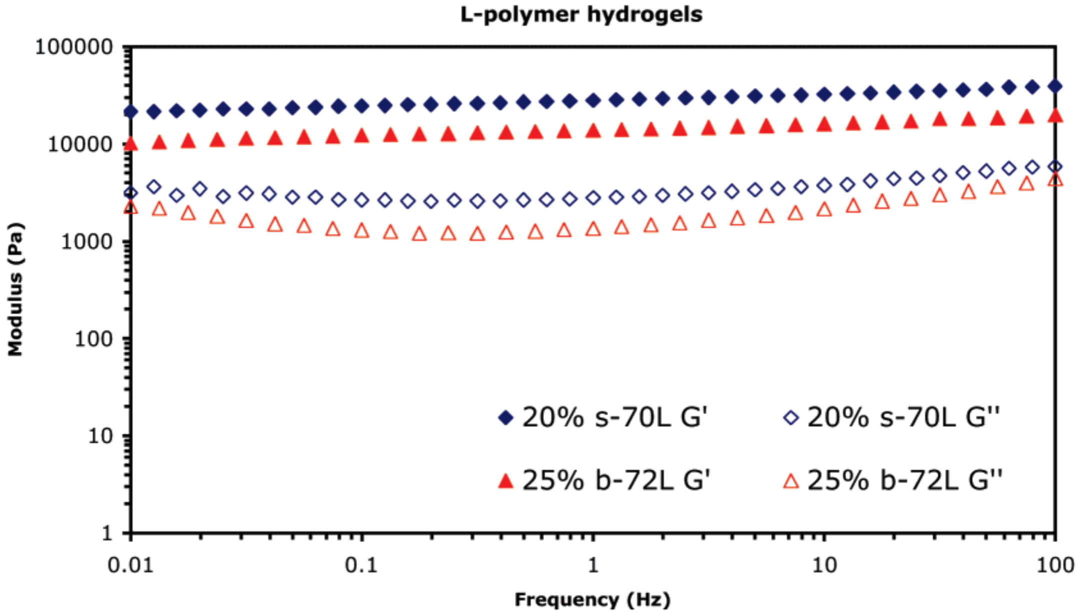

Figure 2. Rheology of bulk- and solution-synthesized L-polymer hydrogels. Mechanical properties of 20 wt % solution-synthesized L-polymer hydrogel and 25 wt % bulk-synthesized L-polymer hydrogel.

General Method for Rheological Data. Rheological measurements were performed using a cone and plate geometry (40 mm diameter cone with a 2° cone angle) on a TA Instruments AR2000 stress controlled rheometer. Water evaporation was minimized at the temperature and time scale investigated by using a solvent trap. A stress sweep at a constant frequency of 1 Hz was first performed to obtain the linear viscoelastic region for collecting subsequent data. Frequency sweep tests over a frequency range 0.01 to 100 Hz were performed at constant strain amplitudes (normally between 0.1% to 0.2% strain) to measure G' and G'' (storage and loss moduli, respectively) corresponding to the linear response.

General Method for Powder X-ray Diffraction. Powder X-ray diffraction measurements of the gels were performed on a Panalytical X'Pert powder diffractometer. The voltage was set at 45 kV, and the current was set at 40 mA. A Ni filter and a $^1/_2$° slit width were used. The samples were scanned at $2\theta = 5-55°$.

Results and Discussion

PLA−PEO−PLA triblock copolymers were synthesized via two different synthetic methods—by bulk polymerization or by solution polymerization (see Table 1). The polymer resulting from polymerization of L-lactide generated triblock copolymers with stereoregular (isotactic) and thus semicrystalline end blocks. Conversely, when D,L-lactide was used, the end blocks were stereorandom (atactic) and thus amorphous. Since all of the triblock copolymers had a PEO midblock with $M_n = 8.8$ kDa, the samples were labeled by the *total* average degree of polymerization (DP) of both PLA end blocks. For example b-72L means the total DP of lactic acid units is 72. The preceding letter signifies the synthetic method used to make the polymer (b = bulk synthesis and s = solution synthesis), while the following letter signifies the stereochemistry of the PLA end blocks (L = semicrystalline PLLA and R = amorphous or stereorandom PRLA). The targeted DP was obtained for both polymerization techniques, and both techniques had high yields (~85−90%) with relatively narrow polydispersity indices (PDI), indicating that the reactions were controlled. However, the solution-synthesized polymers consistently showed a narrower molecular weight distribution. Figure 1 shows typical GPC chromatograms for two polymers, bulk- and solution-synthesized, with similar molecular weights but slightly different PDI's (PDI ≈ 1.16 for bulk-synthesized polymers and 1.07 for solution-synthesized polymers).

Hydrogels from bulk- and solution-synthesized L-polymers were evaluated with a rheometer to characterize their bulk mechanical properties (see Figure 2). The rheological properties of the two polymers were slightly different despite having similar apparent molecular weights and DP values. The solution-synthesized polymer generated a hydrogel that had higher moduli (both storage modulus, G', and loss modulus, G'') than the corresponding bulk-synthesized polymer, despite the fact that the bulk-synthesized polymer hydrogel was more concentrated at 25 wt %. Because solution-synthesized L-polymers consistently showed higher moduli than their corresponding bulk-synthesized polymers, it appeared these findings might have correlated with the solution-synthesized L-polymers having a higher degree of crystallinity.

Impact of Crystallinity on Hydrogel Properties. To understand the impact of crystallinity in these two different hydrogels, we investigated the PLLA crystalline microstructure of the triblock copolymer before and after hydrogel formation with X-ray diffraction (XRD). Figure 3 shows the powder XRD for both bulk- and solution-synthesized L-polymers and their corresponding hydrogels. The peaks at $2\theta \approx 19$ and 23° correspond to crystalline PEO, those at $2\theta \approx 17$, 19, and 22° correspond to crystalline PLLA,[23] and the broad peaks seen in the hydrogels are scattering due to water. It was immediately apparent that the two polymers, in their dry form, had differences at $2\theta \approx 17°$, which is attributed to crystalline PLLA, since the bulk-synthesized polymers had a much sharper peak than the solution-synthesized polymers. Utilizing the Scherrer equation,[24] the mean crystallite length (L_{hkl}) of the PLLA blocks was calculated as follows:

$$L_{hkl} = (K\lambda/\beta_o) \cos \theta \qquad (1)$$

where K is a constant ($K = 1$ is commonly used for these systems and is the value that we have used), λ is the wavelength of the X-rays used (1.54 Å), β_o is the full width at half-maximum of the peak, and θ is the Bragg angle of the reflection. Solution-synthesized polymers had a much shorter mean crystallite length

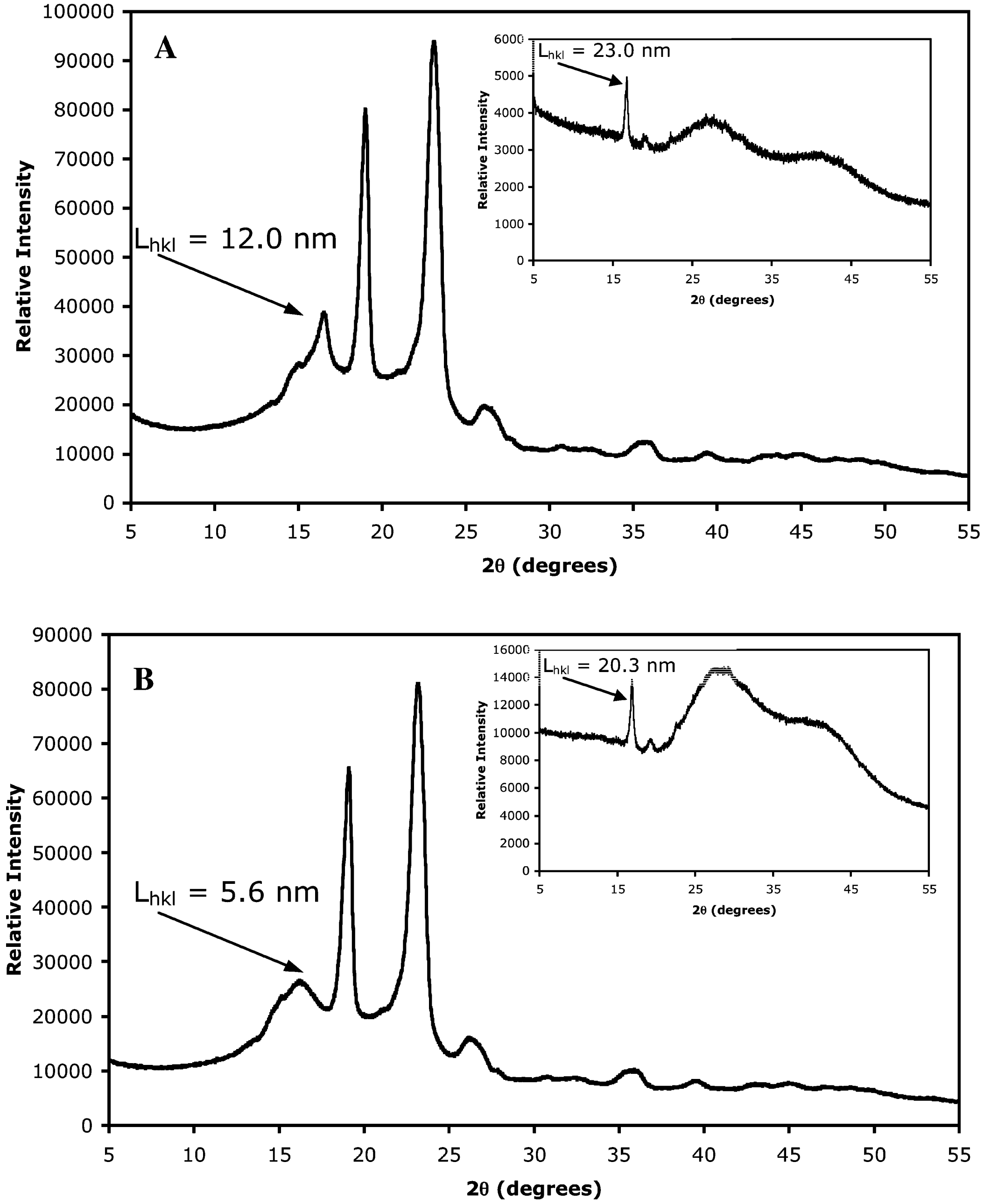

Figure 3. XRD of bulk and solution-made polymers. (A) XRD of bulk-synthesized polymers (b-62L) and the mean crystallite length of crystalline PLLA. (Insets show XRD of gels formed from the polymer.) (B) XRD of solution-synthesized polymers (s-68L) and the mean crystallite length of crystalline PLLA.

Table 2. Crystallite Length and Rheological Properties[a]

bulk-synthesized polymers				solution-synthesized polymers				
sample[a]	L_{hkl} powder (nm)	L_{hkl} gel (nm)	G' (kPa)	sample[a]	L_{hkl} powder (nm)	L_{hkl} gel (nm)	G' (kPa)	increasing modulus
b-62L	13.9	12.8	6.53	**s-68L**	5.56	12.6	17.8	↓
b-62L	13.0	16.2	17.4	**s-68L**	5.56	18.6	19.4	
b-62L	12.0	23.0	21.0	**s-68L**	5.67	9.3	24.9	
b-62L	8.6	25.7	21.2	**s-68L**	5.63	20.3	27.8	
b-62L	10.3	24.2	32.5	s-**68L**	5.63	19.8	29.9	

[a] The same triblock copolymer was used for the bulk- and solution-synthesized polymers, respectively, but was melted and quenched at various temperatures to achieve various mean crystallite lengths. All samples were either refluxed in toluene for 3 h and precipitated in hexanes or was annealed at 180 °C for 3 h and then quenched with one of the following: room temperature, water, ice−water, or liquid nitrogen.

than the bulk-synthesized polymers. For example, from the data shown in Figure 3, the L_{hkl} values are 12.0 and 5.6 nm for bulk- and solution-synthesized, respectively. The different crystallite lengths seen in the starting polymers may have affected the crystallite sizes within the resulting hydrogel structure. Assuming this was true, hydrogels formed from solution-synthesized polymers would have shorter crystallite lengths than hydrogels formed from bulk-synthesized polymers, and this implies that shorter crystallite lengths lead to stiffer gels. To understand why the crystallite length would impact stiffness, one must

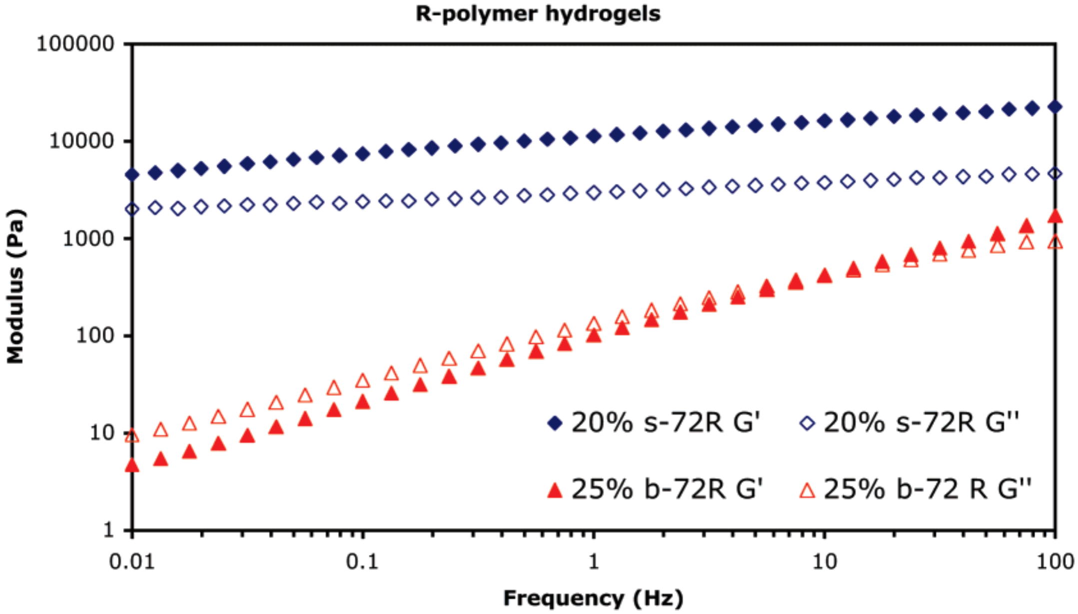

Figure 4. Rheology of bulk- and solution-synthesized R-polymer hydrogels. Mechanical properties of 20 wt % solution-synthesized R-polymer hydrogel and 25 wt % bulk-synthesized R-polymer hydrogel.

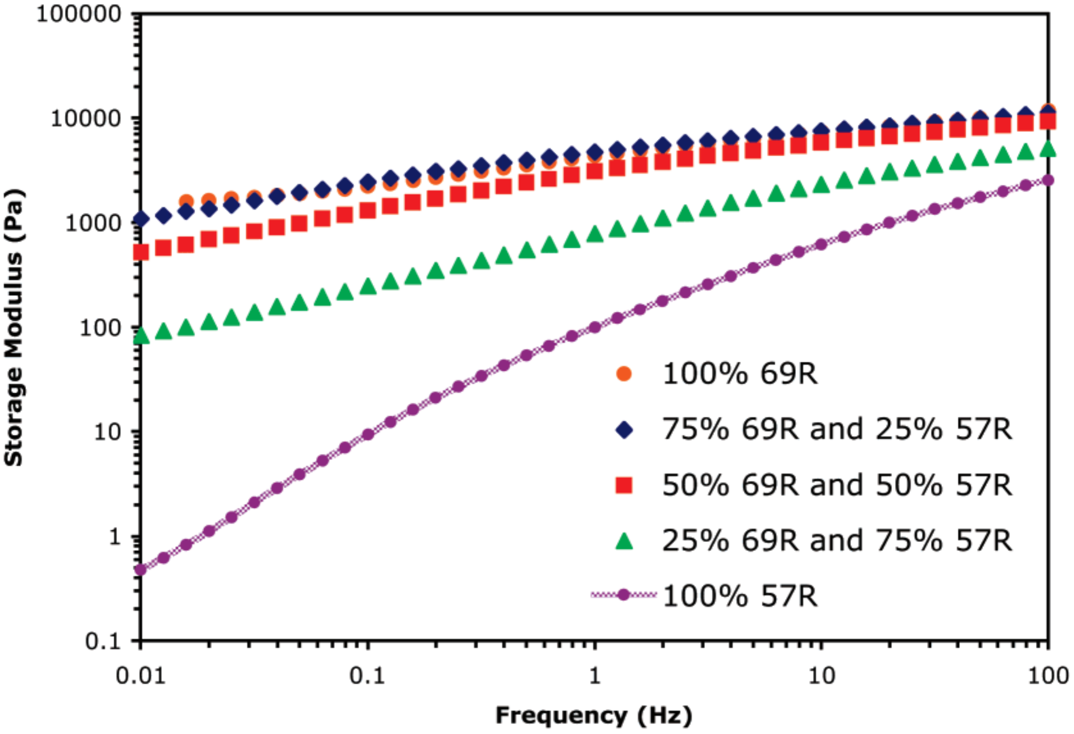

Figure 5. Rheology of mixed molecular weight systems. Solution-synthesized polymers with different molecular weights were mixed, made into gels, and tested rheologically. The graph only displays the storage modulus, G', for clarity.

consider the means by which the network is formed. PLLA end blocks associate through hydrophobic interactions to make a network junction and can crystallize within the hydrophobic core. Shorter crystallite lengths within the hydrogel could mean that fewer PLLA end blocks are associated and crystallizing within any given micelle. Because less PLLA end blocks are within an individual core, this requires a greater number of network junctions since the amount of PLLA in both systems is the same. Therefore, this increase in the number of network junctions would account for the observed increased stiffness.

As a result, polymers with various crystallite lengths, achieved by varying the processing conditions, were studied to elucidate the role of crystallite length on hydrogel modulus. The polymers' and hydrogels' structures were studied using powder XRD, and the hydrogels were tested using rheology. Table 2 lists the mean crystallite length of the starting polymer, the corresponding hydrogel, and the modulus of the hydrogel (reported at 100 Hz

Table 3. Properties of Mixed Molecular Weight Polymer Systems

sample	PDI	G' **at 100 Hz** (kPa)
100% 69R	1.07	11.8
25% 57R and 75% 69R	1.14	11.2
50% 57R and 50% 69R	1.08	9.4
75% 57R and 25% 69R	1.13	5.1
100% 57R	1.07	2.6

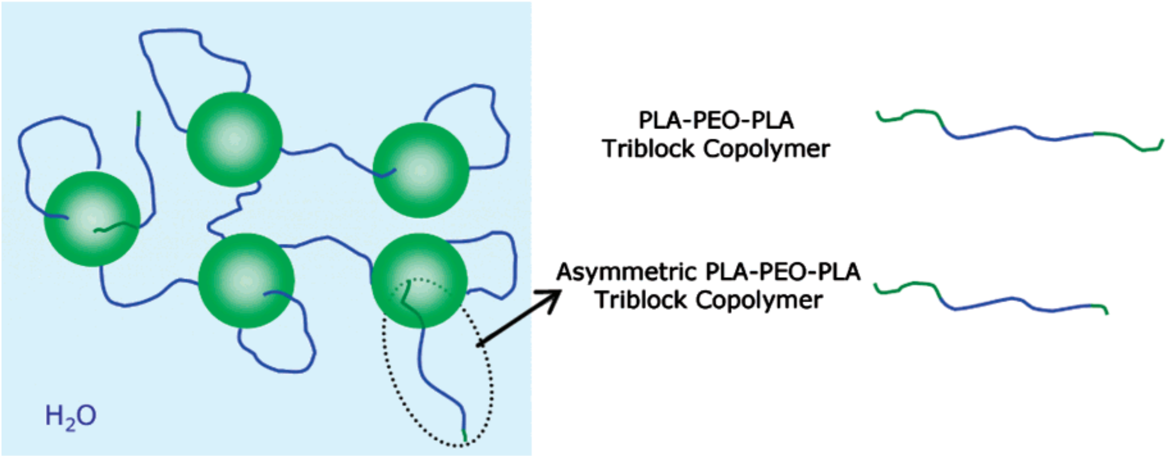

Figure 6. Schematic of mixed triblock/diblock copolymer gel. Green symbolizes the PLA blocks, blue symbolizes the PEO blocks, and the green spheres are the physical cross-links formed by hydrophobic interactions. An asymmetric triblock copolymer would either lead to shorter-lived cross-links or dangling chain ends.

since the gels are less frequency dependent at higher frequencies). In nine of ten cases, the crystallite domain size, L_{hkl}, increases as the powder sample is transformed to the hydrogel. However, no correlation between the crystallite length of the polymer, or hydrogel, and the resulting modulus was found. Therefore, the difference in crystallite lengths for the bulk- and solution-synthesized polymers most likely does not account for the discrepancies in the observed mechanical properties. Furthermore, although the mean crystallite length of the solution-synthesized polymers was shorter in the powder form, the difference was not as pronounced in the resulting hydrogels. So, while bulk- and solution-synthesized polymers started with different crystallite lengths, the resulting crystallite lengths in the hydrogels were more similar (refer to Table 2 and insets of Figure 3). This further suggests that the crystallite length, especially of the neat powder, is not the dominant factor that accounts for the variations in mechanical properties observed in these hydrogels.

Since crystallinity does not appear to be the critical factor, noncrystalline R-polymers were prepared. These new solution-synthesized polymers also produced hydrogels with stiffer modulus than their corresponding bulk-synthesized polymers (see Figure 4). The amorphous systems we previously reported were bulk-synthesized and showed a viscoelastic liquid-like response with G' and G'' varying with frequency over the entire frequency range as shown in Figure 4, but the solution-synthesized amorphous polymer samples did form gels over the whole frequency range, despite being less concentrated, and were much less frequency dependent. These results were unexpected and demonstrated that the polymerization conditions appear to affect the gel properties more strongly than can be explained by crystallinity alone. Aside from crystallinity, the solution-synthesized polymers consistently gave narrower PDI's than the bulk-synthesized molecules and so the impact of PDI was explored.

Impact of Polydispersity. Since the polydispersities of both the crystalline and amorphous polymers were narrower for the solution-synthesized polymers, we investigated the dependence of mechanical properties on polydispersity. We combined solution-synthesized polymers with various molecular weights to obtain mixtures with a range of distributions. Gels were formed with these mixtures and compared to the unmixed systems. Results shown in Figure 5 and Table 3 indicate that polydispersity played little role in the mechanical properties, since two systems with different PDI's (100% 69R, PDI = 1.07 and 25% 57R, 75% 69R, PDI = 1.14) showed almost identical mechanical properties. Rather, the determinant for gel stiffness in this comparison was the PLA block length as previously reported, in which longer PLA blocks yield stiffer gels.[7]

Impact of Diblock Copolymer Impurities. Having shown that crystallinity and PDI do not directly impact gel stiffness, the presence of a "contaminant" was expected to account for the differences in both the amorphous and crystalline polymers. More specifically, the presence of an asymmetric PLA−PEO−PLA triblock copolymer, where one end has a short PLA block, would lower the overall mechanical properties of the hydrogel because networks can only be formed through the bridging of micelles. Highly asymmetric triblocks with short PLA end blocks could act to shorten the lifetime of the junctions formed and weaken the strength of the junction. In certain cases these asymmetric triblocks could also act as "effective diblock copolymers" that lead to dangling ends if the short PLA segments lack the strong hydrophobic character to drive insertion into neighboring micelles. These types of polymers would not contribute toward the network structure and would weaken the overall stiffness of the hydrogel, as illustrated in Figure 6, by an increasing number of "dangling ends". It is also likely that these polymers would decrease the relaxation time of the system, either by decreasing the lifetime of the junction or by allowing for faster dynamics due to a decrease in the number of bridging chains. These effects would be manifest regardless of the presence and/or amount of crystallinity within the network. The higher polydispersity seen in the bulk-synthesized polymers (see Figure 1 again) would be consistent with a side reaction that leads to some asymmetric triblock copolymer, or "effective diblock copolymer", and the loss of mechanical properties. However, these side reactions seem to be suppressed in the solution polymerization, leading to increased mechanical properties and narrower molecular weight distributions. We speculated that asymmetric triblock copolymers were synthesized through chain transfer reactions in which growing PLA polymer chains attacked other growing PLA chains creating two triblock copolymers with one longer and one shorter PLA chain end. Previous literature precedents have demonstrated that both inter- and intramolecular transesterification reactions can occur when using tin catalysts and can lead to partial degradation/depolymerization and cyclic lactide oligomers from chain backbiting at elevated temperatures.[25−28] These reactions are more likely to occur in the bulk than in solution synthesis due to the difference

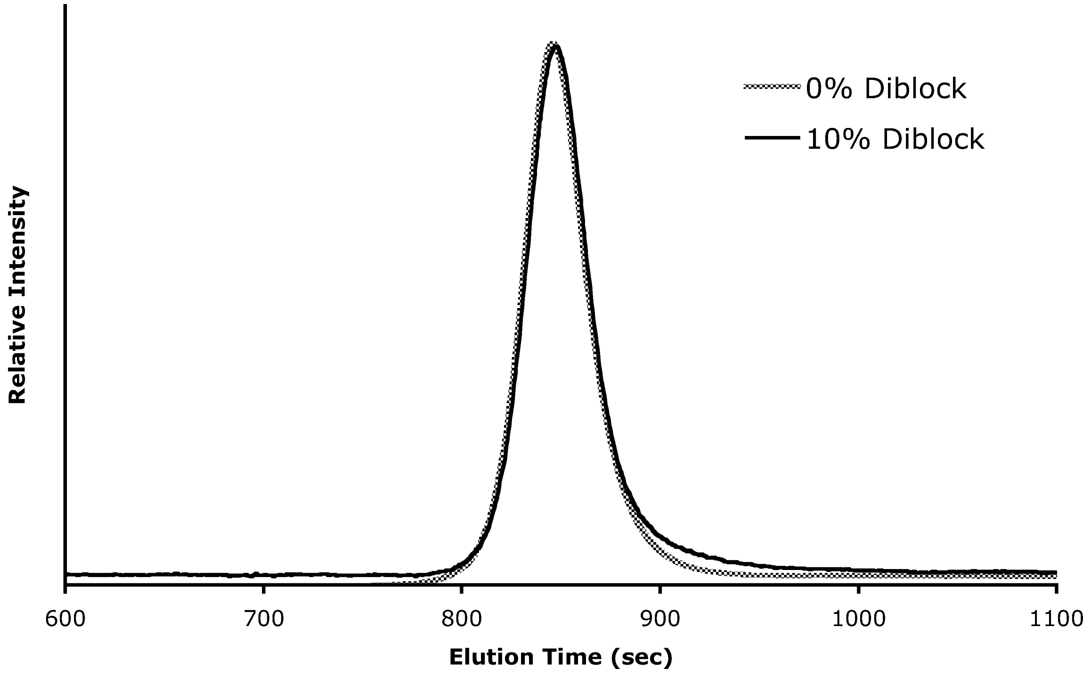

Figure 7. GPC chromatograms of triblock and mixed triblock/diblock copolymers. GPC's of PLA−PEO−PLA triblock copolymer with 0 and 10 wt % incorporation of PLA−PEO diblock copolymer.

in concentrations, since in the bulk there are many more opportunities to interact with other growing chains than when they are diluted in solution.

Previous work by Chang and co-workers demonstrated that PLA−PEO−PLA triblock and PLA−PEO diblock copolymers could have "nonideal" chain growth. Using liquid chromatography at the critical condition (LCCC), they first demonstrated that PLA blocks can have an odd number of lactic acid residues.[29] This was unexpected, since cyclic lactide dimers are used in the polymerization and should ideally produce only even numbers of lactic acid residues; however, their results suggest that the polymer backbone ester bonds may be broken during the polymerization. Further work showed that although the PLA end blocks of PLA−PEO−PLA triblock copolymers are commonly assumed to be symmetric or composed of an equal number of monomer units, this is not always the case.[30,31] There are a number of ways that the lactide monomers may add to either end of the growing telechelic chain that can lead to an asymmetric triblock copolymer, or more specifically, end blocks with different lengths. These findings give credence to the possibility of asymmetric triblock copolymers, or triblocks in which one PLA end block is very short, as contaminants within our triblock copolymer systems.

To investigate this, we synthesized two diblock copolymers, PLLA−PEO and PRLA−PEO, with similar DP's to those found in the triblock copolymer systems. Diblocks were used since asymmetric triblock copolymers cannot be synthesized on command and because they are a good model for highly asymmetric triblock copolymer. The diblock copolymers were combined with the corresponding triblock copolymers (5 wt %:95 wt % diblock:triblock and 10 wt %:90 wt % diblock:triblock) by dissolving them in THF to ensure full mixing, followed by precipitation using hexanes. Although one would expect to see a bimodal molecular weight distribution by GPC when mixing diblock with triblock copolymer, the molecular weight difference between the two (approximately 2.5 kDa) was not significant and was not well resolved by GPC. As shown in Figure 7, this mixing lead to the slightest broadening of the PDI on the low molecular weight distribution side of the peak. This finding appears to be consistent with the GPC data seen for the bulk-synthesized sample previously shown in Figure 1. Both the mixed systems and the bulk-synthesized polymers showed deviation from the solution-synthesized polymer curves with a higher population of lower molecular weight species. Consequently, it appears the GPC data supports the hypothesis of the presence of asymmetric triblock copolymer contaminants. These mixed systems were then used to form hydrogels (20 wt % mixed polymer) as previously described and their stiffness was measured by rheology. Results are shown in Figure 8 for the L-crystalline polymers and in Figure 9 for the R-amorphous polymers. The moduli for both sets of polymers decreased as diblock was added. For example, the storage modulus at 100 Hz for the L-series of polymers decreased from 39.3 to 23.7 kPa for 0% and 10% added diblock, respectively, while the R-series decreased even more from 22.4 to 5.4 kPa for 0% and 10% added diblock, respectively. The PDI's for these mixed systems are also listed and even when 10% diblock copolymer was added, the PDI remained relatively narrow (PDI ≤ 1.10) compared to polymers synthesized by bulk polymerization indicating that the bulk-synthesized samples may have even greater amounts of effective diblock copolymer than was studied here.

Although both systems' mechanical properties were affected by incorporation of diblock, the effect was much less pronounced in the L-systems. This is in line with our previous findings that crystallization within hydrophobic micelle cores increases the network stability. So, although there was a reduction in modulus from the addition of diblock copolymer, these crystalline junctions were more permanent than the R systems so that the overall decrease in modulus, or impact of the asymmetric triblock contaminant was comparatively not as significant. These two opposing forces lead to a softer hydrogel, but one in which the storage modulus still remained greater than the loss modulus over the entire frequency range and the moduli were relatively frequency independent.

On the other hand, the amorphous R-systems were greatly affected by the incorporation of diblock copolymer. The storage

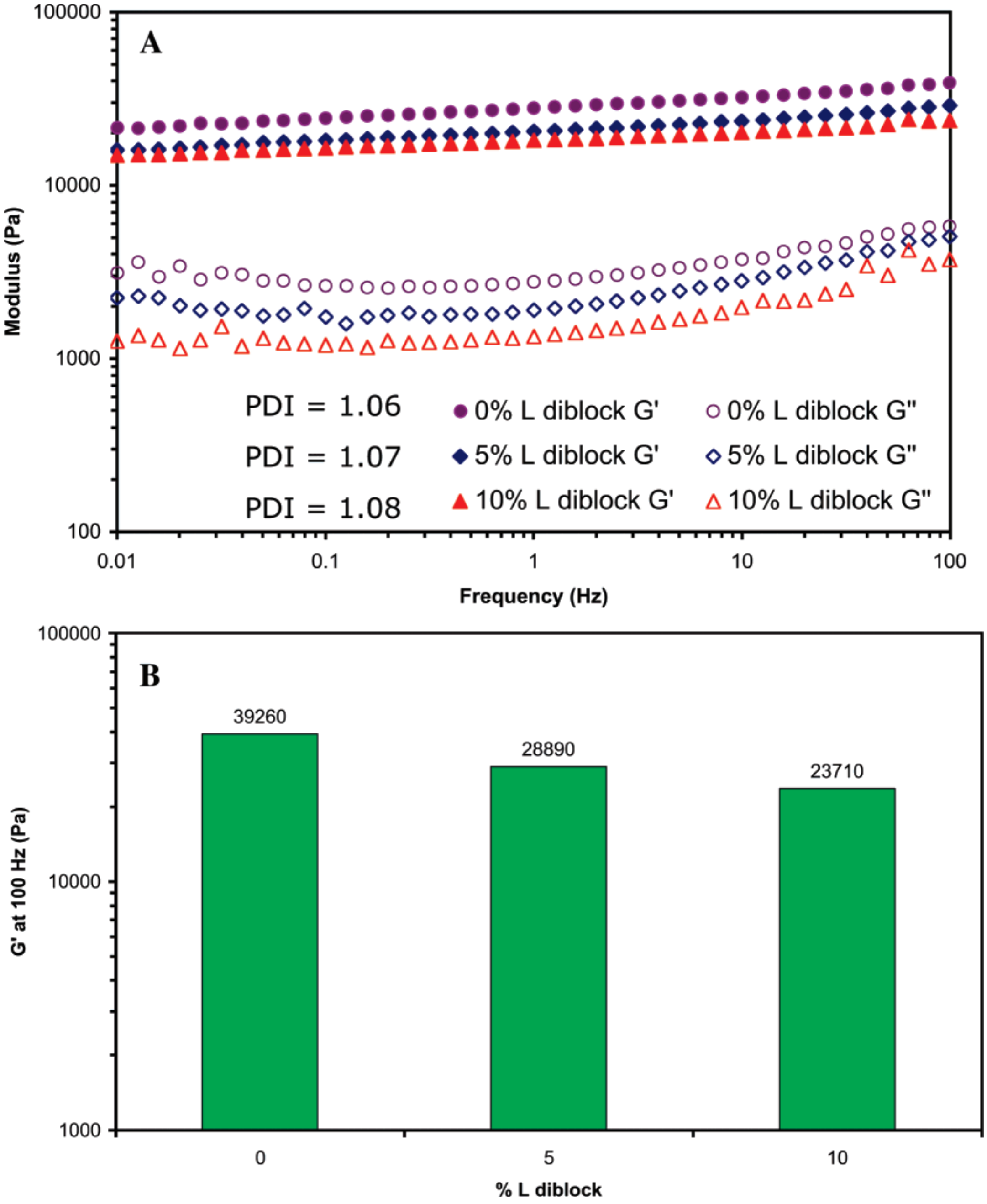

Figure 8. Mechanical properties of L triblock/diblock mixed systems. (A) Rheology of L-systems with 5 and 10 wt % diblock incorporated. (B) Comparison of the storage modulus at 100 Hz.

modulus was reduced by almost an order of magnitude when going from 0% added diblock to 10% added diblock (G' at 100 Hz = 22 and 5.4 kPa, respectively). As a result, the decreased number of network junctions greatly affected the system's stiffness and the network lifetime was shortened. Furthermore, the systems showed viscoelastic liquid character and displayed a crossover point between G' and G'' in the mid-frequency range. These two characteristics were not seen for nonmixed solution-synthesized polymer systems but were evident in the bulk-synthesized polymer systems. This demonstrated that diblock copolymer contaminants clearly affect the mechanical properties of an associative network physical hydrogel, as expected, and supports that the most likely cause for the observed differences between bulk-synthesized and solution-synthesized triblock copolymers is caused by highly asymmetric triblock copolymers.

Conclusions

Properties of physical hydrogel systems made from PLA−PEO−PLA triblock copolymers are influenced by the mode of polymerization used to synthesize the polymers. We found that solution-synthesized polymer hydrogels made stiffer materials than the corresponding bulk-synthesized polymer hydrogels. The bulk-synthesized polymers showed longer crystallite lengths for the L-series, however, once introduced to water, the self-assembly can drive further crystallization or assembly of previously crystallized domains in the solution-synthesized polymers, resulting in more similar crystallite lengths for the gels formed from the bulk- and solution-synthesized polymers. Therefore, differences in crystallinity do not account for the different mechanical properties seen with the synthetic technique used, and although the two polymers showed slight variations

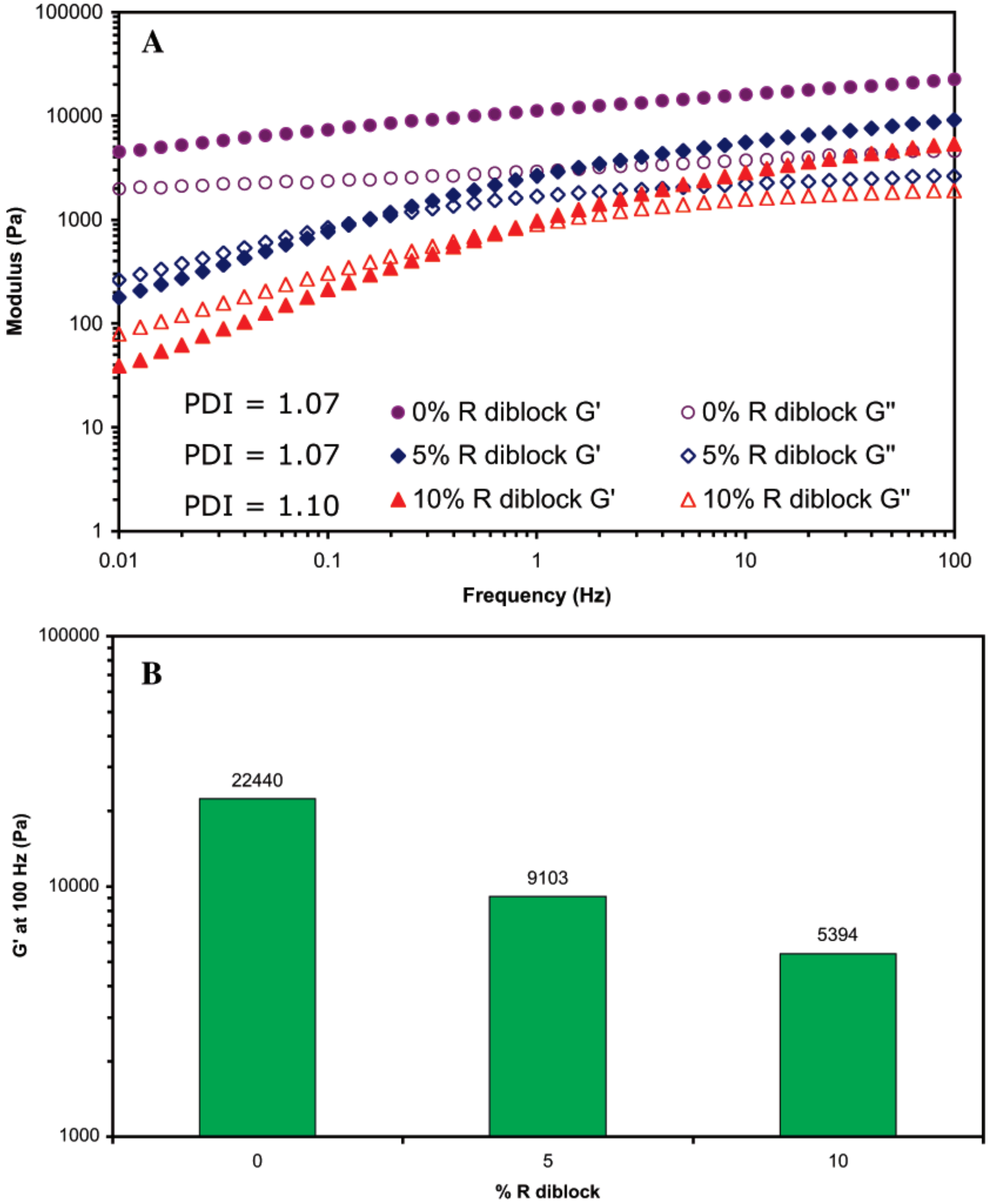

Figure 9. Mechanical properties of R triblock/diblock mixed systems. (A) Rheology of R-systems with 5 and 10 wt % diblock incorporated. (B) Comparison of the storage modulus at 100 Hz.

in molecular weight distribution, it was determined that PDI alone was not the sole cause for the discrepancies in hydrogel stiffness. We believe that the differences are more likely due to contamination of highly asymmetric triblock copolymer in the bulk-synthesized polymers. In the bulk at high temperatures side reactions may be more prevalent leading to polymer chains with short PLA blocks which can reduce the lifetime of the network junction, reduce the number of physical cross-links in the system, or both. The most significant effects on mechanical properties were seen in the amorphous triblock copolymer systems and demonstrated that even a small amount of diblock contaminant can greatly change the mechanical properties of a physical hydrogel. This demonstrated impact on mechanical properties of diblock copolymers was surprising given the GPC data presented in Figure 1, showing a relatively narrow molecular weight distribution for bulk-synthesized polymers. It highlights the limitations of GPC and provides additional motivation for more sophisticated polymer characterization methods. Finally, crystallization can still be used to influence modulus as we previously reported (though not to the same extent). Furthermore, amorphous micelles with shorter network junction lifetimes are more susceptible to forming dangling ends than crystalline network junctions, as one might expect. These principles may be used to design hydrogel stiffness in general but are certainly pertinent within the range of moduli reported here.

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