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Aqueous electrospinning of wheat gluten fibers with thiolated additives

Jing Dong [a], Alexandru D. Asandei [b,c], Richard S. Parnas [a,b,*]

[a] University of Connecticut, Department of Chemical, Materials and Biomolecular Engineering, Storrs, CT 06269, USA
[b] University of Connecticut, Institute of Materials Science, Storrs, CT 06269, USA
[c] Department of Chemistry, University of Connecticut, Storrs, CT 06269, USA

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ABSTRACT

The molecular weight distribution (MWD), rheology and electrospinning of a series of wheat gluten (WG) mixtures with poly(vinyl alcohol) (PVA), dithiothreitol (DTT), and thiolated poly(vinyl alcohol) (TPVA) in water/1-propanol (1/1) were investigated by size-exclusion chromatography, steady-shear viscosity measurements and scanning electron microscopy. Thiolated additives reduce disulfide bonds between protein subunits and thus increase WG solubility. Accordingly, Newtonian behavior is observed for pure components and PVA/WG, and shear-thinning for DTT/WG and TPVA/WG. Concentration, viscosity and additive type affect WG electrospinnability. At higher concentrations, PVA/WG fibers are thicker than WG ones, whereas DTT/WG and TPVA/WG fibers are thinner and beadless. While at low concentrations both DTT/WG and TPVA/WG generate poor fibers, lowering TPVA thiolation level results in better fibers, unobtainable with DTT. Thus, although using only the lower end of the WG MWD, reasonably good fibers can nonetheless be obtained with an inexpensive aqueous system and very low additive amounts.

1. Introduction

In addition to conventional compression molding and film casting, electrospinning is a novel, very convenient and useful avenue for engineering new material structures, including production of a variety of nano/micro scale fibers [1–7]. Typical experiments require only gram quantities of raw materials and, in the basic process, a jet of a polymer solution/suspension is accelerated through an electric field gradient towards a grounded collector where it undergoes a stretching and whipping process, leading to the elongation of the thread. After solvent evaporation, a nonwoven mat of either micrometer- or nanometer-sized fibers is thus produced.

Polymer electrospinning is affected by both system (molecular weight (M_n), molecular weight distribution (PDI), viscosity, concentration, conductivity and surface tension) and process (flow rate, applied voltage, air gap distance, temperature and humidity) parameters. Among these variables, the concentration and viscosity of the polymer solution, applied voltage, air gap distance and delivery rate are critical in determining the fiber shape and size [8,9]. For synthetic polymers, semi-empirical models have been developed to predict fiber morphology (beads, beaded or uniform fibers) with knowledge of solution concentration, and entanglement molecular weight [10,11]. Fiber diameters were also predictable when a good solvent was employed, no intermolecular interactions were involved and only viscosity and concentration were considered as variables [8,12,13].

Many biopolymers, such as zein [14–16], dextran [17], wheat gluten (WG) [18,19], collagen [20], chitosan [21] and gelatin [22] have potential uses as tissue engineering scaffolds, wound dressings, drug delivery, medical implants and others. The very abundant WG is especially competitive as a potential substitute for conventional plastics due to its unique ability to form cohesive blends with viscoelastic properties [23–28]. WG is mainly composed of a low M_n gliadin fraction and a high M_n glutenin fraction [23,29]. Gliadins have predominantly intra-molecular disulfide linkages and are readily soluble, while glutenins have both inter- and intra-molecular disulfide linkages and are almost insoluble in aqueous alcohols [30–33]. Thus, disulphide bonds play a key role in determining the structure and properties of WG proteins as exemplified by the well-known effects of redox agents on the rheological properties of dough and gluten [34,35].

Since water/1-propanol (1/1 v/v) mixtures are known to solubilize wheat proteins [36,37], we have tested them as WG electrospinning solvents and obtained flat, ribbon-like fibers with diameters less than 1 μm and with limited bead formation [38]. Although WG electrospinning can also be performed from

* Corresponding author. University of Connecticut, Department of Chemical, Materials and Biomolecular Engineering, Storrs, CT 06269, USA. Tel.: +1 860 486 9060; fax: +1 860 486 4745.
E-mail address: rparnas@ims.uconn.edu (R.S. Parnas).

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1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) [18], this solvent is corrosive and expensive, and its toxic traces in final electrospun products may limit potential biomedical applications of WG fibers. Therefore, using environmentally friendly aqueous solvents is an important issue for WG electrospinning. While commercial WG is not suitable for direct electrospinning from aqueous mixtures as only the gliadin fraction dissolves, glutenin subunits obtained by reducing the disulfide bonds, show a similar solubility in aqueous alcohols as gliadin [31] and thus, aqueous WG solubility can be improved by reducing the wheat glutenin fraction.

Thiolated derivatives such as dithiothreitol (DTT) are effective protein reducing agents and the effect of DTT on wheat flour rheology is widely thought to involve the thiol/disulfide interchange reaction [39–42]. We have recently shown that thiolated poly(vinyl alcohol) (TPVA) synthesized by the esterification of poly (vinyl alcohol) (PVA) with 3-mercaptopropionic acid, also behaves as a WG reactive bulk modifier [43,44], and have reported the mechanical and rheological properties [43] of WG blends with PVA and TPVA, as well as related imaging and thermal studies [44].

In this study, the electrospinning of WG with thiolated additives from a water/1-propanol mixture was investigated. Small amounts of TPVA or DTT were added to WG aqueous solutions to improve the WG solubility. PVA was also used for comparison purposes. In addition, chain entanglements and an increased number of intermolecular disulfide bridges *via* the thiol/disulfide interchange reaction are believed to control the WG spinnability because the average molecular weight of WG was increased as well. Thus, the effects of additives on WG solution rheology, fiber formation and morphology were also investigated. Due to the rather complex and heterogeneous nature of commercial WG, traditional characterization methods, such as NMR and FTIR, are not easily applicable. Thus, the formation of new disulfide bonds between TPVA and WG was inferred by 3-point bending mechanical tests, SE-HPLC, rheology and morphology results from our earlier studies [43,44].

2. Experimental

2.1. Materials

American vital wheat gluten (WG) from Arrowhead Mills, Hereford, TX USA, dithiothreitol (DTT) and 3-mercaptopropionic acid from Sigma–Aldrich, poly(vinyl alcohol) (PVA) ($M_w = 30{,}000$–$70{,}000$, PDI = 1.8) and 1-propanol from Acros Organics, all were used as-received. Thiolated poly(vinyl alcohol) (TPVA) was synthesized by the esterification of PVA with 3-mercaptopropionic acid in the presence of HCl at 80 °C. ^{1}H NMR was employed to determine the thiolation level (TL) of the OH groups in PVA with the 3-mercaptopropionate, as previously reported [43]. All experiments that used TPVA with the same TL used TPVA from the same batch.

2.2. Techniques

2.2.1. Preparation of WG solutions

2 g (Series I) or 3 g (Series II) of WG was dispersed in 20 mL of a 1:1 (v/v) mixture of distilled water and 1-propanol at 25 °C (Table 1). Since 3.0 g of WG is about the maximum amount that can be dispersed in 20 mL of aqueous solvent before gelation occurs, higher concentration samples were not prepared. TPVA or DTT were added to the WG suspension at different ratios, relating the amount of thiols in DTT or TPVA to those in WG. Control samples were formulated by adding PVA to the suspensions in amounts equal to TPVA. Pure PVA and TPVA solutions were prepared as well. The WG mixtures were stirred overnight, and then centrifuged at 5000*g* for 15 min ($T = 25$ °C). The supernatant layer was separated and used for rheology and electrospinning studies. The insoluble fraction was dried and its weight compared with the initial weight of the added gluten and additives to determine the solubility of WG and the total concentration of each blend, assuming that only the insoluble WG

Table 1
Characterization of the electrospinning solutions.

#	Additive	Additive/WG thiol ratio[a]	Wt% additive[b]	Solubility of WG (g/dL) (±0.2)	Wt% WG dissolved	Total Conc. (g/dL) (± 0.2)	Aver. fiber diameter (nm)
Series I							
1	None	0	0	6.8	55.2 ± 2.9	6.8	189 ± 9
2	PVA	–	100	0	–	6.8	36 ± 2
3	TPVA[c]	–	100	0	–	6.8	66 ± 3
4	PVA	1:5	9.06	6.9	54.2 ± 0.9	7.7	224 ± 9
5	PVA	1:10	4.66	7.2	55.3 ± 1.5	7.6	185 ± 4
6	DTT	1:1	3.03	8.2	73.6 ± 3.9	8.4	–
7	DTT	1:2	1.51	8.3	74.9 ± 2.6	8.5	–
8	DTT	1:5	0.63	8.7	79.4 ± 3.4	8.8	–
9	DTT	1:10	0.28	9.4	88.1 ± 2.3	9.5	178 ± 8
10	TPVA[d]	1:5	6.90	8.4	72.9 ± 3.9	9.0	101 ± 3
11	TPVA[d]	1:10	3.52	8.4	73.9 ± 0.9	8.7	91 ± 4
12	TPVA[e]	1:5	11.35	8.5	75.8 ± 2.6	9.6	175 ± 6
13	TPVA[e]	1:10	6.00	8.5	76.0 ± 3.0	9.0	424 ± 19
14	TPVA[e]	1:18	3.60	8.3	72.4 ± 1.0	8.6	269 ± 6
Series II							
15	None	0	0	10.8	54.2 ± 2.0	10.8	647 ± 28
16	PVA	–	100	0	–	10.8	76 ± 3
17	TPVA[c]	–	100	0	–	10.8	392 ± 17
18	DTT	1:10	0.29	13.3	81.3 ± 1.5	13.3	802 ± 32
19	PVA	1:10	4.72	10.7	54.5 ± 2.5	11.2	1225 ± 25
20	TPVA[d]	1:10	3.27	13.2	79.8 ± 1.8	13.6	791 ± 29
21	TPVA[e]	1:10	6.06	12.9	72.8 ± 1.0	13.8	905 ± 21

[a] 1:1, 1:2, 1:5 and 1:10 refer to the mole ratio of the SH bonds in DTT or TPVA to those in WG; As there is no thiol in PVA, these samples (# 4, 5, 19) were prepared to match the corresponding TPVA/WG blends with the same WG wt fraction.
[b] Weight fraction of additives in the final, soluble WG mixture, assuming no additive precipitation.
[c] TL (thiolation level) = 4%.
[d] TL = 5%.
[e] TL = 2.8%.

fractions precipitated. Since the wt fraction of additives is very small (<5%) and they are soluble in water/1-propanol, the calculation is representative for the WG solubility even though the precipitated part could conceivably contain some additive residues. The solubility from at least three experiments is presented as the average ± standard error in Table 1.

2.2.2. Rheological measurements

The viscosities of WG, polymeric additives, and WG/additive solutions were measured using an AR-G2 Rheometer (TA Instruments Inc) with Couette fixtures at 25 °C. The bob and cup radii employed for rheological measurements were 14 and 15 mm, respectively. The viscosities were measured as shear rate swept from 1 to 1000 s^{-1}. At least three replicates were prepared of each solution.

2.2.3. Size-exclusion HPLC of DTT/WG solutions [23]

All DTT/WG solutions from Series I were diluted with the water/1-propanol to the same concentration as that of pure WG solution (6.8 g/dL). To prepare a 1 mg protein/1 mL buffer mixture, 14.7 μL of the diluted DTT/WG solution was added to 1 mL of 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% (w/v) SDS. The mixture was stirred for 1 h at room temperature and centrifuged (10 min, 10,000*g*). Supernatants were filtered (0.45 μm) and loaded (20 μL) on a Phenomenex BioSep-SEC-S4000 (300 × 7.8 mm) column (Phenomenex, Torrance, CA). The proteins were eluted at room temperature with 50.0% (v/v) acetonitrile containing 0.05% (v/v) trifluoroacetic acid (flow rate, 0.5 mL/min). The detection was performed with a Milton Roy SpectroMonitor 3100 detector at 210 nm. All SE-HPLC analyses were performed in triplicate.

2.2.4. Electrospinning

WG and WG/additive solutions were forced through a 1.0 mL syringe using a syringe pump (model 100, KD Scientific Inc. New Hope, PA), and a high voltage (20 kV) applied between the tip (20-gauge blunt needle) and a grounded collection target. The syringe pump was set to deliver the solution at 0.5 mL/h, while the distance between the tip and collector was 10 cm.

2.2.5. Microscopy

Electrospun fibers were sputter-coated with Au/Pd, and the fiber morphology was examined with a JEOL 6335F field emission scanning electron microscope (FESEM) at an accelerating voltage of 5.0 kV. The average diameter of electrospun fibers was determined by measuring it at 50 different points in the SEM images (×5000 magnification) using ImageJ software. The diameters are represented as the average ± standard error.

3. Results and discussion

3.1. Solubility of WG in water/1-propanol mixture with and without additives

Table 1 presents the solubility of WG, the percentage of WG dissolved in the solvent and the total concentration of solutions as prepared. To ensure reproducibility, at least 3 replicates of each solution were prepared and tested for the solubility, solution concentration, viscosity, and fiber spinning reported below. The amount of additive is provided in terms of both wt fraction as well as thiol groups mole ratio in the additive *vs.* WG (*i.e.* DTT/WG = 1:2 indicates a solution where WG has twice the amount of SH groups *vs.* DTT). TPVA/WG solutions were prepared only with ratios up to 1:5, since the 1:2 and 1:1 were too viscous to electrospin and quickly formed gels.

Commercial dry WG usually contains 75–80% protein, 10–15% starch and non-starch polysaccharides, 5–8% moisture, about 5% lipid and <1% minerals [18,29]. In addition, according to their solubility in alcohol–water solvents, gluten proteins consist of roughly equal fractions of gliadins and glutenins [45]. Thus, since the majority of starch is water-soluble we expect that both gliadin and starch fractions will dissolve [46,47], leading to an expected solubilized WG percentage in the range of 52–63%. This is verified by the experimental data for pure WG solutions from both Series I and Series II (55.2% and respectively 54.2%) and indicates that most starch and non-starch polysaccharides dissolved.

While PVA does not affect WG solubility by more than 1%, (within experimental error), DTT increases WG solubility by up to 38%, and TPVA by up to 25%. This indicates that only TPVA and DTT interact with WG proteins *via* the thiol groups to release soluble fractions from glutenins and thus increase overall WG solubility. This is consistent with previous findings that DTT reduces wheat protein and increase its solubility [38–41], and with our previous TPVA/WG SE-HPLC data [43].

Interestingly, WG solubility increases with decreasing the DTT amount. As more DTT is added, more disulfide bonds are reduced, leading to the production of smaller protein fractions which should be more soluble. However, exposure of such proteins with a tightly packed hydrophobic amino acid core to a reducing environment (DTT/TPVA), leads to the breaking of the disulfide bonds which together with protein unfolding under continuous shear stress (overnight stirring) probably exposes more hydrophobic groups to the aqueous solution [48] thus lower WG solubility.

This hypothesis was confirmed by both visual observations and SE-HPLC results (Fig. 1). Thus, during the preparation of DTT/WG solutions, it was found that the amount of insoluble fractions after centrifugation increased with increasing the amount of DTT. Fig. 1 presents the SE-HPLC profiles of WG and DTT/WG solutions (all from Series I, at 6.8 g/dL), where increasing time on the *x*-axis corresponds to lower protein molecular weight. As shown in Fig. 1, the high molecular weight peak at ~11 min significantly diminishes and lower molecular weight fractions appear in solution as more DTT is added to WG. Thus, while for the 1:10 and 1:5 ratios, a small amount of higher molecular weight fractions were observed, the 1:2 and 1:1 DTT/WG solutions consist mostly of low molecular weight protein subunits.

Although desired for comparison purposes, it is not possible to concurrently match both thiol levels and additive amounts when the thiolation level of TPVA is changed. Thus, the 1:10 ratio is comparable for PVA/WG (#5), DTT/WG (#9) and TPVA/WG (#11 (TL = 5%) and #13 (TL = 2.8%)). However, trying to obtain a similar wt fraction of the additive using a lower thiolation level, leads to larger thiol ratio (#14, is 1/18 for ~3.6%). Nonetheless, in the TPVA/

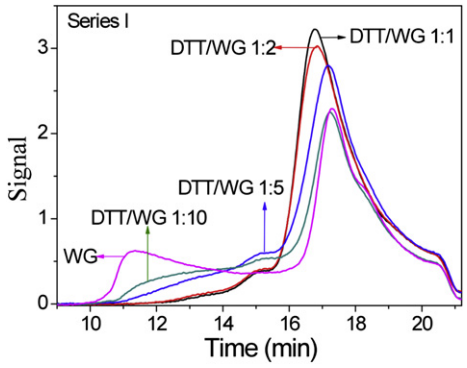

Fig. 1. SE-HPLC chromatograms of diluted DTT/WG solutions (6.8 g/dL) (Series I).

WG Series I, neither the thiolation level (TL) nor the thiol ratio significantly affected WG solubility. This weak dependence may indicate that TPVA is not as strong a reducing agent as DTT. However, the hydrophilic nature of TPVA, and its interactions with WG *via* thiol/disulfide interchange reactions complicate the interpretation of the solubility data.

Conversely, for both Series I and II, all WG/additive solutions have higher concentrations than pure WG, due to the combined effect of more glutenin fractions being dissolved after disulfide reduction and to contribution of polymer additives [38–40,41,43]. Since more WG is dispersed, the concentrations in Series II are higher than those of corresponding solutions from Series I. Thus, concentration can be manipulated by adjusting WG solubility (*e.g.* adding denaturizing agents) or the amount of WG added, and further optimized for electrospinning.

3.2. Rheology of WG aqueous solutions

Solution viscosity is a critical factor affecting its spinnability and fiber morphology [10]. The dependence of the solution viscosity on shear rate is presented in Fig. 2 for all samples and can be explained by the combined effect of the molecular weight, weight fraction and additive functionality (*e.g.* SH groups). Two types of rheological behavior are clearly emerging.

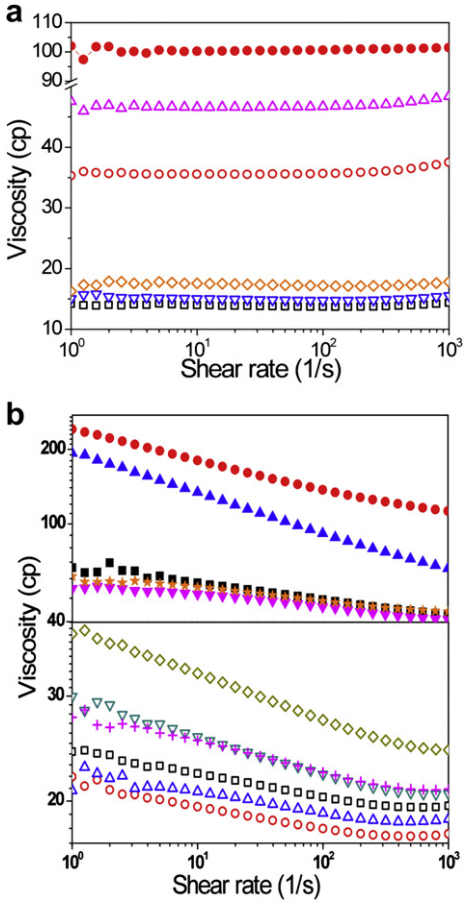

Fig. 2. Dependence of the solution viscosity on shear rate: (a) Newtonian fluids: (●) PVA (#16), (△) TPVA (#3), (○) PVA (#2), (◇) PVA/WG = 1:5 (#4), (▽) PVA/WG = 1:10 (#5), (□) WG (#1). (b) Non-Newtonian fluids: (●) TPVA/WG = 1:10 (#21), (▲) TPVA/WG = 1:10 (#20), (■) DTT/WG = 1:10 (#18), (★) PVA/WG = 1:10 (#19), (▼) WG (#15), (◇) TPVA/WG = 1:5 (#12), (▽) TPVA/WG = 1:5 (#10), (+) TPVA/WG = 1:10 (#13), (□) DTT/WG = 1:10 (#9), (△) TPVA/WG = 1:10 (#11), (●) DTT/WG = 1:5 (#8).

First, pure systems (WG, PVA, TPVA) and all PVA/WG mixtures at low concentrations (Series I) provide a Newtonian, shear independent viscosity profile (Fig. 2a). Thus, upon adding PVA to WG, (1:5 and 1:10) the viscosity not only remains Newtonian but also changes very little (~15 cP). The higher viscosity of PVA and TPVA (35 and 45 cP) is simply an expression of their better solubility and higher molecular weight (M_n ~ 50 K) than that of the gliadin WG soluble fractions. TPVA provides larger viscosity than PVA due to the additional formation of intermolecular disulfide bonds [49]. Thus, while a higher concentration PVA (10.8 g/dL) has the largest Newtonian viscosity in this set, a similar TPVA solution could not even be studied due to its fast gelation (<30 min). Moreover, due to formation of intermolecular disulfide bonds, TPVA viscosity is consistently higher than that of PVA, although both solutions have the concentration and molecular weight. This indicates that even a small amount of –SH groups introduced to the PVA chain can significantly change its rheology.

Second and by contrast, all DTT/WG and TPVA/WG samples, irrespective of concentration, as well as the higher concentration WG and PVA/WG display a non-Newtonian, shear-thinning behavior in the 1–1000 s^{-1} range (Fig. 2b). As expected from higher concentrations, the viscosities of Series II samples (filled symbols in Fig. 2b) are consistently greater than those of Series I (open symbols). Moreover, within a given blend series (*e.g.* TPVA/WG, Series II), viscosity increases with increasing additive weight fraction. These viscosity trends are the combined result of the additive weight fraction and its effect on the molecular weight distribution of the soluble WG.

Interestingly, DTT/WG = 1:10 (#18, 13.3 g/dL), PVA/WG = 1:10 (#19, 11.2 g/dL) and WG (#15, 10.8 g/dL), have similar viscosity values. As PVA does not change the molecular weight distribution of WG, and the PVA/WG and WG solutions are of very similar concentrations, the similarity in viscosity is expected. In the case of DTT, lower molecular weight glutenin subunits are released in solution and the viscosity should decrease. This is however compensated by the higher DTT/WG solution concentration to generate a viscosity close to that of WG.

For Series I, a similar trend is observed, and the additive molecular weight effect becomes apparent. Thus, using TPVA and DTT at the same thiol ratio (1:5 and 1:10), a higher viscosity is obtained for TPVA, due to its higher molecular weight and its ability to act as a macromolecular branching agent for soluble gliadins and reduced glutenin fractions.

Pure WG solutions are similar to gliadin since all insoluble materials including glutenins were removed by centrifugation. As gliadin and glutenin rheology depends strongly on concentration [50,51], the shear-thinning behavior of DTT/WG and TPVA/WG solutions is consistent with the enhanced contribution of solubilized glutenin fractions [51,52] and with possible shear induced structural rearrangements (larger aggregates broken and/or oriented by shear) [53]. In addition, since the DTT/WG systems also exhibit a time dependent viscosity [54–56] we expect a similar behavior for the TPVA/WG blends.

3.3. Electrospinning of WG and WG/additive aqueous solutions

As TPVA/WG 1:10 (TL = 2.8%) mixtures from Series I and II have time dependent viscosities which are also the largest in each group, they were selected for evaluating the electrospinning solution stability. As seen in Fig. 3, while the average fiber diameter (d_{aver}) increases very slightly with time, it is relatively constant, especially in the first 2 h. Therefore, to minimize potential effects of time dependent viscosity in DTT/WG and TPVA/WG solutions, all other electrospinning experiments were carried out within 2 h of sample preparation.

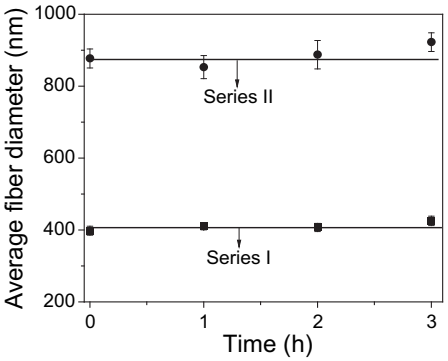

Fig. 3. Dependence of the diameter of fibers electrospun within 5 min, 1h, 2h and 3 h of solution preparation (TPVA/WG = 1:10 TL = 2.8%, exp. #13 and #21).

Pure WG, PVA and TPVA Series I solutions (6.8 g/dL) were successfully electrospun (Fig. 4). WG provided fibers with $d_{aver} \sim 190$ nm, although with bead-on-string morphology. While big beads (~1.5 μm) and thinner fibers ($d_{aver} = 36$ nm) were obtained for PVA, as a consequence of higher TPVA viscosity due to −SH interactions, TPVA ($M_w = 50$ k TL = 4%) displayed the best overall spinnability ($d_{aver} \sim 66$ nm).

FESEM images of PVA/WG and DTT/WG fibers are shown in Figs. 5 and 6. As a result of chain entanglements and H-bonding between PVA and WG [18,19], both PVA/WG = 1:10 and 1:5 solutions were electrospun into continuous fibers with $d_{aver} \sim 185$ nm and respectively 225 nm (Fig. 5). By contrast, the spinnability of DTT/WG solutions although more concentrated than pure WG, decreases greatly with increasing DTT. This is in line with their different molecular weight distributions (Fig. 1) and indicates that due to lower molecular weight subunits as well as fewer intermolecular disulfide bonds, there just are not enough chain entanglements in DTT/WG solutions. Consequently, the lowest DTT content (DTT/WG = 1:10) barely affords beaded fibers, ($d_{aver} \sim 180$ nm), while very limited fiber formation occurs from DTT/WG = 1:5 and none from 1:2 and 1:1.

Fig. 7 presents the morphology of TPVA/WG fibers electrospun from Series I. Although both DTT and TPVA increase the solubility of WG and viscosity of WG solutions, they had different effects on fiber formation and morphology. In addition, for TPVA/WG, the TL was also found to significantly affect fiber formation. Thus, the quality of TPVA/WG fibers increases with decreasing both TL from 5% to 2.8% and TPVA amount down to TPVA/WG = 1/10 and 1/18, which provides smoother fibers ($d_{aver} \sim 430$ nm and respectively ~270 nm) with limited bead formation, whereas a bead-on-string morphology occurs in all other cases. This is consistent with rheological results and indicates that rearrangement of disulfide bonds in TPVA/WG solutions influences fiber morphology. The different effects of DTT and TPVA on fiber aspect also correlate with their different chemical structures. Thus, similarly to the PVA/WG case, TPVA also contributes to fiber formation by increased chain entanglements and hydrogen bonding.

In order to further understand the effects of chain entanglement on fiber formation, the best spinnability solutions of each additive from Series I (#5, #9, and #13, PVA/WG = DTT/WG = TPVA (TL = 2.8%)/WG = 1:10) were diluted to the same concentration as pure WG (6.8 g/dL) and were electrospun. As seen in Fig. 8, by comparison with parent solutions, spinnability decreases in all cases, and especially for DTT/WG and TPVA/WG, which being composed of mainly lower M_n protein subunits, cannot provide sufficient chain overlap at low concentrations. Thus, although TPVA and DTT increase WG solubility, they also change WG molecular weight distribution by reducing the WG disulfide bonds, and the amount of chain entanglements in the solution, which eventually affects fiber formation.

Since increasing WG solubility/concentration increases solution viscosity, which should facilitate formation of smooth fibers [57], the effect of higher concentrations was further explored with Series II solutions (Fig. 9) where indeed, smooth and uniform fibers with greatly reduced bead formation were obtained, albeit at the expense of increasing fiber diameter. As observed in Series II, the diameter of PVA/WG fibers (1225 ± 25 nm) is larger than that of TPVA/WG fibers (905 ± 21 and 791 ± 29 nm), which indicates the different behavior of PVA and TPVA with respect to WG. Thus, PVA interacts with WG only by physical means (H-bonding, etc) while TPVA provides the additional chemical bonding of the disulfide linkages. Interestingly, it appears that additives do not affect fiber quality at higher concentrations (Series II) as much as they do at lower concentrations (Series I). This indicates that both solution concentration and the interactions between additives and WG determine fiber morphology, and that each factor may predominate within a certain concentration range.

While up to 26 wt% PVA ($M_w = 85$ k–124 k) was required to blend with WG in HFIP for electrospinning, the resulting fibers were rather large (~10 μm in diameter) [18,19]. By contrast, WG aqueous solutions with only 4.7 wt% PVA ($M_w = 50$ k) noted above can be electrospun into fibers with d_{aver} of only 1.3 μm. Thus, WG fibers electrospun from water/1-propanol have a similar appearance to, but a narrower size distribution than those obtained from HFIP (Table 2), although the electrospinning parameters (concentration, applied voltage and delivery rate) were not identical. The

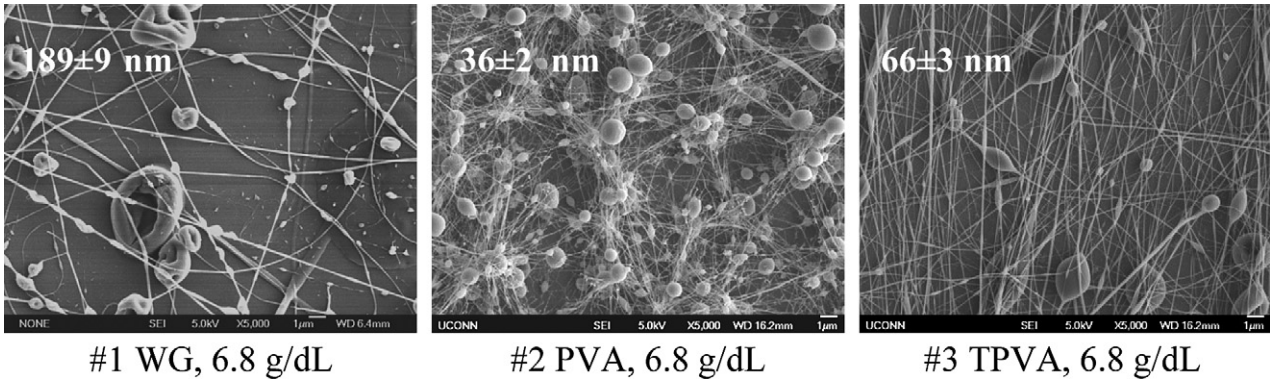

Fig. 4. FESEM images of WG, PVA and TPVA fibers electrospun from Series I.

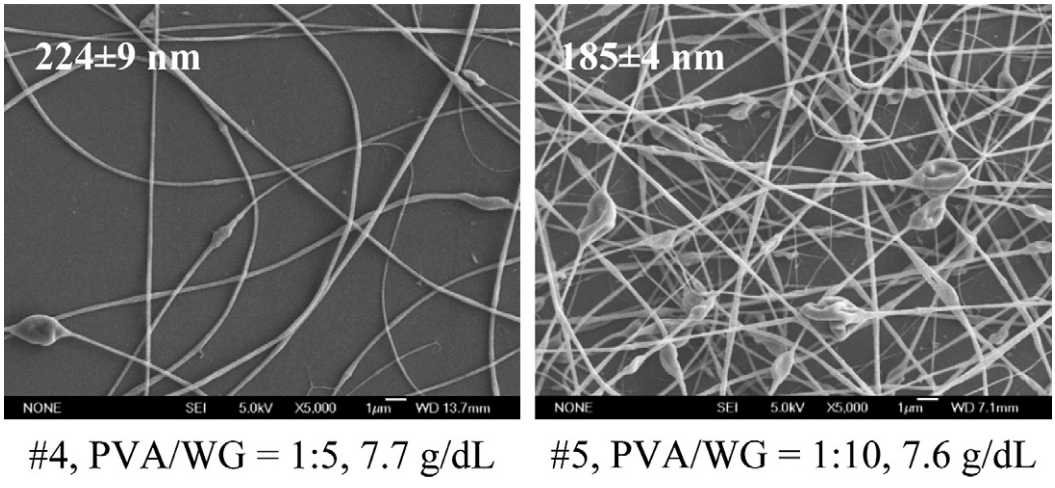

Fig. 5. FESEM images of PVA/WG fibers electrospun from Series I.

size distribution is characterized in Table 2 as the relative standard deviation of 50 fiber diameter measurements taken from the images presented in Figs. 4–9 and from images presented in [18,19]. Most likely, the WG molecular weight distribution in water/1-propanol is responsible for the diameter difference since the high molecular weight WG fraction is removed before electrospinning. These results indicate that spinnability of WG aqueous solutions is more than acceptable despite the removal of high molecular weight glutenins from the suspensions. Moreover, a protein reducing agent (DTT and TPVA) increases the solubility of WG in aqueous mixtures and contributes to fiber formation *via* the thiol/disulfide exchange reactions.

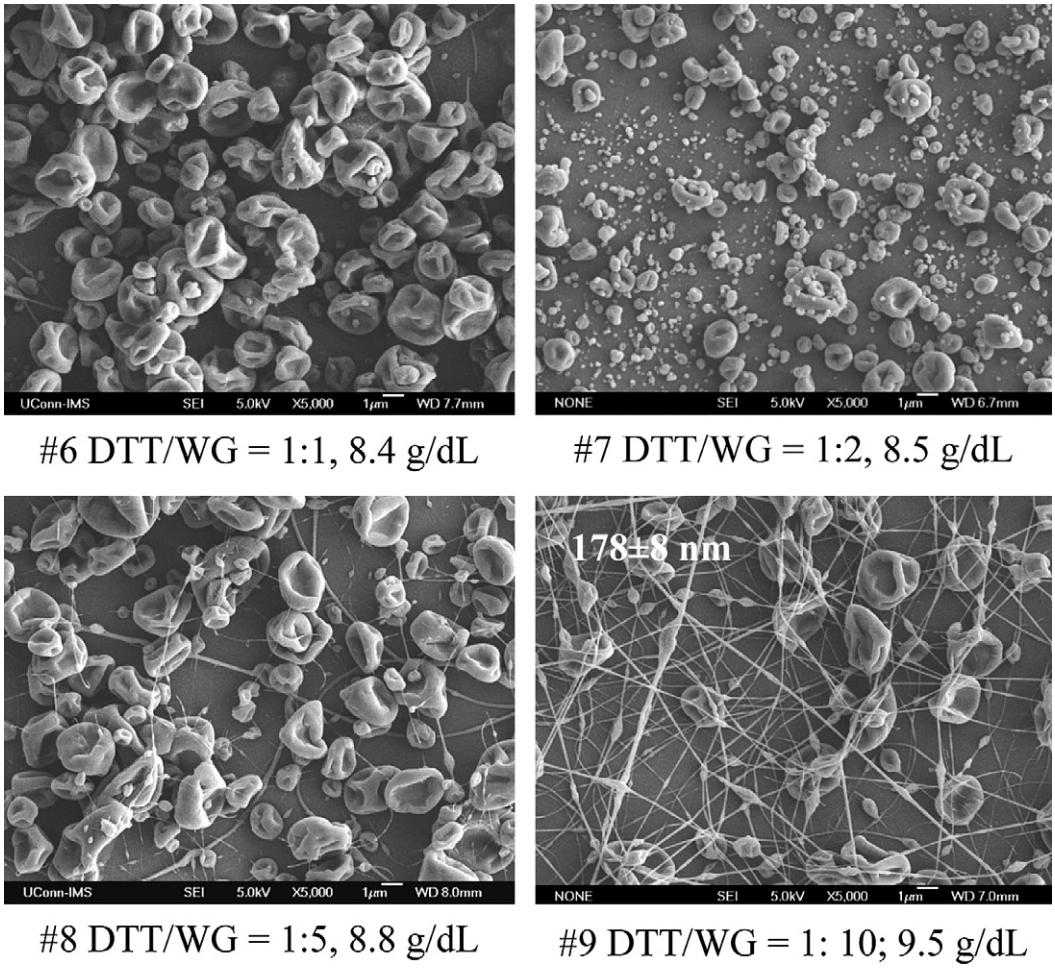

Fig. 6. FESEM images of DTT/WG fibers electrospun from Series I.

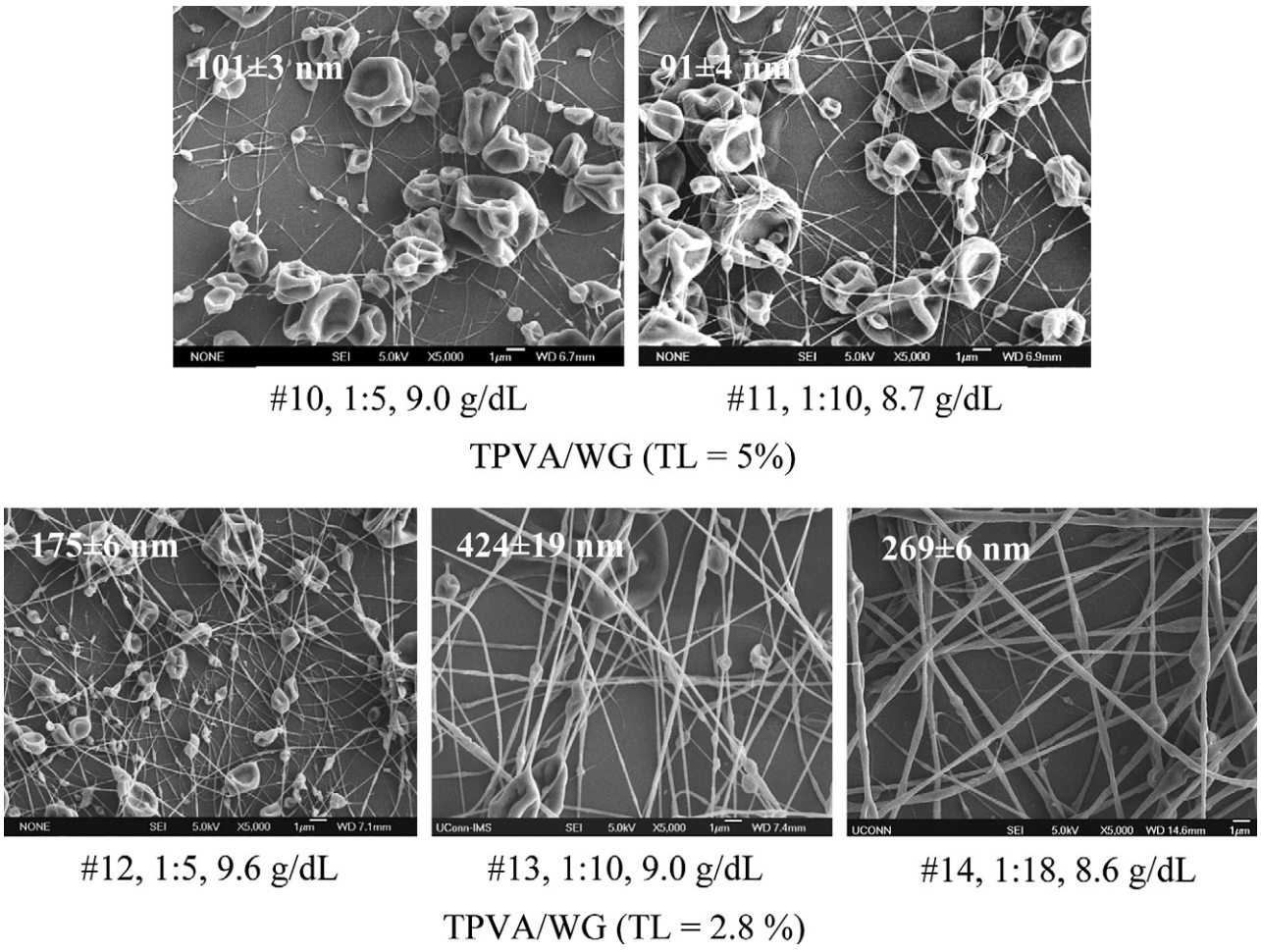

Fig. 7. FESEM images of TPVA/WG fibers electrospun from Series I.

Unlike synthetic polymers, commercial WG is a highly complex and heterogeneous protein mixture. Besides electrospinning system parameters (including polymer and solution properties), the presence of reversible junctions through thiol/disulfide interchange reactions seems to play a very important role in determining fiber formation. Thus, the relationship between solution rheology and fiber diameter and morphology is rather complicated and current available experimental and semi-empirical models developed based on synthetic polymers [6,11] may not be directly applicable to WG. Here, concentration, chain entanglements as well as the amount of disulfide bonds determine fiber quality. Further investigations on different formulations and the possible chemical reactions between additives and wheat protein will help to clarify the relationship between WG solution properties and electrospun fiber formation and characteristics.

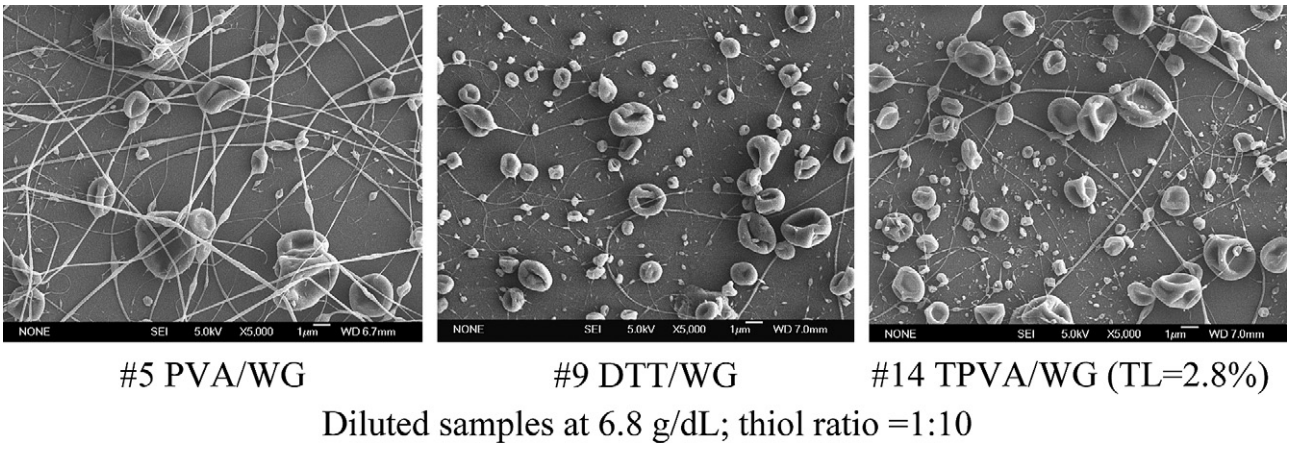

Fig. 8. FESEM images of fibers electrospun from diluted (6.8 g/dL) PVA/WG (#5), DTT/WG (#9) and TPVA/WG (#14) solutions from Series I.

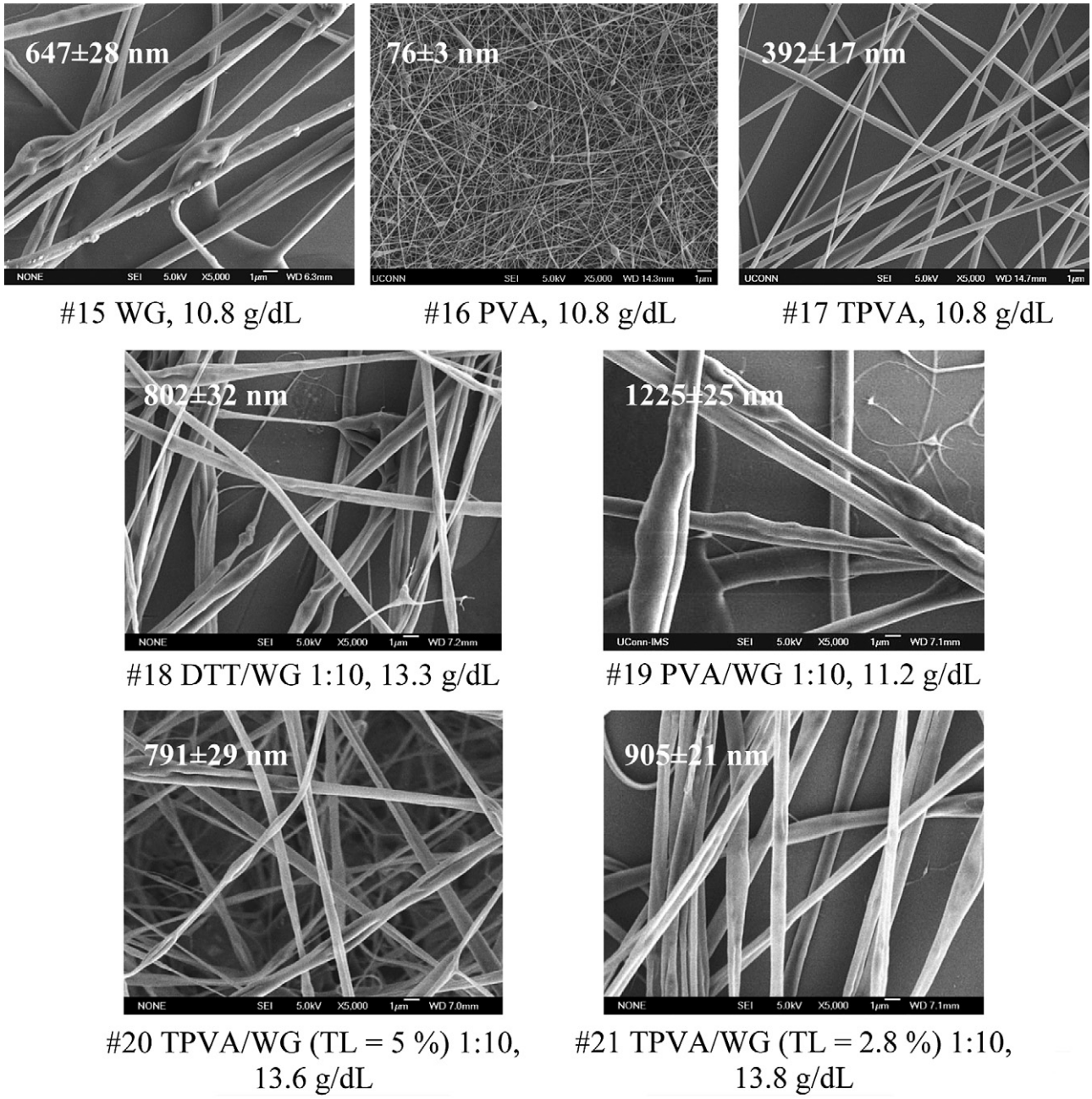

Fig. 9. FESEM images of fibers electrospun from Series II solutions.

Table 2
Comparison of WG Electrospinning from HFIP and Aqueous Solutions.

#	Sample	Conc. (g/dL)	Size distribution (μm)	
			HFIP	Water/1-propanol (1/1 v/v)
1	WG[a],[18]	5.0	0.41 ± 61%	–
2	WG[b],[18]	10	0.82 ± 45%	–
3	WG (#1)	6.8	–	0.19 ± 34%
4	WG (#15)	10.8	–	0.65 ± 31%
5	PVA (26 wt%)/WG [19]	5.0	9.37 ± 23%	–
6	PVA/WG (#19)	11.2	–	1.23 ± 14%
7	TPVA/WG(#20)	13.6	–	0.79 ± 26%
8	TPVA/WG(#21)	13.8	–	0.91 ± 16%

[a] WG specimen A from Ref. [18], fibers from as-received commercial WG.
[b] WG specimen C from Ref. [18], fibers from the 0.05 M acetic acid-extractable fraction of commercial WG.

4. Conclusions

The rheology and electrospinning behavior of a series of water/1-propanol (1/1 v/v) solution mixtures of WG with additives (DTT, PVA and TPVA) were investigated by SE-HPLC, steady-shear viscosity measurements and FESEM. DTT and TPVA increased the WG solubility by reducing WG disulfide bonds. The solution rheology was also changed where a Newtonian behavior is observed for each pure component and for the chemically non-interacting mixtures (PVA/WG), whereas a non-Newtonian, shear-thinning profile is displayed by all DTT/WG and TPVA/WG solutions.

As expected, concentration and viscosity affect the electrospinnability of WG mixtures. In addition, the reactivity of the additive also affects the electrospinnability of WG mixtures. PVA

does not reduce WG, and acts more like a macromolecular physical crosslinker *via* H-bonding to give good fibers. But PVA/WG fibers are twice as thick as WG at higher concentrations (~11 g/dL) while both DTT and TPVA provide thinner fibers and very limited bead formation. By contrast, DTT and TPVA provide poor WG fibers at low concentrations (~9 g/dL) by reducing the WG molecular weight below the level required to sustain spinnability. However, lowering the TPVA thiolation level results in better fibers, unobtainable with DTT, indicating TPVA combines the advantages of PVA (macromolecular nature and ability to promote spinnability *via* H-bonding with WG) with those of DTT (reducing the disulfide bonds).

Thus, even though the water/1-propanol mixtures contain only the lower end of the WG molecular weight distribution compared with the corresponding HIFP solutions, comparably good fibers can nonetheless be obtained. The advantages of this green, environmentally friendly system make it thus preferable to its expensive and toxic alternative.

The effects of the molecular weight and MWD of the macromolecular additives, PVA and TPVA, were not investigated in this work, but may be quite important. For example, low molecular weight TPVA may be more effective at reducing disulfide bonds in the protein due to increased ability to diffuse into protein aggregates, but higher molecular weight polymer may provide better entanglement properties to support superior spinning.

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