Electrospun Zein Fibers Using Glyoxal as the Crosslinking Reagent

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ABSTRACT: After reaction of zein with glyoxal the resulting electrospun fibers have improved resistance to known zein solvents. Durable fibers with diameters of 0.6 μm could be produced. The reaction between zein and glyoxal was carried out in acetic acid (AcOH) at temperatures between 25 and 60°C at various lengths of time. Gelation would occur after higher extents of reaction. During the course of reaction, solution viscosity increased which increased the diameter of the electrospun fibers produced from these solutions. Gel electrophoresis showed increased molecular weight as the reaction progressed. When 6% glyoxal was allowed to react with zein at 25°C for 6 h, the resulting fibers were durable to AcOH as spun. Other formulations required a second thermal treatment to provide solvent durable fabrics. Fibers displayed different secondary structure utilizing far-UV circular dichroism spectroscopy. The infrared spectra displayed peaks in the C—O region supporting the reaction of glyoxal with hydroxyl groups present on zein. Zein fabrics incorporating glyoxal had modestly improved tensile strength. © 2011 Wiley Periodicals, Inc.[†] J Appl Polym Sci 123: 2651–2661, 2012

Key words: zein; glyoxal; electrospinning; fiber; crosslinking

INTRODUCTION

Ethanol (EtOH) production from corn is one of the largest industrial processes using renewable materials. Both dry- and wet-milling processes can be used to produce EtOH.[1] In both of these processes, one of the main coproducts produced contains a large amount of protein. The main protein in these coproducts is zein. By developing higher valued products using zein, the economics of these industries will improve. One of the leading historic uses of zein was as fibers in the textile market.[2] Textile fibers are put through many different treatments to be transformed into a garment. These treatments can be physically demanding as the fabric is pulled through various machines during production. In addition, the fibers need to be durable to the various chemicals and solvents used during dyeing and finishing. Because of these requirements, the fibers were crosslinked with formaldehyde.[2,3] Through crosslinking, the fibers had

improved tensile strength and solvent resistance as well as reduced tendency to degrade due to downstream fabric treatments (such as dyeing).[3,4] To crosslink the zein, the fiber was drawn through a number of baths containing formaldehyde and various salts.[5] Significant investment is needed by the industry to safely perform chemistry using formaldehyde. Because of this requirement, other techniques have been explored to provide a process requiring lower investment and lower production costs. The techniques have been developed to improve the properties of zein-based materials where the chemistry is performed before the formation of solution cast films or molded samples.[6–12] Wu et al. have used polycaprolactone diol/hexamethylene diisocyanate prepolymers to modify zein.[6,7] Although some improvements were obtained in physical properties and resistance to water, the amount of diisocyanate required was between 20 and 50%. 1-[3-Dimethylaminopropyl]-3-ethyl-carbodiimide hydrochloride has been used to modify zein and deliver improved tensile strength.[8] However, solvent resistance was not determined for the solution cast films using this approach. Glutaraldehyde (GDA),[9,10] glyoxal (GLY),[11,12] and cinnamaldehyde[9] have been used to modify zein and provide improved tensile properties of cast films and molded articles. In this work, the solvent resistance of zein GDA or cinnamaldehyde articles was not determined.[9,10] GLY-modified zein was shown to provide solution cast films and compression-molded articles that were resistant to acetic acid (AcOH, a good solvent for zein).[11,12] The solvent resistance was

attributed to increased molecular weight through branching and crosslinking reactions.

Although films and molded articles have value, the re-introduction of zein into the fiber market would be beneficial. Electrospinning[13] is a useful technique to produce lab quantities of fibers to evaluate certain properties that may support further research in producing fibers. This requires only small amounts of polymer solution. If the polymer is of a suitable molecular weight (to give chain entanglements) and has a certain minimum extensional viscosity, fibers will form and provide a nonwoven fabric. To date, this technique has been used to produce zein fibers.[14–24] In most of these studies, the zein has not been altered, and the exposure to various solvents will lead to significant property changes or even dissolution. Electrospun zein fibers have been post-treated through the use of hexamethylenediisocyanate, which could react with the reactive moieties on zein and result in altered properties.[14] However, chemical post-treatments have some limitations when compared to derivatization of the zein before spinning. There have been studies undertaken where the chemical reaction was performed before spinning.[16,21,23,24] In one study, GDA was used to crosslink the zein before spinning.[16] To determine the degree of crosslinking, the fibers were evaluated for resistance to AcOH.[16] In this work, it was found that an additional thermal treatment improved solvent resistance of the fibers. This thermal treatment further increased the molecular weight of the soluble protein. Zein-based fibers were produced that could not be dissolved by AcOH using this technology. Other studies have been carried out using citric acid as the crosslinking reagent where it was proposed that it would react with the alcohol moieties on zein to provide esters.[21,23] Although citric acid has significant advantages from a safety perspective, it was not determined in these studies, if the fibers could retain their fibrous nature on exposure to a good zein solvent. GLY has been used to modify zein before spinning to produce fibers suitable for growing fibroblast cells.[24] However, the solvent durability studies in known solvents for zein were not performed on these fibers.

There is still a need to develop additional techniques that will provide solvent durable zein fibers where the chemical modifications are performed before spinning. GLY can react with cysteine[25] (through the thiol) and argenine[26] (through the guanidine) residues as well as alcohol[27] containing compounds (present on serine and threonine) and amine[28] containing compounds (present on lysine and the amine terminus). GLY has been shown to crosslink poly(vinyl alcohol),[29] chitosan,[30] and proteins,[31] such as soy protein,[32] cotton-seed protein,[33] gliadin,[34] wheat protein,[35] and zein. α-Zein (>80% of commercial zein) has relatively few cysteines, and essentially no lysines so that their contribution to

the reaction of GLY with zein may be minimal.[36] The amine terminus will play a role, but the reaction of this group will lead to dimerization. Given that zein has many serines and threonines, then the alcohol functionalities present on these residues will play a dominant role in the chemistry of zein with GLY. Here, we report our efforts in modifying zein using GLY in AcOH under a variety of conditions. The resulting solutions were electrospun and solvent durability of these fabrics was determined as well as characterizing the fibers using a variety of techniques.

EXPERIMENTAL

Materials and equipment

Zein Lot F40004081C (14.1% nitrogen [92.8% protein dry basis] and 5% moisture) was obtained from Flo Chemical (Ashburnham, MA). Other reagents, GLY (40% w/w aqueous solution), N,N-dimethylformamide (DMF), glacial AcOH, and EtOH were obtained from Sigma-Aldrich. Solution elastic modulus (G') was determined using a TA Instruments ARES LS-1 (New Castle, DE) controlled strain rheometer using a 50-mm cone and plate geometry where the cone angle was 0.0401 radians with a gap of 0.046 mm. The electrospinning power supply was a Gamma Model ES50P-10W (Gamma High Voltage Research, Ormond Beach, FL). The syringe pump was a KDS 100 Series (KD Scientific, Holliston, MA) using 5 mL syringes with 1″ long flat-tipped 17 gauge needles. A quick connector, DR18-250-C (Panduit, Tinley Park, IL), was used to connect the hot wire from the power supply to the needle. The target was an aluminum driven roll having a 30.5 cm circumference that was covered with nonstick aluminum foil and driven by a Gear Motor (Bodine Electric Company type 24A4BBEPM-3F, Chicago, IL). The motor was controlled using a DC Motor Speed Control (Bodine Electric Company type WPM-2109EI, Chicago, IL). Scanning electron microscopy (SEM) was performed after sputter coating the fibers with Au–Pd and then examination by a JEOL 6400 V (JEOL USA, Peabody, MA). The oven used to thermally treat the samples was a Precision 170 oven (Winchester, VA). The microbalance used to measure the mass in fiber dissolution studies was a Mettler Toledo AB265-S/FACT (Columbus, OH). Fabric mat samples that would be used for evaluating physical properties were conditioned in a 50% relative humidity and temperature-controlled (23°C) room. Sample thicknesses were measured at five different locations with a micrometer (Model No. 49-63, Testing Machines Amityville, NY). Physical property testing was performed using an Instron Universal Testing Machine (Model 4021, Canton, MA) to determine the tensile strength, Young's Modulus (YM) and percent elongation at

TABLE I
Zein Formulations and Reaction Conditions*

Sample	% Solids	% GLY	Temp (°C)	Time (min)	Sample	% Solids	% GLY	Temp (°C)	Time (min)
27-0-24	27	0	24	30	23-2-45	23	2	45	30
19-0-24	19	0	24	30	19-2-45	19	2	45	30
27-0-45	27	0	45	30	27-4-24	27	4	24	30
25-0-45	25	0	45	30	19-4-24	19	4	24	30
23-0-45	23	0	45	30	27-6-24	27	6	24	30
21-0-45	21	0	45	30	19-6-24	19	6	24	30
19-0-45	19	0	45	30	27-6-24-60	27	6	24	60
27-0-60	27	0	60	30	19-6-24-60	19	6	24	60
19-0-60	19	0	60	30	27-6-24-90	27	6	24	90
27-1-24	27	1	24	30	19-6-24-90	19	6	24	90
19-1-24	19	1	24	30	27-6-24-120	27	6	24	120
27-1-45	27	1	45	30	19-6-24-120	19	6	24	120
25-1-45	25	1	45	30	27-6-24-180	27	6	24	180
23-1-45	23	1	45	30	19-6-24-180	19	6	24	180
21-1-45	21	1	45	30	27-6-24-240	27	6	24	240
19-1-45	19	1	45	30	19-6-24-240	19	6	24	240
23-1-60	23	1	60	30	27-6-24-300	27	6	24	300
19-1-60	19	1	60	30	19-6-24-300	19	6	24	300
27-2-24	27	2	24	30	27-6-24-360	27	6	24	360
19-2-24	19	2	24	30	19-6-24-360	19	6	24	360
27-2-45	27	2	45	30	19-6-24-420	19	6	24	420

* The first value represents the % solids, the second value represents the % GLY, and the third value represents the temperature. If a fourth value is present, it represents the number of minutes the reaction was allowed to be carried out (if there is no forth value, then the reaction carried out for 30 min).

break (%E). Optical microscopy using polarizing light was performed using an Olympus CX31-P microscope (Center Valley, PA) fitted with a ×10 eyepiece and ×40 strain-free objectives with a polarizing intermediate attachment. Microscope pictures were taken using an Olympus DP72 camera (Center Valley, PA). Infrared spectra were obtained on a Thermo Nicolet Avatar 370 FTIR (Thermo Scientific, Waltham, MA) spectrophotometer. Far-UV circular dichroism (CD) spectra were obtained on an AVIV Model 215 CD Spectrometer (Aviv Biomedical, Lakewood, NJ). NuPAGE Novex 10% Bis-Tris gels, and lithium dodecyl sulfate sample buffer were obtained from Invitrogen (Carlsbad, CA). Protein molecular weight standard was obtained from Lonza (Walkersville, MD).

Preparation of zein solutions

For the reaction of zein with GLY, the zein was slowly added to AcOH to give 27% solids (i.e., 7.3 g AcOH/2.7 g zein). Appropriate amounts of GLY (40% w/w) were then added to each solution so that the final concentration of GLY in the zein fiber was 0, 1, 2, 4, and 6%. The resulting % zein solids for the 1, 2, 4, and 6% GLY AcOH solutions were 26.9, 26.6, 26.0, and 25.3, respectively. These solutions were allowed to stir at 24, 45, or 60°C for the desired length of time before spinning (Table I). The reaction time was 30 min for all samples except for a series of experiments when using 6% GLY at 24°C where aliquots were removed at times between

30 and 420 min (Table I). After reaction and before spinning, additional AcOH was added to aliquots of the initial solution to determine the impact of spinning solids (25, 23, 21, and 19% prepared) on the fiber properties. The solutions were then loaded into a 5 mL syringe and electrospun at room temperature.

Preparation of electrospun zein fibers

The fibers were prepared in a fashion similar to that performed previously when using AcOH as solvent.[16] The hot wire from the power supply was attached to the quick disconnect terminal attached to the needle. The grounded target roll was driven at 1000 rpm. The distance from the target to the needle tip was set to 10 cm, and the syringe pump was set to 12 mL h^{-1}. The power supply was set to 25 kV. At times during production, the solution would dry on the tip of the needle, and this dried polymer solution would need to be wiped off using a paper towel. After processing a total of 3 mL of solution, spinning was halted, and the fabric was removed from the roll and allowed to fully dry overnight. As detailed in Table I, the samples are coded to signify the % solids at which they were spun, the percent GLY used, and the temperature at which the reaction was carried out. For select items, a fourth value is provided indicating the amount of time the reaction was allowed to proceed. For example, sample 23-1-24 describes a sample that was spun at 23% solids using 1% GLY and carried out at 24°C for
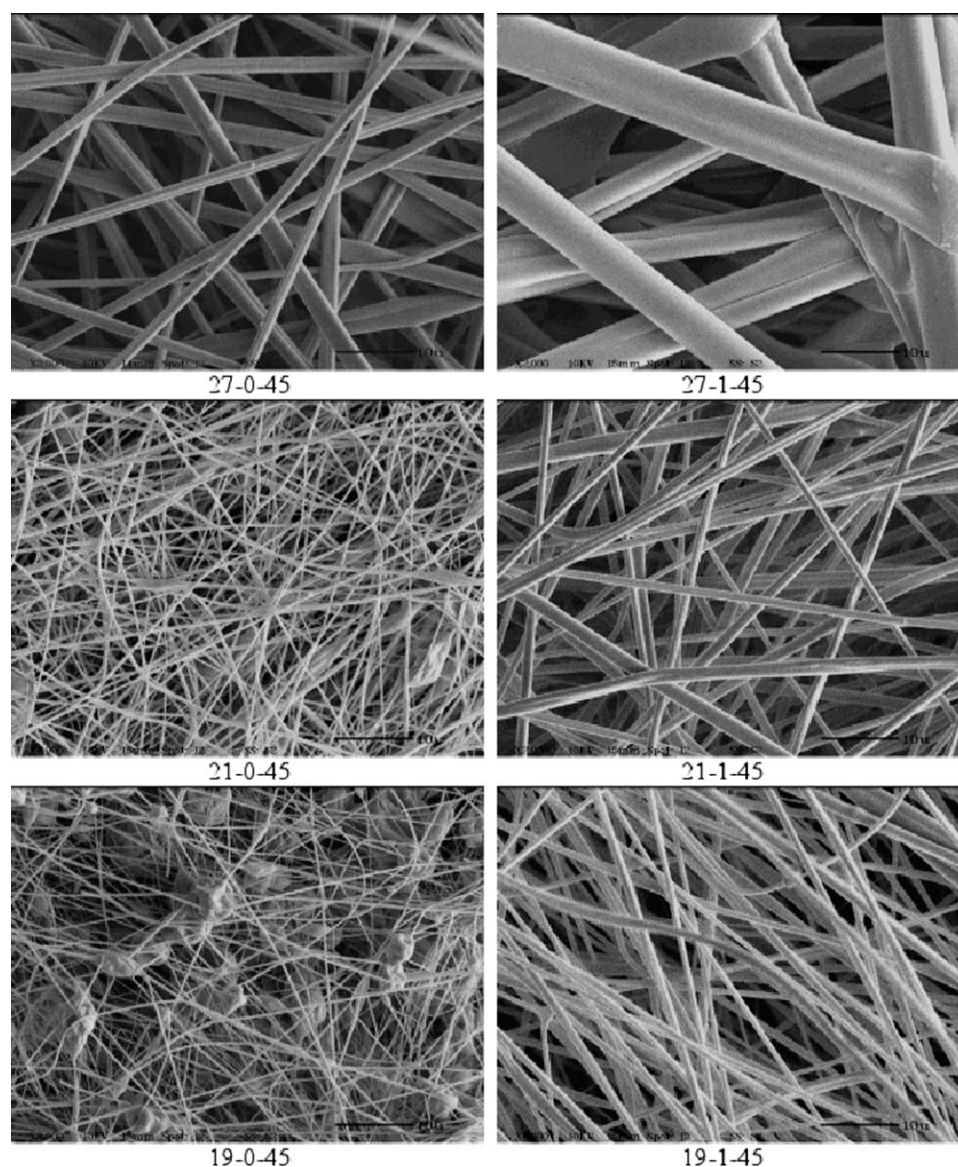
Figure 1 SEM pictures (2000×) of electrospun control zein and zein-1% GLY fibers spun at solution concentrations of 25, 21, and 19% having either 0 or 1% GLY and reacted at 45°C. The first value represents the spinning solids, the second value represents the % GLY and the third value represents the reaction temperature: 19-1-45 = 19% solids, 1% GLY, 45°C. Scale bar in lower right corner measures 10 μm.

30 min. Similarly, sample 19-6-24-90 represents a sample spun at 19% solids, using 6% GLY, performed at 24°C for 90 min.

Fabric post-treatment

The samples of the fabric were tested as is or after an additional heat treatment (known to improve solvent resistance[16]) for various lengths of times. For heated samples, the desired amount of fabric was placed in an aluminum weigh boat and placed in a preheated oven at the desired temperature for the desired time. The temperatures reported were the temperature of the oven, and fabric temperatures were not measured. The fabric samples were weighed ($\sim$ 30 mg, recorded to the

nearest 0.01 mg) and then allowed to soak in 10 mL of AcOH for 2 h. The AcOH solution was then carefully removed via pipette. The undissolved fabric was rinsed with AcOH once more to remove additional soluble material, then with acetone twice to remove residual AcOH. The amount of fabric that did not dissolve was determined gravimetrically after drying in a 120°C oven for 2 h. All samples were done in triplicate. The average standard deviation for the percentage of fabric sample that dissolved in all of these runs was $\sim$ 2.

Fabric analyses

Fabric samples were cut from the mats with a die conforming to the ASTM D-638-V Standard for

TABLE II
Fiber Dimensions of Zein-GLY Formulations

Sample	Fiber diameter (µm)			Sample	Fiber diameter (µm)		
	Ave	Min.	Max.		Ave	Min.	Max.
27-0-24	1.5	1.0	1.8	23-2-45	4.4	2.1	6.9
25-0-24	0.6	0.4	0.9	19-2-45	1.6	1.0	2.0
19-0-24	0.3	0.2	0.5	27-4-24	1.6	1.0	2.6
27-0-45	1.5	0.8	2.4	19-4-24	0.4	0.3	0.8
25-0-45	0.9	0.5	1.4	27-6-24	1.5	0.8	2.9
23-0-45	0.5	0.2	0.9	19-6-24	0.5	0.2	0.7
21-0-45	0.4	0.3	0.6	27-6-24-60	2.0	1.2	3.5
19-0-45	0.3	0.1	0.5	19-6-24-60	0.4	0.3	0.7
27-0-60	1.6	0.5	2.8	27-6-24-90	2.3	1.5	3.6
19-0-60	0.4	0.2	0.6	19-6-24-90	0.5	0.3	0.6
27-1-24	2.5	1.2	4.5	27-6-24-120	3.4	1.7	5.7
23-1-24	0.8	0.5	1.2	19-6-24-120	0.6	0.4	0.9
19-1-24	0.4	0.3	0.6	27-6-24-180	4.6	2.5	6.2
27-1-45	5.0	1.4	8.1	19-6-24-180	0.7	0.5	1.0
25-1-45	2.2	1.6	3.1	27-6-24-240	6.0	4.9	9.0
23-1-45	1.7	1.1	2.6	19-6-24-240	0.9	0.5	1.5
21-1-45	1.2	0.7	2.4	27-6-24-300	30	5.5	48
19-1-45	0.9	0.7	1.3	19-6-24-300	1.5	0.6	2.9
23-1-60	67	43	99	27-6-24-360	64	24	123
19-1-60	8.2	5.5	14.6	19-6-24-360	2.6	0.8	4.8
27-2-24	2.0	1.0	3.2	19-6-24-420	10.6	5.4	16.4
19-2-24	0.4	0.2	0.7				

physical property testing.[37] Samples were then conditioned at 50% RH (23° C) for 5 days. Physical property testing was done using an Instron Universal Testing Machine at a crosshead speed of 10 mm min^{-1} and a gauge length of 7.62 mm using a minimum of six samples. Denaturing gel electrophoresis, using sodium dodecyl sulfate and polyacrylamide gel (SDS-PAGE), was performed to monitor changes in molecular weight of the protein.[38] The proteins were dissolved in DMF to insure that the most material possible would go into solution and still provide a quality analysis.[39] The presence of the SDS denatures the protein so that the depth that the protein penetrates the gel is proportional to its molecular volume and the relative molecular weight can be determined when compared to standards. Fabrics having higher extent of reaction with GLY (higher amounts of GLY, higher temperature, longer reaction times), had limited solubility in DMF, and the gel traces were not representative. The zein samples in DMF were then diluted to an appropriate concentration with water and loading buffer and loaded onto a NuPAGE Novex 10% Bis-Tris gel and run at 200 V for ~ 40 min. Gels were stained with Coomassie Blue. IR spectra were obtained and analyzed in a fashion determined previously.[16] CD spectra were obtained in the manner described previously.[40] CD samples were prepared by adding 90% EtOH/water to ~ 7.5 mg/15 mL (0.5 mg mL^{-1}) of sample and filtering through a 0.45-µm syringe filter (Whatman, Piscataway, NJ). The actual sample concentration present after dissolution and filtering was determined gravimetrically after testing.

RESULTS AND DISCUSSION

Spinning performance

Zein was allowed to react with GLY under a variety of conditions (Table I). The control fibers (0% GLY) were predominantly round and had diameters between 0.3 and 1.5 µm depending on spinning concentration (Fig. 1 and Table II). As the spinning concentration was reduced, the fiber diameter decreased. The largest reduction in fiber diameter occurred between 27 and 25% zein % solids (Table II). It has been reported that the largest changes in viscosity occur at higher polymer concentrations.[39,41] Changes in polymer viscosity will alter fiber diameter (*vide infra*).[15] For the control fiber (0% GLY) spun at 27% solids and allowed to stir at 24°C for 30 min before spinning (sample 27-0-24), the average fiber diameter was 1.5 µm. Lowering the solids to 25% (sample 25-0-24)-generated fibers that had an average diameter of 0.6 µm. Further reductions in spinning solids reduced the diameter more slowly with the 19% solids item (19-0-24) having a fiber diameter of 0.3 µm. This slower reduction in fiber diameter with decreasing spinning solids is seen in other polymer systems.[42] The average fiber diameter and morphology of the control fibers spun at 27% solids did not change significantly as the temperature of
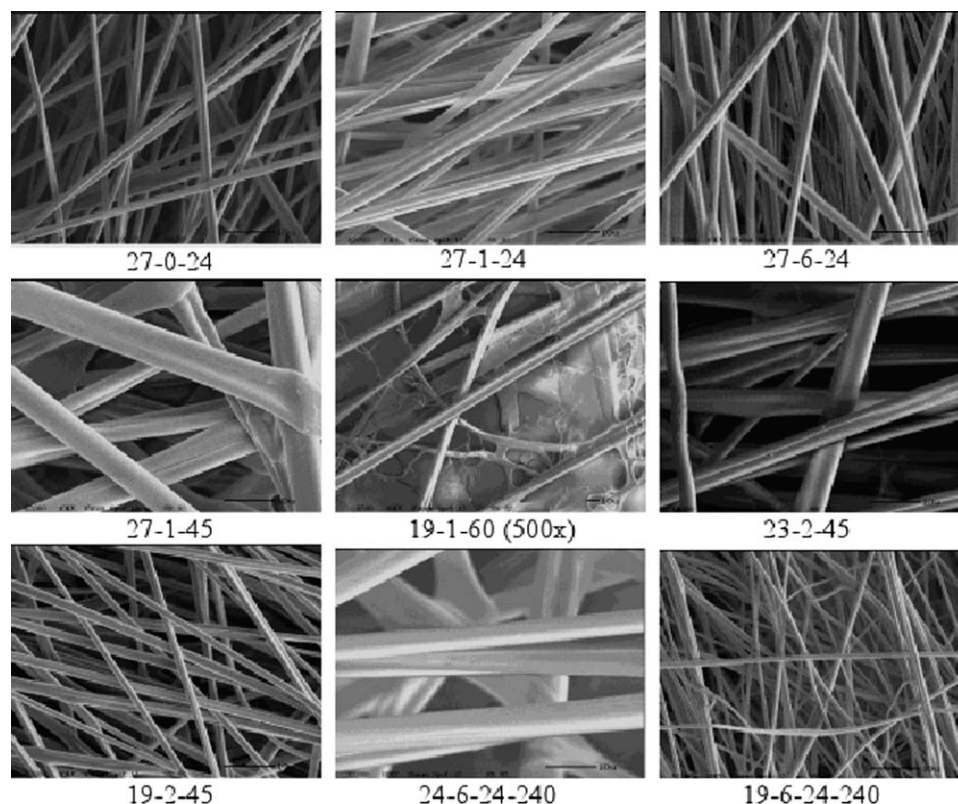
Figure 2 SEM pictures (2000× except where noted) of electrospun zein fibers with various amounts of GLY and prepared at various conditions (see Table I). Scale bar in lower right corner measures 10 µm.

the solution was heated between 24 and 60°C for 30 min before spinning (Samples 27-0-24 and 27-0-60, Table II). The range in fiber diameters became larger as the temperature increased. This may have been due to nonuniform solution temperature during spinning as the temperature of the solution was not controlled. At 19% solids, the impact of temperature on the average fiber diameter is smaller with the 19-0-24, 19-0-45, and 19-0-60 samples having average diameters of 0.3, 0.3, and 0.4 µm, respectively (Table II). At this concentration, the range in fiber diameter is also very similar. As seen previously, the fiber deformities (such as beads) also become more prevalent with reduced solids,[15] in addition to the reduction in fiber diameter. Figure 1 shows SEM pictures of control (0% GLY, 27-0-45, 21-0-45, 19-0-45) and 1% GLY fibers (27-1-45, 21-1-45, and 19-1-45) spun after reacting at 45°C for 30 min and spun at 27, 21, and 19% spinning solids. Samples spun at 25% and 23% solids had fiber diameters between those obtained at 27 and 21% solids (Table II). There are two factors that are quickly noticed, at 27% solids, the diameter of the fiber produced from zein treated with 1% GLY (27-1-45, 5 µm average diameter) are much larger than control fibers (27-0-45, 1.5 µm average diameter). When spun at 21% solids, deformities begin to appear (Fig. 1) in the control sample (21-0-45) which are not present in the 1% GLY sample (21-1-45). These

deformities, which contain a significant amount of material, drive the average diameter of the control fibers to smaller values. Samples 21-0-45 and 21-1-45 had fiber diameters of 0.4 and 1.2 µm, respectively. At 19% solids, the control fiber (19-0-45) has large deformities (10 × 6 µm), whereas the 1% GLY sample is made up of 1 µm defect-free fibers. The deformities along the fiber are caused by limited chain entanglements which lower extensional viscosity leading to poor spinning performance.[15,16,43]

The reaction of zein with GLY results in changes in fiber morphology (Table II). Morphology changes also occur with changes in temperature, reaction time, and solution concentration (Table II and Figs. 1 and 2). As the % spinning solids for the zein-GLY-containing solution is reduced, the diameter of the electrospun fiber decreases (Table II and Fig. 1). The impact of zein concentration on viscosity has been studied before in dimethylformamide[39] and EtOH/water[41] solutions where viscosity increased in the expected power law fashion. Such a relationship suggests that viscosity would increase significantly on going from 19 to 27% solids without including the impact of increased molecular weight on viscosity (which with reaction time and temperature would become the dominating factor on viscosity growth). The relationship between the extent of reaction, solution rheology, and spinning properties are

under current investigation. Viscosity differences taking place during zein crosslinking reactions have been observed before[16] and were attributed to differences in solution viscosity which arise as the concentration of the spinning solution changes.[15] To produce uniform fiber, the control solutions had to be spun above 23% solids (Fig. 1). With the incorporation of 1% GLY and spun after reacting at 45°C for 30 min, uniform fibers could be obtained at 19% solids (19-1-45, Fig. 1). This is indicative of increased chain entanglements that results from protein branching and crosslinking reactions. With increased extent of reaction between zein and GLY, obtained through higher % GLY incorporation, higher temperature or longer reaction time, large changes in fiber morphology and diameter occur (Table II and Fig. 2). Increased extent of reaction results in ribbon fibers being produced with larger average diameters. It has been shown that GLY can increase the molecular weight of zein[9] and other proteins[44,45] through branching and crosslinking reactions. Therefore, these morphological changes in the fiber are driven by increased molecular weight which results in increased chain entanglements which then impact solution rheology. The relationship between molecular weight and/or concentration and fiber morphology (round vs. ribbon) has been studied previously in polyvinyl alcohol systems.[46] A transition from round to ribbon morphologies took place as the concentration or molecular weight of polyvinylalcohol was increased in solution.

When zein was allowed to react with 1% GLY at 24°C (27-1-24), the average fiber diameter was 2.5 μm (Table II and Fig. 2). When the amount of GLY is increased from 1 to 6% (27-6-24) under the same reaction conditions, the fiber diameter decreases from 2.5 to 1.5 μm (Table II and Fig. 2). With increasing % GLY, the % protein solids in the solution is reduced slightly (*vide supra*) as the % spinning solids (GLY is considered as solid) was controlled. With decreasing % protein solids, the solution viscosity will decrease because protein is the main contributor to viscosity in the early stages of reaction.[15,39] As the % GLY is changed from 1 to 6% (Samples 27-1-24 to 27-6-24), the % protein solids will be reduced which will result in fewer chain entanglements[43] and cause a downward trend in fiber diameter (Table II). At 19% spinning solids, where small changes in % protein solids had a reduced impact on viscosity,[39] fibers containing 0 to 6% GLY had similar average diameters (Table II).

As the reaction temperature is increased, the extent of the GLY-zein reaction is increased. The increasing reaction temperature had a large effect on fiber diameter which was further exacerbated by % spinning solids. When 1% GLY was used to modify the zein at temperatures of 24 and 45°C and spun at 27% solids, the fiber diameters increased from 2.5 μm for the 24°C item (27-1-24) to 5.0 μm for 45°C item (27-1-45,

Fig. 2). The fibers produced after reacting at 45°C were also predominantly ribbon shaped. The solutions produced after reacting at 60°C could not be spun at 27% solids at the spinning conditions used due to the high viscosity. Decreasing the % solids to 23% for these 1% GLY solutions (23-1-24, 23-1-45, 23-1-60) resulted in fibers having fiber diameters of 0.8, 1.7, and 67 μm, respectively (Table II). The higher reaction temperature results in more protein branch points, generating higher bulk and extensional viscosity resulting in larger diameter fibers. For these same chemical compositions spun at 19% solids, the fiber diameters were reduced to 0.4, 0.9, and 8.2 μm, respectively, for the 24, 45, and 60°C items (Fig. 2 and Table II). When 2% GLY was used to modify the zein and allowed to react at 24°C, the diameter of the fibers was similar to control. When the reaction temperature of the 2% GLY formulation is increased to 45°C, solutions of 27% and 25% solids would not produce fibers as the viscosity was too high to spin at conditions used. At 23% solids, fibers could be produced (23-2-45) that had an average diameter of 4.4 μm (Table II, Fig. 2). When spun at 19% solids (19-2-45), fiber average diameter was further reduced to 1.6 μm (Fig. 2, Table II). At lower spinning solids, the fibers went from being predominantly ribbon shaped to a mix of ribbon and round fibers (Fig. 2). The impact of temperature could not be readily tested when either 4 or 6% GLY was used as they would gel in less than 30 min at 45°C.

The impact of reaction time was accessed on formulations using 6% GLY at 24°C. As the reaction time is increased from 30 to 360 min (27-6-24 to 27-6-24-360), the fiber diameter increases (Table II, Figs. 2 and 3). On going from a reaction time of 30 to 240 min, the average fiber diameter increases from 1.5 to 6.0 μm (Samples 27-6-24 and 27-6-24-240, respectively). This increases to 30 and 64 μm after 300 and 360 min of reaction, respectively (Samples 27-6-24-300 and 27-6-24-360). Solution rheology was performed on 27% solids having 6% GLY and monitoring solution viscosity with time at 25°C. The initial G' was 0.04 Pa. Over the first 2 h, G' increases slowly. G' after 300 min was 0.07 Pa. Beyond 6 h, G' increases quickly at 400 and 800 min, G' is 0.26 and 1278 Pa, respectively. These changes in viscosity drive differences in fiber diameter and if G' increases too much, the solution will not spin. The fiber could not be produced after a reaction time of 420 min at 27% solids using conditions used due to high viscosity. When these 6% GLY-zein solutions were spun at 19% solids, the fiber diameters are reduced after all reaction times relative to fibers produced at 27% spinning solids (Table II and Figs. 2 and 3). However, the diameter of 6% GLY fibers spun at 19% spinning solids increases with longer reaction times; albeit at a lower rate (Fig. 3). With increased reaction time, increased bulk viscosity was observed,
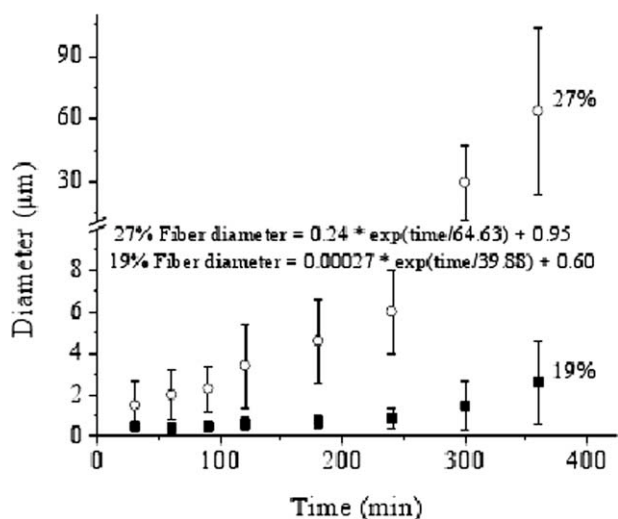
Figure 3 Relationship between diameter of zein-6% GLY (27-6-24) fibers after various reaction times (min) spun at 27% (○) and 19% (■) solids. Bars represent min and max diameters. Equations are for fitted lines using average diameters, $R^2 \sim 0.9$.

which could lead to increased extensional viscosity and larger fiber diameters. Fibers spun at earlier reaction times or when spun at 19% solids were predominantly round. The fibers produced at 27% spinning solids, or after longer reaction times were predominantly ribbons (Fig. 2). Many of the differences in fiber morphology are driven by solution rheology. A complete study of the reaction of GLY with zein using rheological techniques and how this affects spinning performance is in progress.

As was seen in other electrospun zein studies,[15,16] the zein-GLY fibers produced are birefringent. The birefringence results from stresses imposed on the protein during spinning resulting in orientation. The orientated protein will interact with polarized light leading to birefringence which can be observed using microscopes fitted with a pair of polarizing filters. Seen in Figure 4 is sample 27-6-24 under cross-polarizing filters. The white color of the fibers illustrate that order is present in the fibers; as seen in other electrospun zein fibers.[15,16]

Resistance to dissolution

In order for zein-based fibers to be considered for use in industrial applications, the solvent resistance is important. It has been shown that GDA-modified zein electrospun fabrics were resistant to dissolution by AcOH.[16] Similar studies were undertaken for these GLY-modified zein fabrics (Table III). As expected, more than 95% of the control fabric dissolved on exposure to AcOH. Fibers produced from the reaction of zein with 1% GLY (27-1-24) proved to be resistant to dissolution by AcOH after applying a heat treatment to the fabrics (125°C for 30 min). Such a heating step was

Figure 4 Sample 27-6-24 (27% solids, 6% GLY, 24°C, 30 min) at 400× using cross-polarizing filters detailing birefringence of fibers.

required when using GDA as the crosslinking reagent.[16] When the 1% GLY reaction was carried out at 45°C (27-1-45), there was no improvement in solvent resistance relative to when the reaction was carried out at 24°C; apparently, the additional GLY reactions that take place when the temperature was increased from 24 to 45 were not sufficient to provide improved solvent resistance. By carrying out this reaction at 60°C (19-1-60), sufficient GLY reactions occur to deliver improved fabric dissolution resistance. By carrying out the reaction at 60°C, the additional thermal treatment could be reduced from 125°C to 75°C and still provide moderate solvent resistance. On increasing the amount of GLY to 2% (27-2-24), further improvements in solvent resistance were observed. When fabric sample 27-2-24 was heated at 125°C for 30 min, only 21% of the sample dissolved on exposure to AcOH (Table III).

TABLE III
Fiber Loss on Exposure to AcOH of Various Zein Formulations after Various Heat Treatments*

Sample	% Fiber lost				
	*125/30	100/30	75/30	50/30	Unheated
27-0-24	98	100			
27-0-60	95				
27-1-24	44	96			
27-1-45	51	98			
19-1-60	36	46	63		
27-2-24	21	37	96		
23-2-45	19	30	56		
27-4-24	21	43	91		
27-6-24		15	47	93	
27-6-24-90		16	45	95	
27-6-24-240		14	24	41	47

* First number represents oven temperature, second number represents heating time (125/30 means an oven temperature of 125°C and heating for 30 min), and average standard deviation for values was 2.
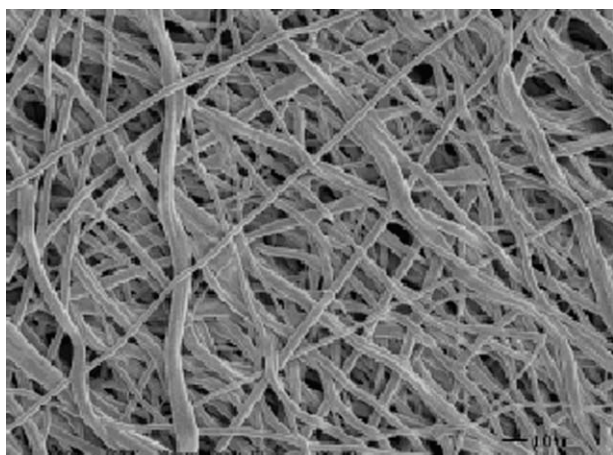
Figure 5 SEM (500×) of 27-6-24 fibers after application of water to surface of fabric and followed by drying. Scale bar in lower right corner measures 10 µm.

Figure 6 IR spectra of 27-0-24 (thin line) and 27-6-24-30 (thick line) electrospun fabrics. Arrow highlights absorbance of interest.

When 2% GDA was used to modify zein,[16] the fabric had to be heated to 180°C to provide durable fabrics, demonstrating the superiority of GLY relative to GDA. Carrying out the 2% GLY reaction at 45°C (23-2-45) provides fabrics with even higher degrees of solvent resistance. After this reaction, and thermally treating the 23-2-45 fabric at 75°C for 30 min, the fabric only loses 56% of its mass on exposure to AcOH. For the same chemical composition carried out at 24°C (27-2-24), such a thermal treatment provided no benefit. This further demonstrates that additional reaction is taking place at the higher temperature. Increasing the amount of GLY to 4 or 6% continued this trend in improved solvent resistance with increasing GLY level (Table III). When using 6% GLY and allowing the reaction to take place for 240 min, the resulting fabric provided moderate resistance to dissolution by AcOH without the thermal treatment. The reaction of GLY with zein continues while the fiber samples are in storage. After storing sample 27-6-24-90 at atmospheric conditions for ∼ 90 days the % fiber lost on exposure to AcOH dropped from 95 to 37% after a 50°C at 30 min oven treatment.

It has been previously shown that when water is applied to heated or unheated electrospun fibers produced from noncrosslinked zein, a crude film immediately forms.[16] In this same study, it was shown that crosslinking with GDA provided fibers that after an additional heat treatment were resistant to film formation with the application of water. When sample 27-6-24 had water applied to it, the fibers did not undergo film formation (Fig. 5). Unlike GDA zein fibers, the fibers produced using GLY modified zein did not require the additional heat treatment to provide water resistant fibers.

Fiber properties

To help understand how GLY reacts with zein, IR analysis was undertaken. The IR spectra of 27-0-24
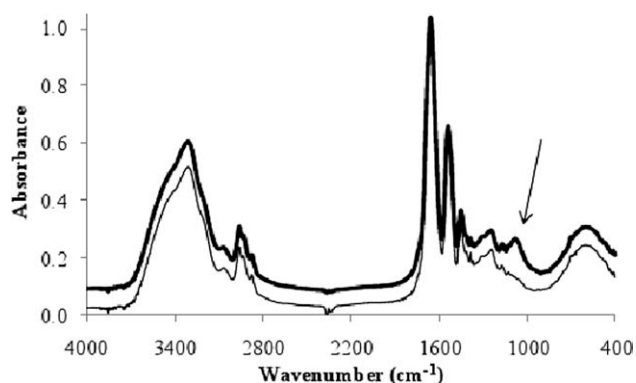
and 27-6-24 are shown in Figure 6. Differences were observed between 1000 and 1100 cm^{-1}. In this area, C—O stretching vibrations are typically found.[47] The addition of these types of bonds are expected given that the reaction of glyoxal with alcohols has been shown to give acetals and hemiacetals (Scheme 1).[48] Zein has many serines and threonines[49] which could undergo this type of chemistry leading to chain extension, branching and ultimately a fully crosslinked network. Other functionalities, such as thiols (cysteine) and amino (lysine and amine terminus) will also play a role forming zein branch points and crosslinking as detailed earlier. However, given their low abundance and the expected location of the IR absorbencies of the products of the reaction of these moieties with GLY,[50] it would be hard to observe new peaks in the region of 1650–1470 cm^{-1} due to the many peaks already present in this region.

Electrospun control (27-0-24) and GLY (27-6-24) fabrics had their physical properties measured. It was found that sample 27-0-24 had a tensile strength of 3.1 MPa ($\sigma = 0.7$), elongation of 6.5% ($\sigma = 1.3$) and Young's modulus of 58 MPa ($\sigma = 7$). With the incorporation of 6% GLY (27-6-24) tensile strength of 4.8 MPa ($\sigma = 0.9$) and YM 84 MPa ($\sigma = 14$) were modestly
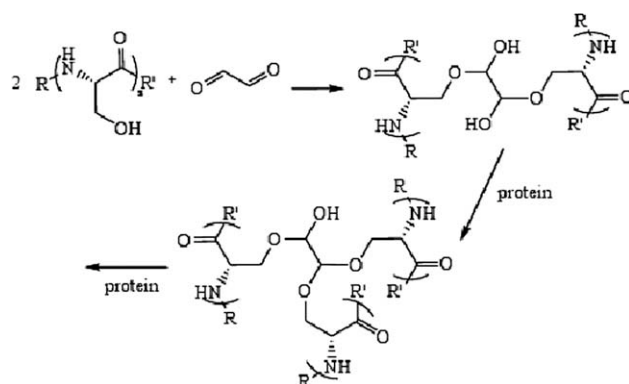
Scheme 1 Reaction of zein amino acids bearing alcohols with glyoxal.

higher, whereas elongation was equivalent at 6.8% (σ = 0.6). The impact of crosslinking on the physical properties of zein is consistent with previous studies of zein, where the crosslinking allows the polymer to bear higher stresses until failure.[9,10] These improvements are needed to utilize zein in textile markets.

The use of SDS-PAGE is a well-established technique to monitor changes in the molecular weight of a protein.[16,38,51] When GLY[11] or GDA[16] were used to react with zein the molecular weight of the protein increased as measured by SDS-PAGE. When SDS-PAGE was carried out on 27-0-24 and 27-6-24 and 27-6-24-60 an increase in m.w. was evident by the increased smearing above 100 kDa (Fig. 7). As seen previously[16] for samples with higher extents of reaction, most of the sample does not dissolve in the DMF, and the resulting SDS-PAGE gel is not representative of the sample.

The secondary structure of the protein is formed by intramolecular hydrogen bonds to provide α-helices, β-sheets, and other conformations.[52] As the functionalities on the zein protein react with GLY, the intricate hydrogen bonding network of the protein changes which results in changes in secondary structure. CD measures how the protein solution interacts with circularly polarized light. The resulting molar ellipticity (expressed in millidegrees cm^2 per decimole) details the amount of rotation that circularly polarized far-UV light undergoes as it interacts with the chiral protein and provides information on the secondary structure of the protein.[53] For a protein with a completely random structure, the
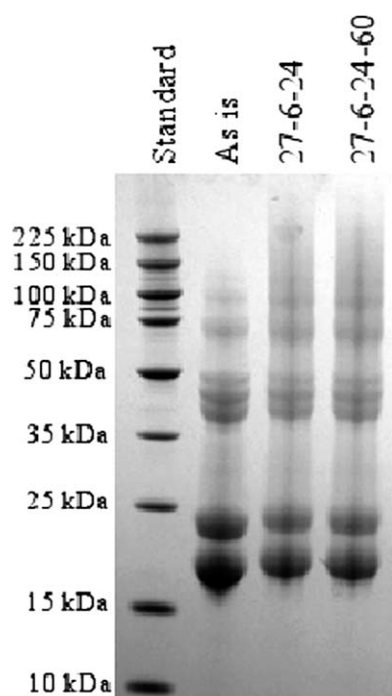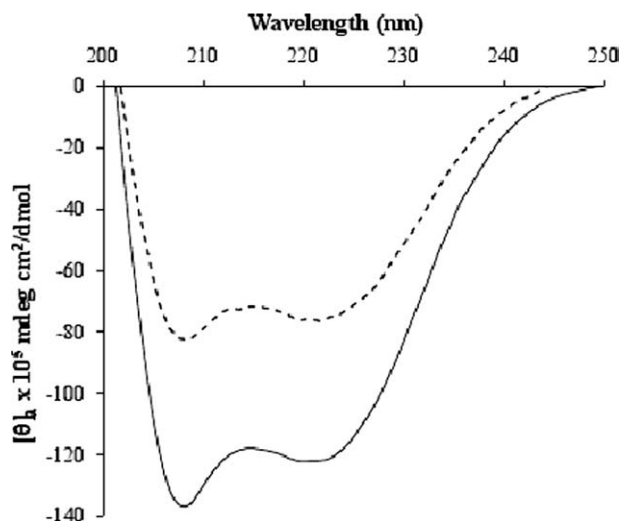

Figure 8   Far-UV CD spectra of samples 27-0-60 (——) and 27-1-60 (- - - ; soluble fraction) in 90% EtOH/water.

molar ellipticity would be close to zero. Proteins with α-helix and β-sheet structures will have molar ellipticities that are negative with their highest absolute magnitudes around 208 and 222 nm, respectively. As observed previously, when zein is allowed to react with crosslinking reagents, the secondary structure of zein becomes more random.[16] The far-UV CD spectra were measured in 90% EtOH/water on fiber Samples 27-0-60 and 27-1-60 (Fig. 8). As seen, the absolute magnitude of the molar ellipticity at 208 and 222 nm are reduced after reaction with GLY. This is indicative of a reduction in the amount of α-helix and β-sheet present in the proteins.[54–56] The use of 70–90% EtOH/water has been successfully used to determine the structure of zein using CD,[40,55] ORD,[56] or small-angle X-ray scattering.[57] While this solvent system readily dissolved the control fabric sample 27-0-60, for the GLY modified zein, 27-1-60, only 42% of the desired fabric sample went into solution. Given this, the spectra shown in Figure 8 is representative of only the soluble portion of the protein present in fabric sample 27-1-60.

CONCLUSIONS

GLY has been shown to be an effective reagent at crosslinking zein in AcOH solution to provide fibers that are durable to dissolution by AcOH. Round and ribbon fibers were produced whose dimensions depended on the viscosity of the spinning solution. Durable fibers with average diameters of 1 to 2 μm could be produced. The fiber diameter and morphology were driven by the concentration of the spinning solution, the amount of GLY used and the extent of reaction (which was driven by reaction time and temperature). Ribbon fibers were predominantly formed if spun at higher spinning solids or higher extent of reaction. The impact of reaction

Figure 7   SDS-PAGE of samples 27-0-24, 27-6-24, and 27-6-24-60.

time on the reaction of zein with 6% GLY showed that the relationship between fiber diameter and reaction time was exponential in nature. The elastic modulus of GLY solutions increased with time which resulted in increased fiber diameter. IR spectra of the spun fibers had an additional peak at 1075 cm^{-1}, which supports the presence of additional C—O bonds; which is consistent with the reaction of GLY with the alcohol functionalities present on zein. An increase in m.w. was observed by SDS-PAGE. Fabrics having 6% GLY had modestly improved tensile strength. The secondary structure of the protein becomes more random (loss in α-helix and β-sheet character) on reaction with GLY. Solvent durable fibers could be produced from zein solution which had been treated with 1 to 6% GLY. Fibers made from solutions having undergone lower extents of reaction, required additional heating of the fabric to become solvent durable. It was found that GLY was superior to GDA in terms of solvent durability. The use of 6% GLY followed by reaction at 24°C for 6 h and spinning at 19% protein solids provides the most durable fibers with diameters of ∼ 1 μm.

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