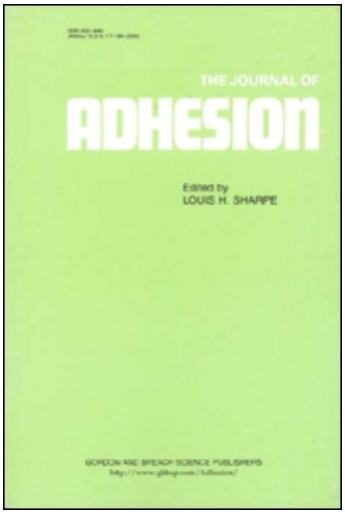

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Role of Chain Entanglements in the Electrospinning of Wheat Protein-Poly(Vinyl Alcohol) Blends

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The aim of this investigation was to determine if the rapid solvent removal evaporation that occurs during electrospinning enabled the gluten protein and poly-(vinyl alcohol) (PVOH) chains to remain at least partially entangled in the final product. Natural and synthetic biopolymer blends are known to phase separate in the melt. Differential scanning calorimetry (DSC) was used to test our hypothesis, which we achieved by systematically comparing the thermal profiles of the nonwoven fibrous sheets comprising: 1) 100% commercial wheat gluten, 2) 100% PVOH, and 3) the (75/25) wheat gluten/PVOH blend. The DSC scans of the two PVOH-containing, nonwoven fibrous sheets exhibited differences in the characteristics and positions of the melting peaks (T_m) of the PVOH crystalline phase, while the DSC scans of the nonwoven fibrous sheets comprising either 100% commercial wheat gluten or the wheat gluten/PVOH blend yielded neither a measurable glass transition temperature (T_g) nor a T_m. Energy dispersive spectroscopy (EDS) was used to compare the elemental compositions of the individual fibers with the compositions of the spherical domains found in the nonwoven fibrous mats. These scans revealed that the mineral matter found in commercial wheat gluten (roughly 1% by weight) had phase-separated from the bulk gluten protein as a result of electrospinning.

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One of a Collection of papers honoring Liliane Léger, the recipient in February 2007 of *The Adhesion Society Award For Excellence in Adhesion Science, Sponsored by 3M*.

In honor of Professor Liliane Léger, a wonderful scientist, mentor, and friend.

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Keywords: Biopolymer blends; Chain entanglements; Electrospinning; Fiber-forming mechanisms; Gluten fibers; Irreversible reactions; Plant protein polymers; Wheat gluten

INTRODUCTION

Gluten is the protein, starch, and lipid nutrient storage component of grain endosperm cells. It has been known for some time that gluten, when mixed with water or another plasticizer, can be transformed into a biobased polymer with thermoplastic properties. Studies have shown that standard petrochemical polymer molding processes can be used to form high-quality biobased films and plastics derived from wheat gluten [1–6]. More recent efforts have led to investigations of conditions under which fibers can be formed from wheat gluten [7,8]. Electrospinning is widely recognized in the scientific community as a convenient technique for producing nonwoven mats of micron to nanometer-sized fibers [9,10–22]. In the basic process, a reservoir of polymer fluid is charged and a fluid jet is accelerated through an electric field gradient toward a grounded target or collector. As the conical jet of polymer fluid propagates through the air, the solvent evaporates and a nonwoven mat of submicrometer-diameter fibers is produced on the collector (Figure 1a). Earlier work on the electrospinning behavior of homogeneous, synthetic polymers has shown that the presence of chain entanglements will ultimately dictate whether or not fibers will form, as illustrated in Figure 1b [23].

The molecular-scale behavior of wheat gluten protein has been investigated extensively over the last several decades [24–27]. Commercial wheat gluten protein is highly complex and heterogeneous, in terms of both its molecular weight (ranging from 10^4–10^6 Da) and overall composition (comprising roughly 75% protein, 10% starch, 5% lipids, 5–10% water, and $<1\%$ minerals). Dough strength is normally attributed to the high molecular weight component of wheat gluten, namely, the glutenin polymers [24–28]. The main proteins, gliadin and glutenin, exist as coiled or folded chains; the proteins are stabilized *via* disulfide bonds between cysteine residues. Mixing the gliadin and glutenin protein chains in solution or in a dough-like state promotes stretching of these molecules and simultaneously disrupts the weak bonds in the system. When chemical and physical bonds reform between the individual protein chains (*e.g.*, by removing unbound solvent or by allowing molecular relaxation to occur in the case of the dough), the disulfide bonds have the potential to form both intramolecular and intermolecular bonds between different protein molecules.

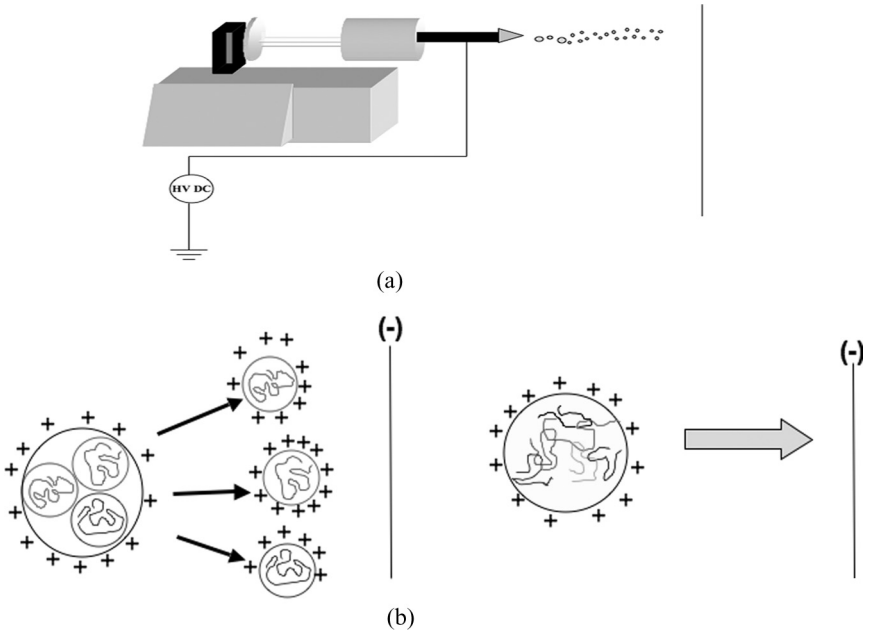


FIGURE 1 (a) Schematic representation of the electrostatic process. In the basic process, a reservoir of polymer fluid is charged. If the electrostatic forces exceed the surface tension of the fluid, the fluid jet is accelerated through an electric field gradient toward a grounded target or collector. (b) In the electrospinning process, droplet fragmentation is limited by the presence of polymer (or protein) chain entanglements. Chain stretching is accompanied by the rapid evaporation of the solvent. Dry fibers accumulate on the surface of the collection plate in the form of a nonwoven mat.

Intermolecular bond formation *via* these disulfide bonds enables the gluten protein chains to achieve very high molecular weights.

Dobraszczyk and Morgenstern [29] have observed that the rheological response of hydrated gluten can vary depending on the precise deformation conditions, exhibiting either shear thinning or strain hardening behavior. In spectroscopic studies by Popineau and coworkers [30,31], the exact conformations of wheat gluten proteins were found to depend on the physical state in which they were analyzed. For example, infrared spectra of the glutenin proteins in the viscoelastic, hydrated dough exhibited a higher amount of intra- and intermolecular β-sheet conformations than the glutenin proteins in solution, which yielded a higher ratio of β-turns and α-helices [30,31].

The goal of this study was to determine if the rapid solvent removal evaporation that occurs during electrospinning enabled the gluten protein and poly(vinyl alcohol) (PVOH) chains to remain at least partially entangled in the solid state. We use differential scanning calorimetry (DSC) to compare the thermal characteristics of electrospun nonwoven fibrous sheets comprising: 1) 100% commercial wheat gluten, 2) 100% PVOH, and 3) the (75/25) wheat gluten/PVOH blend. Energy dispersive spectroscopy (EDS) is used to compare the elemental compositions of the individual fibers with the compositions of the spherical domains found in the nonwoven fibrous sheets.

EXPERIMENTAL

Materials

Commercial wheat gluten [70.2% protein on an "as is basis" as determined by the Dumas Method ($N \times 5.7$)] from Amylum (Aalst, Belgium) was used in this study. PVOH (Catalog # 363146; average $M_w = 85{,}000–124{,}000$, 99+ % hydrolyzed) was obtained from Aldrich Chemical, Inc., Milwaukee, WI, USA.

Electrospinning

Wheat gluten was dissolved at a concentration of 5% (w/v) in 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP) [7,8]. PVOH was added to the suspensions on a weight basis relative to wheat gluten [13% or 26% (w/w)]. Suspensions of wheat gluten were forced through a 5.0 mL syringe using a syringe pump (Model 100, KD Scientific Inc., New Hope, PA, USA), forming a bead of solution at the tip of the syringe. A high voltage was applied between the tip (an 18-gauge blunt needle) and a grounded collection target. The positive lead from a high voltage supply unit, Spellman CZE 1000 R (Plainview, NY, USA), was attached to the external surface of the metal syringe needle, and the rotating mandrel was positioned 10 cm from the tip of the syringe. The syringe pump was set to deliver the protein suspension at rates up to 5 ml/hr, and the applied voltage was fixed at 25 kV.

Microtensile Tests

A tensiometer (Model Mini 55 with 10 N static load cell, Instron Corp., Canton, MA, USA) was used to characterize the tensile properties of

electrospun nonwoven fibrous mats [8]. 4.5 cm × 1.0 cm × 2 mm (length × width × thickness) test coupons were cut, and subsequently reinforced with 1.0 cm strips of tape on either end prior to mounting them vertically between two mechanical gripping units, leaving a 3.5 mm gauge length for mechanical loading. An extension rate of 2 mm/min was used in the tensile tests.

Scanning Electron Microscopy and Energy Dispersive Spectroscopy

The specimens were mounted on stubs as-is, and viewed in a Zeiss Supra 50 field emission scanning electron microscope (Carl Zeiss SMT, Bonn, Germany) at 20 kV. The specimens were analyzed in variable pressure mode, and the micrographs were acquired with the backscatter detector. The energy dispersive spectra were obtained with the X-ray detector.

Differential Scanning Calorimetry

DSC measurements were performed on both powdered samples and electrospun nonwoven fabrics packed into aluminum pans, hermetically sealed, using a Differential Scanning Calorimeter 7 (Perkin Elmer, Norwalk, CT, USA) that was calibrated against an indium standard, and all experiments were conducted under a nitrogen atmosphere. Samples and empty aluminum pans, as reference, were heated and cooled from 25°C up to 250°C at a scan rate of 10°C/min, for two cycles. The peak melting and recrystallization temperatures of PVOH were then recorded.

RESULTS AND DISCUSSION

In our earlier work, the fiber-forming characteristics of wheat gluten were found to improve as more PVOH was introduced into the electrospinning system [8]. The compositions of the nonwoven electrospun fibrous sheets included the following: as-received wheat gluten and wheat gluten with either 13% (w/w) or 26% (w/w) PVOH [8]. Relatively large amounts of the high molecular weight polymer (PVOH) were used to ensure that the effects of PVOH on the electrospinning behavior of wheat gluten would be measurable. Our current aim is to gain a deeper understanding of how the molecular structure of the wheat gluten protein and PVOH polymer chains in the nonwoven electrospun fibrous sheets are dictated, at least in part, by the rapid

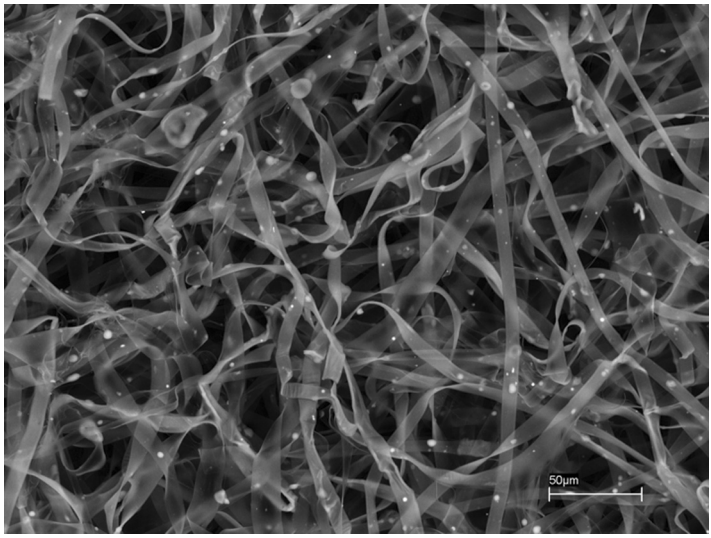


FIGURE 2 Scanning electron micrograph (SEM) of electrospun nonwoven mat made of wheat gluten and 26% (w/w) PVOH. Adapted from [8] with permission.

removal of solvent during electrospinning. An electrospun nonwoven fibrous sheet, depicting ribbon-like fibers, is presented in Figure 2.

Researchers have shown for synthetic polymers how, with knowledge of the entanglement molecular weight and weight-average molecular weight of the polymer in a good solvent, one can predict the critical polymer concentration needed to cross the transition between electrospraying and electrospinning [23]. Liquid jet stabilization and the subsequent formation of fibers are generally attributed to molecular-scale phenomena, such as physical entanglements and thermally reversible junctions [32–33]. However, in the electrospinning of wheat gluten protein, the intermolecular disulfide bridges formed through sulfhydryl/disulfide interchange reactions are known to play an important role in increasing the overall molecular weight of the protein polymer [7].

A summary of the micromechanical results can be found in Figure 3. Micromechanical tests were conducted on the nonwoven electrospun fibrous sheets to assess the impact of PVOH on the strength and elongational properties of the nonwoven electrospun fibrous sheets (Figure 3a). A similar plot was constructed for percentage elongation-at-break (Figure 3b). These results were in-line with those presented earlier, which yielded substantial increases in both the tensile

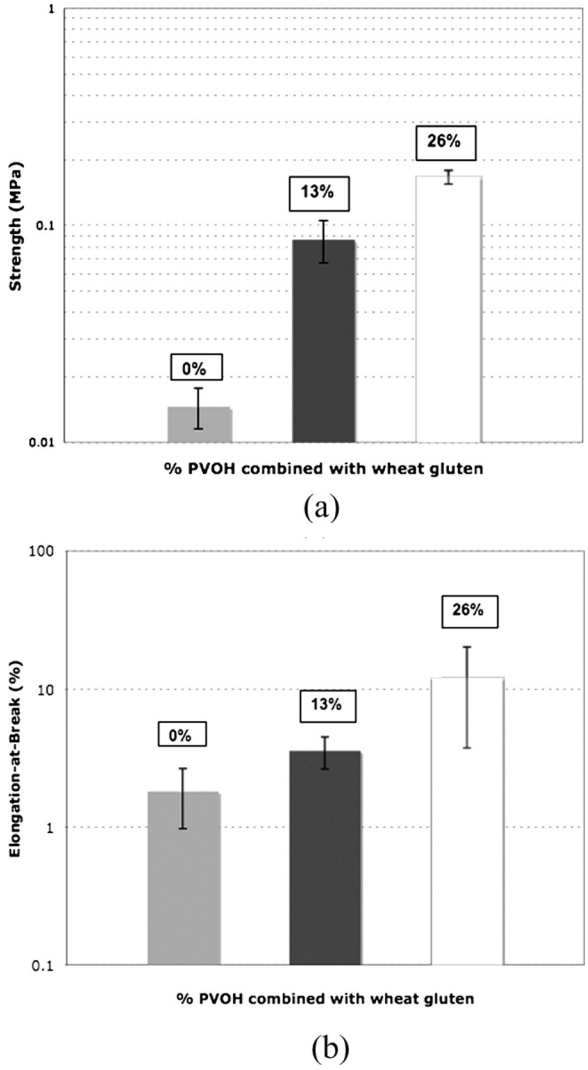


FIGURE 3 Mechanical properties (a) Tensile Stress (MPa) and (b) % Elongation-at-break of nonwoven fibrous mats derived from wheat gluten as a function of PVOH content: 0% (w/w) PVOH, 13% (w/w) PVOH, 26% (w/w) PVOH. The error bars represent ± 1 standard deviation.

strength and the percentage elongation-at-break as increasing amounts of PVOH were introduced to the system [8].

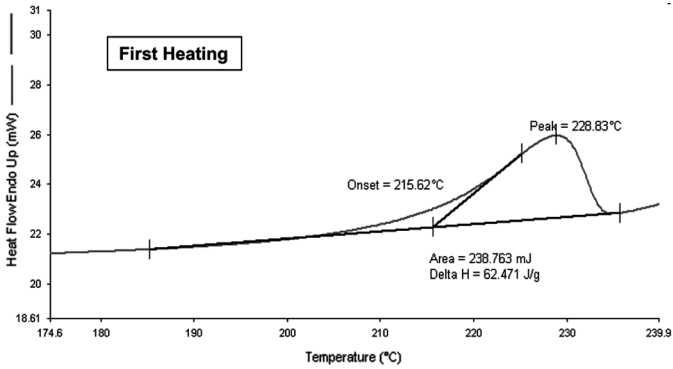
The next step involved investigation of whether the dramatic increase in the tensile properties of the PVOH-modified-electrospun

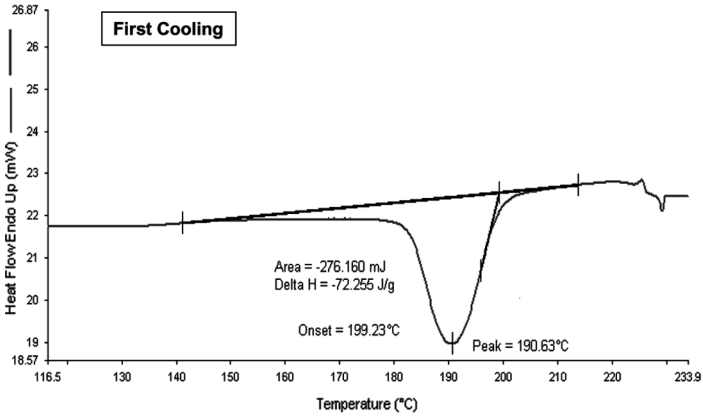
fibrous sheets was a result of chain entanglements in the wheat gluten protein/PVOH blend. Moreover, the thermal properties of these materials were evaluated *via* DSC, and the heating and cooling profiles were compared with PVOH and wheat gluten controls. We hypothesized that there would be insufficient time for the polymeric chains to unentangle and separate (relaxation times being so large) during the solvent evaporation step.

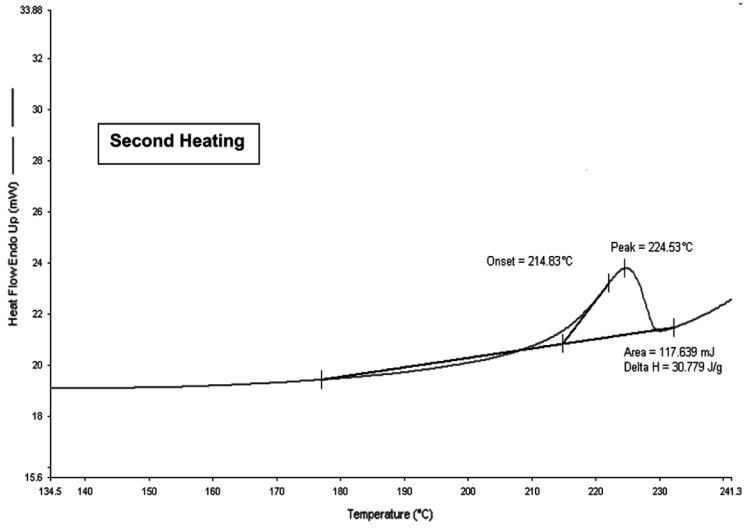
Presented in Figures 4a, b, and c are the DSC thermograms of pure PVOH (our first control), a blend of commercial wheat gluten protein and 26% (w/w) PVOH, and pure commercial wheat gluten protein (our second control), respectively. Figure 4a depicts the phase transitions of the semi-crystalline polymer, PVOH, during two heating and cooling cycles. In the first heating, the melting endotherm was at 228°C, while the first cooling exhibited a recrystallization exothermic peak at 190°C; in the second cycle, the melting endotherm was at 224.5°C. This behavior differs from that observed with the nonwoven electrospun fibrous sheet comprising the commercial wheat gluten/PVOH blend (Figure 4b): in these thermograms, a melting peak corresponding to the PVOH was observed at 229°C, but its measured area was considerably lower than that of the PVOH control. Similarly, a much smaller recrystallization peak area and temperature (146°C) was present in the thermogram upon cooling the sample from 250°C to 25°C. However, no melting peak was observed in the second heating ramp (Figure 4b), which led us to wonder whether the gluten protein chains could have had a direct influence on the crystallization. Furthermore, no transitions appeared in the thermograms of plain, commercial wheat gluten (Figure 4c). We believe these data can be interpreted in a number of different ways:

1) In the case of the wheat gluten/PVOH blend, PVOH is diluted to the point at which recrystallization is no longer possible.

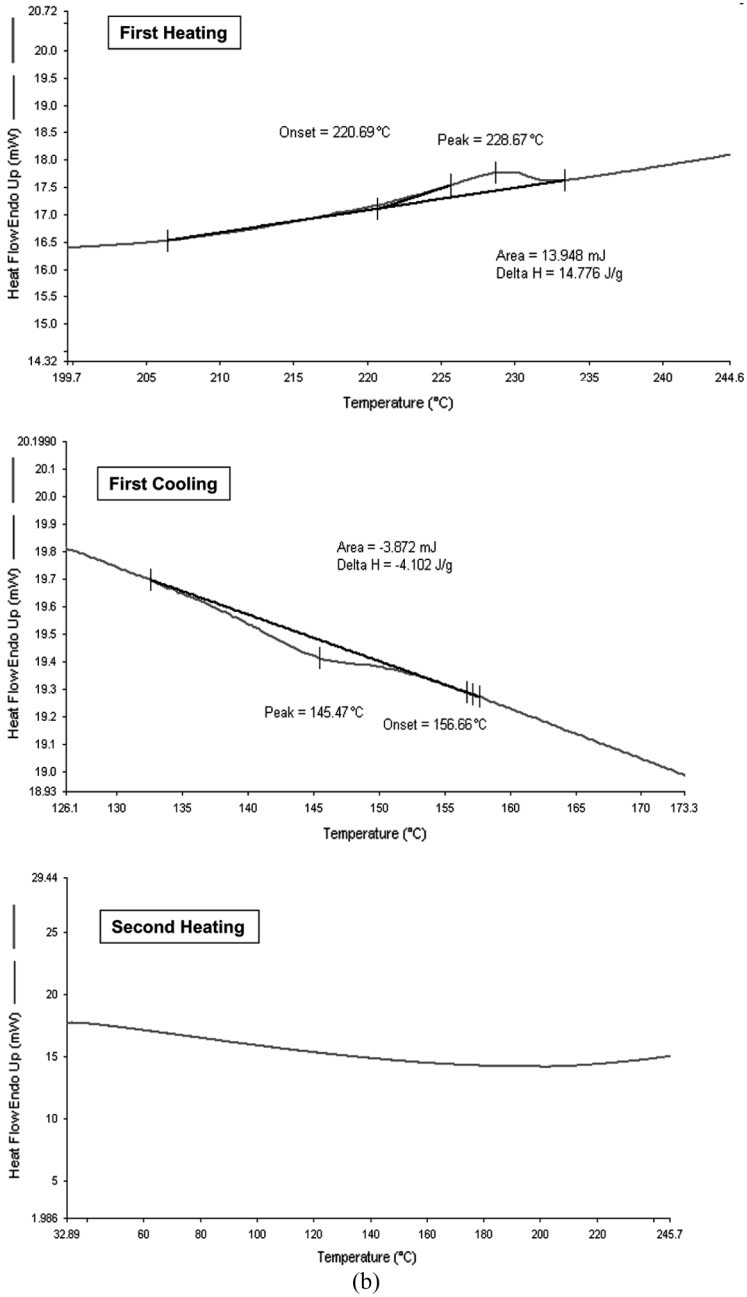
FIGURE 4 DSC thermograms of semi-crystalline (PVOH) and amorphous (wheat gluten) biopolymers. (a) First heating, first cooling, and second heating ramp of pure PVOH powder (control). The thermograms exhibited the characteristic melting endotherms and recrystallization exotherm of the crystallites in PVOH. (b) First heating, first cooling, and second heating ramp of an electrospun fibrous mat comprising a blend of commercial wheat gluten protein and PVOH. These scans yielded a melting peak during the first heating ramp *only*. (c) First heating, first cooling, and second heating ramp of an electrospun fibrous mat of pure commercial wheat gluten protein. This series of thermograms exhibited no thermal transitions at all.







(a)



(b)

FIGURE 4 Continued.

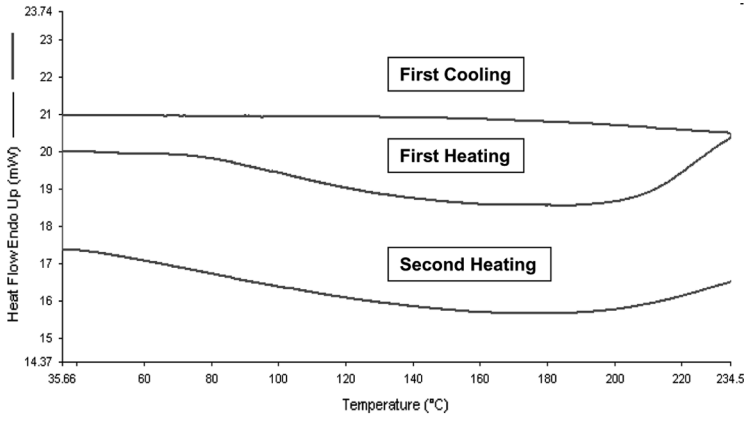


FIGURE 4 Continued.

2) There are physical interactions between wheat gluten protein and PVOH, and hence crystallization has been either slowed or prevented.
3) An irreversible chemical reaction has taken place between the wheat gluten protein and the alcohol groups in PVOH, thereby preventing any crystallization at all.
4) The wheat gluten protein has begun to break down at temperatures above its degradation temperature (roughly 200°C), and the degradation products act to prevent crystallization from taking place.

In the course of this study, we observed other interesting things. Figure 2 revealed the presence of small inorganic domains (bright spots) inter-dispersed throughout the nonwoven electrospun fabrics. EDS was conducted to determine the elemental composition of these domains, which were then compared directly with compositions at localized regions along the length of the fibers (Figure 5). Figure 5a depicts an EDS spectrum of a micron-sized fiber surface, revealing the presence of carbon, nitrogen, oxygen, and sulfur, consistent with those elements found in a cysteine-containing protein polymer. The micrometer-sized spherical domains, on the other hand, yielded a much broader spectrum of elements, including: carbon, nitrogen, oxygen, magnesium, phosphorous, sulfur, potassium, and calcium (Figure 5b). These findings suggest that while the minerals are preferentially excluded from the fibers that form on the collector, they appear

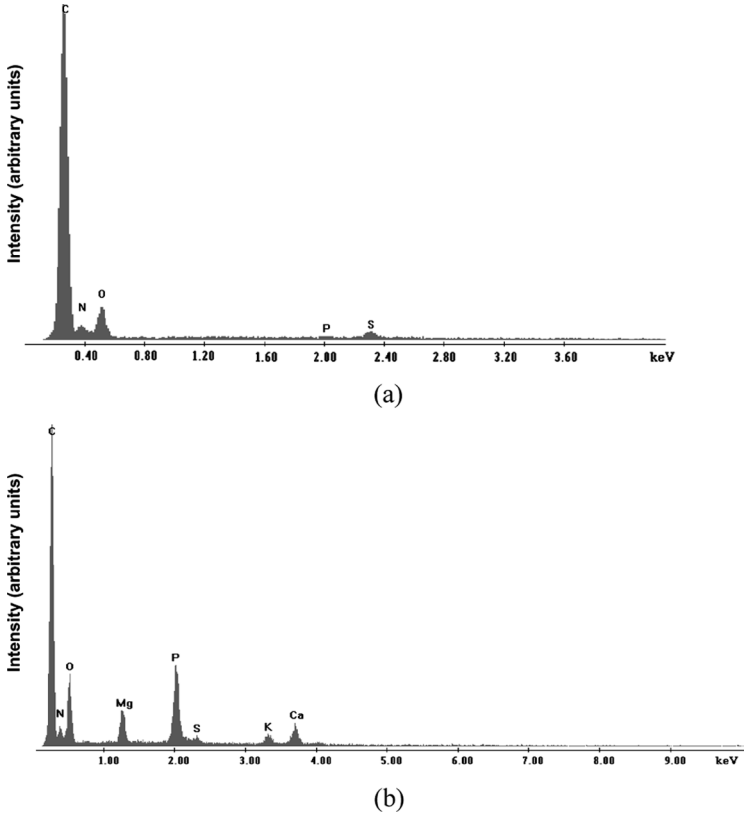


FIGURE 5 Energy dispersive spectroscopy (EDS) performed at different locations on a nonwoven, electrospun fibrous mat depicted in Figure 2. (a) Localized spot on one of the fibers. (b) Localized spot on a 2 μm spherical domain.

to have little influence on the ability of the gluten protein molecules to form chain entanglements during electrospinning.

CONCLUSIONS

The main objective of this study was to determine whether the rapid removal of solvent that occurs during electrospinning enabled the biodegradable copolymer blend to remain entangled in the solid state. Our preliminary results suggest that electrospinning gives rise to a unique result in the processing of wheat gluten and PVOH blended fibers. The apparent synergy between the wheat gluten protein and PVOH chains is supported by the observed ten-fold increase in the

tensile strength of the modified specimens in relation to plain wheat gluten control specimens. Due to the complex changes that occur in the wheat gluten protein at elevated temperatures, several different interpretations of the DSC data are possible, all of which were enumerated above. Important to note, however, is that the melting peak of PVOH disappears completely after the second heating ramp, which could suggest that the wheat gluten constituents in the system prevented the PVOH chains from recrystallizing. The precise mechanism behind this event will be investigated in a future study. As a side note, the minerals in the wheat gluten system appeared to neither hinder nor enhance the formation of fibers: SEM and EDS analysis of the nonwoven electrospun fibrous sheets revealed that the inorganic constituents exist as spherical, micrometer-sized domains in the final product.

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