Water Absorbance and Thermal Properties of Sulfated Wheat Gluten Films

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ABSTRACT: Wheat gluten films of various thicknesses formed at 30–70°C were treated with cold sulfuric acid to produce sulfated gluten films. Chemical, thermal, thermal stability, and water uptake properties were characterized for neat and sulfated films. The sulfated gluten films were able to absorb up to 30 times their weight in deionized water. However, this value dropped to 3.5 when the film was soaked in a 0.9% (w/w) NaCl solution. The films were also soaked 4 times in deionized water, and each soaking resulted in a reduced water uptake capacity. The temperature of film formation had no effect on the final water uptake properties. Also, thinner films had higher concentrations of sulfate groups than thicker films; this resulted in higher water uptake values. In addition, sulfated gluten films had comparable glass-transition temperatures but lower thermal stabilities than the neat gluten films. © 2010 Wiley Periodicals, Inc.† J Appl Polym Sci 116: 2638–2644, 2010

Key words: biomaterials; films; proteins

INTRODUCTION

The majority of superabsorbent polymers currently produced are derived from synthetic monomers, such as acrylic acid and acrylamide.[1] The most successful product is poly(acrylic acid) sodium salt (also known as crosslinked polyacrylate). These polymers can absorb hundreds of times their original weight in water and are widely used in the hygiene industry. However, poly(acrylic acid) has several drawbacks. It is not biodegradable, which leads to persistence and accumulation in the environment. In addition, it is derived from petroleum products, which are not renewable.

To provide alternatives to petroleum-based superabsorbent polymers, various authors have modified natural polymers, including polysaccharides, such as starch,[1–9] cellulose,[10–14] chitosan,[15–19] carrageenan,[20] guar gum,[21] pectin,[22] and alginate,[23] and proteins, such as collagen,[24] soy protein isolate,[25,26] and fish protein.[27] The modification of these natural polymers has usually involved the grafting of poly (acrylic acid), polyacrylamide, polyacrylonitrile, or their copolymers onto a polymer backbone. In fact, one of the first commercial superabsorbent polymers was developed by the U.S. Department of Agriculture and involved polyacrylonitrile grafted onto starch. These natural polymers have also usually been crosslinked to improve their mechanical properties. Recently, different types of clays have been incorporated into these polymers to improve their strength and reduce their cost.[3,5–8,17,19,23] Although these modified polymers have relied on natural polymers, their nonbiodegradable and petroleum-based content could be substantial. In the case of starch/polyacrylonitrile grafted polymers, the petroleum-based component could be as high as 50%.[28]

There have been few superabsorbent polymers developed from just natural polymers. These have usually involved the crosslinking of natural polymers.[11–13,18,22] Another approach involved the sulfating of wheat gluten powder by the formation of sulfuric acid esters with gluten amino acids that contained reactive hydroxyl groups, such as serine and tyrosine. The sulfate incorporation was inferred from a strong linear correlation between the concentration of sulfate added and the concentration of hydroxyl groups present in a series of natural proteins with various amounts of hydroxyl groups.[29,30] However, this process involved large quantities of solvents, such as acetone, during the washing and purification steps. In addition, the yields reached only approximately 50% because of material loss during filtration and washing and possible solubilizing reactions.

In this study, we produced superabsorbent wheat gluten films by a simple reaction with cold sulfuric

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acid and subsequent washing with deionized water. We characterized the films' chemical, thermal stability, and thermal properties. We also examined water uptake after repeated soakings in deionized water and after soaking in a saline solution.

EXPERIMENTAL

Gluten film preparation

Wheat gluten was extracted from unbleached flour (Giusto, San Francisco, CA) containing 14.4% protein (5.7 X N, Leco Corp., St. Joseph, MI). The flour was a blend of dark northern spring wheat and hard red winter wheat from Montana. We used a batter method to produce wheat gluten by first mixing 700 g of flour (12.02% moisture) with 630 mL of deionized water in a Hobart mixer (model A120, Troy, OH), equipped with a McDuffie bowl pin mixer (National Manufacturing, Inc., Lincoln, NE), for 15 min at 60 rpm. The batter was then relaxed for 15 min. After this, the batter was dispersed in 1 L of distilled water at 22°C with continued mixing for an additional 5 min. The concentrated protein fraction was then collected on a 213-μm screen with a vibratory separator (Pharmacep PH12 vibratory separator, Sweco, Florence, KY). This step was repeated three more times with each concentrated gluten fraction. The final wheat gluten sample contained 79.6% protein (5.7 X N, Leco) and was mixed with deionized water in a 1 : 2 gluten-to-water ratio. The part of the sample not immediately used was stored in a freezer. Approximately 3.5 g of the sample was placed between two steel plates covered with Mylar film to prevent adhesion. Three different spacers (Aloma Shim and Manufacturing, Oakmont, PA), 0.01, 0.025, and 0.05 inch thick, were used to control the sample thickness. The steel plates were then clamped together and placed in an oven. After 3 h, the plates were pulled apart, and the sample was allowed to remain in the oven for an additional 2 h. Three different temperatures, 30, 50, and 70°C, were used in the experiments.

Sulfated gluten films

Each film was soaked in concentrated sulfuric acid (Fisher Scientific, Pittsburgh, PA) for 3 h at −2°C. The sample was thoroughly rinsed with deionized water to remove excess acid and was then soaked in deionized water at room temperature (23°C) for 1 h. Afterward, the sample was dried overnight under ambient conditions.

Moisture content

Each film was dried *in vacuo* at 60°C for 24 h. The moisture content was calculated from the sample weights before and after drying.

Fourier transform infrared (FTIR) spectroscopy

A PerkinElmer 2000 FTIR spectrometer (Waltham, MA) was used to characterize the chemical changes of the gluten samples. The samples were ground into powder with a ball mill for 1 min before they were placed in a DuraSamplIR attenuated total reflectance attachment (ASI SensIR Technology, Danbury, CT). Each IR spectrum contained an average of 50 scans over a 10-min period with a resolution of 4 cm^{-1}. The asymmetric SO_2 stretching in the sulfate ($R{-}O{-}S{-}O_3^-$) peak occurred at 1210 cm^{-1}. For absorbance calculations, the area under the amide I peak at 1637 cm^{-1} was used as an internal standard to normalize the area under the sulfate peak for each film.

Scanning electron microscopy (SEM)

The wheat gluten samples were first affixed to stubs with carbon adhesives. A Polaron E5100 sputter coater (Hatfield, PA) was then used to apply a gold coating. The samples were sputter-coated for 90 s at a voltage of 1.5 kV and a discharge current of 20 mA. The vacuum chamber was set to a pressure of 10 Pa. A Hitachi S-4700 scanning electron microscope (Pleasanton, CA) was used to observe the samples under 1000× magnification. The voltage setting was 15.0 kV, and the current setting was 10 μA.

Thermogravimetric analysis (TGA)

A TA Instruments TGA 2950 (New Castle, DE) was used to characterize the thermal stability of the gluten samples. Each 11-mg sample was heated from 30 to 800°C at a rate of 10°C/min. The sample was maintained in a nitrogen environment with a nitrogen gas flow rate of 40 cm^3/min. Each sample was conditioned in a 50% relative humidity chamber at 23°C for at least 48 h before each measurement. The chamber was maintained at this humidity with a saturated solution of calcium nitrate tetrahydrate ($Ca(NO_3)\cdot 4H_2O$; Fisher Scientific) in deionized water.

Differential scanning calorimetry (DSC)

A TA Instruments DSC 2910 was used to measure the thermal properties of the gluten samples. Each 10–11-mg sample was heated from 0 to 140°C at a rate of 5°C/min in a sealed stainless steel pan. The sample chamber was purged with nitrogen gas at a flow rate of 75 cm^3/min. Each sample was conditioned in a 50% relative humidity chamber at 23°C for at least 48 h before each measurement.

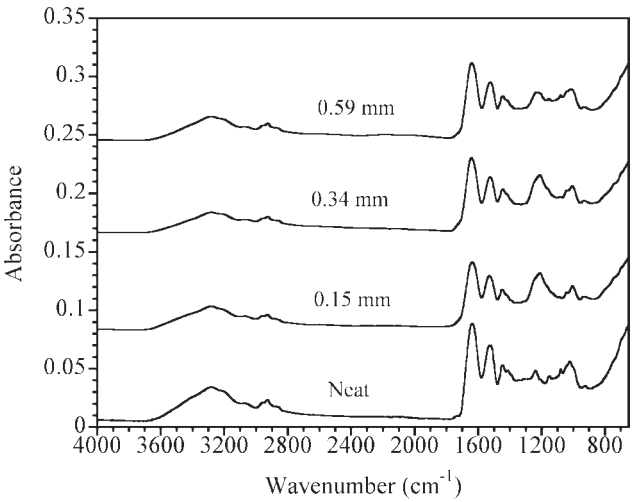

Figure 1 FTIR spectra of the neat and sulfated gluten films with different thicknesses. Each spectrum shifted 0.08 absorbance units up from the one beneath it.

Water uptake

Each film was initially weighed before it was soaked in 30 mL of deionized water for 2 h at room temperature. In addition, some films were soaked in 30 mL of a 0.9% (w/w) NaCl (Fisher Scientific) solution as a comparison. After soaking, the sample was placed on a paper towel and gently blotted dry with another paper towel. The sample was then reweighed. The water uptake was calculated as follows:

$$W = \frac{w_s - w_o}{w_o} \quad (1)$$

where W is the water uptake (g of water/g dry gluten), w_s is the weight of the soaked sample, and w_o is weight of the dry sample before soaking.

Statistical analysis

Data were analyzed by three- and two-way analyses of variance and paired t tests at a significance level of less than 0.05 with Minitab version 14.12.0 statistical software (Minitab, Inc., State College, PA).

RESULTS AND DISCUSSION

FTIR spectroscopy

The FTIR spectroscopy results indicated the presence of sulfate groups on all of the gluten films treated with sulfuric acid. This is shown in Figure 1, where we plotted the FTIR spectra of neat and sulfated films of different thicknesses. The sulfate peak at 1210 cm^{-1} appeared in all of the sulfated films. In contrast, no sulfate peak appeared in the neat gluten film. These results indicate that sulfuric acid reacted with the hydroxyl groups on the amino acids, such as serine and tyrosine, in wheat gluten to form acid sulfate esters.[30]

Thinner gluten films had more sulfate groups than thicker films. This is shown in Figure 2. The sulfate content was directly proportional to the film thickness over the range of thicknesses examined in this study. These results suggest that sulfuric acid did not react homogeneously with hydroxyl groups throughout the entire cross section of each film. Apparently, hydroxyl groups near the film surface were more readily accessible to sulfuric acid. Consequently, more hydroxyl groups in thin films were converted into sulfate groups per volume of gluten.

SEM

The neat gluten films had a smooth surface but became somewhat blotchy after treatment with sulfuric acid. This is shown in Figure 3, where we present the SEM micrographs of the neat and sulfated gluten films. The neat gluten film [Fig. 3(a)] exhibited a smooth and featureless surface. However, the sulfated gluten films displayed uneven spots on their surfaces [Fig. 3(b,c)]. These spots appeared to be shallow and did not penetrate the surface to an appreciable depth. Also, we observed no differences in the surface features on sulfated gluten films of different thicknesses.

Thermal stability

The sulfated gluten films were slightly less thermally stable than the neat gluten films. This is shown in Figure 4. Film thickness had little effect on the TGA curves for either the neat or sulfated gluten films. Each film had a constant weight until the

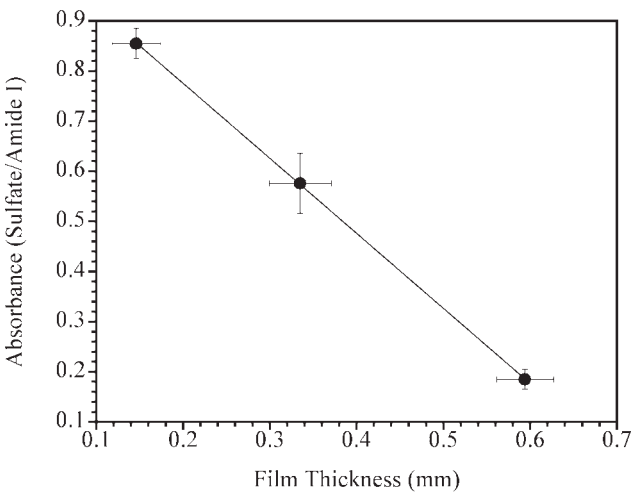

Figure 2 Normalized sulfate absorbance area as a function of the film thickness for films processed at 50°C. The line is a linear fit to the data and has an R^2 value of 1.00.

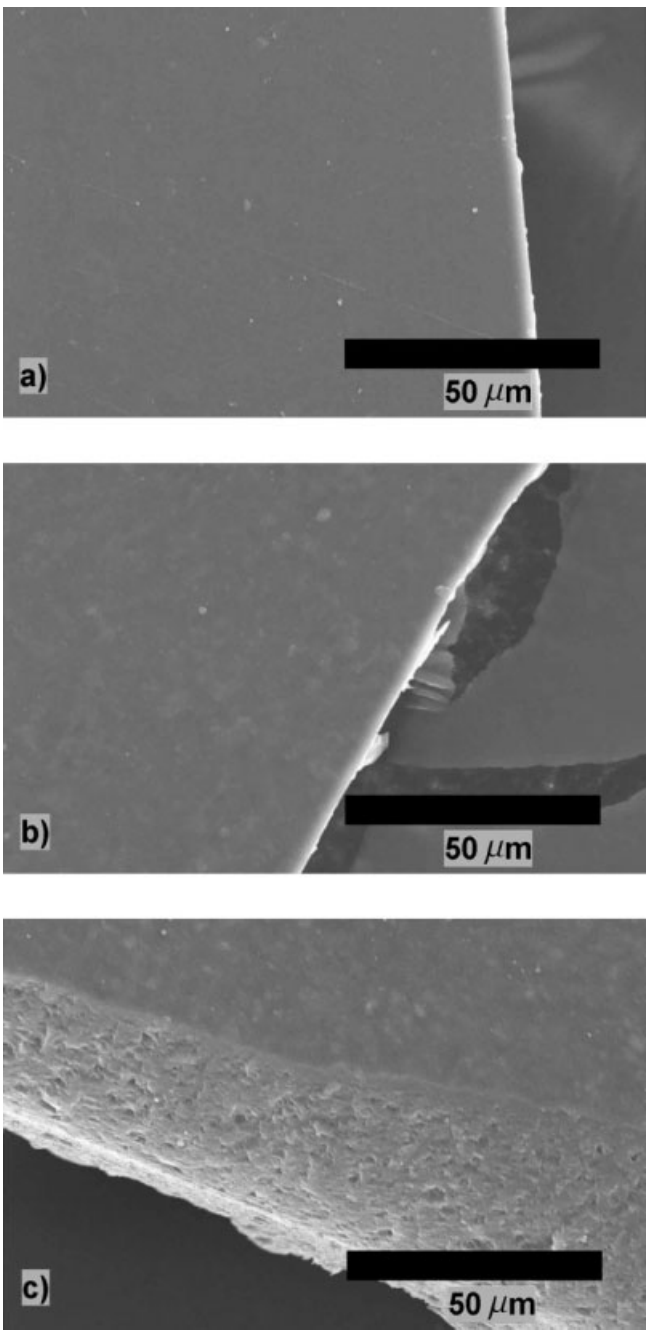

Figure 3 SEM micrographs of the (a) 0.15 mm thick neat gluten film, (b) 0.15 mm thick sulfated gluten film, and (c) 0.6 mm thick sulfated gluten film. All of the films were processed at 50°C.

temperature reached approximately 100°C. At this point, water in the film volatized, and the film decreased in weight. After this, the weight for the sulfated gluten films began to decrease rapidly at approximately 230°C. In contrast, the weight of the neat gluten films did not decrease rapidly until approximately 250°C. These results were most likely due to the lower thermal stability of the sulfate groups. This decrease in thermal stability was observed more clearly in a plot of the derivative of weight loss as a function of temperature, as shown in Figure 4(b). The sulfated gluten curves began to rise rapidly at lower temperatures than the neat gluten curves. In addition, the sulfated gluten curves became more jagged, unlike the neat gluten curves, which had a relatively sharp peak. These results indicate the sulfated gluten films contained more heterogeneous components than the neat gluten films.

Thermal properties

The incorporation of sulfate groups slightly reduced the glass-transition temperatures of the gluten films. This is shown in Table I, where we present the glass-transition temperatures of the neat and sulfated gluten films. The glass-transition temperatures ranged from 46 to 54°C; this was consistent with values reported in the literature for gluten containing approximately 7–9% (w/w) moisture.[31–34] In addition, the range of processing temperatures used in

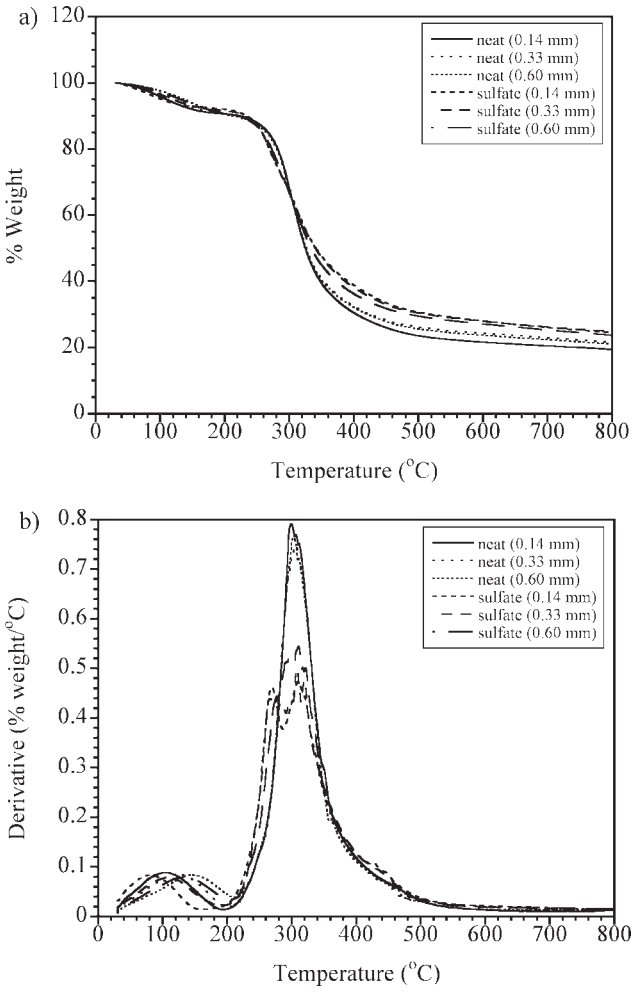

Figure 4 TGA curves of the (a) weight percentage and (b) derivative of the weight percentage as a function of temperature for the films processed at 30°C.

TABLE I
Glass-Transition Temperatures of the Wheat Gluten Films

Process temperature (°C)	Spacer (in.)	Glass-transition temperature (°C)	
		Neat[B]	Sulfated[A]
30[NS]	0.01	52.2 ± 8.1[NS]	53.0 ± 1.4[NS]
	0.025	50.7 ± 2.0	49.4 ± 1.2
	0.05	53.2 ± 2.1	51.3 ± 2.0
50	0.01	54.3 ± 2.3[b]	51.1 ± 2.8[b]
	0.025	50.5 ± 1.7[a]	45.8 ± 1.9[a]
	0.05	52.8 ± 1.4[b]	51.2 ± 2.4[b]
70	0.01	51.8 ± 1.5[b]	51.1 ± 2.7[b]
	0.025	50.1 ± 0.3[a]	46.7 ± 1.3[a]
	0.05	52.6 ± 1.6[b]	51.1 ± 0.7[b]

NS = no significant difference.
[A,B] Significant difference between both samples.
[a,b] Significant difference among the spacers at each process temperature.

this study did not alter the glass-transition temperatures of the films.

Water uptake

Sulfated gluten films were able to absorb up to 30 times their weight in deionized water. Also, thin gluten films had higher water uptake values than thick films. This is shown in Table II, where we present the water uptake values of films processed at different temperatures after each of four soakings. The 0.15, 0.3, and 0.6 mm thick films had water uptake values of approximately 29, 18, and 8, respectively. In comparison, a 0.15 mm thick neat gluten film had a water uptake value of 1.5 ± 0.2. A semilogarithmic plot of water uptake as a function of film thickness is shown in Figure 5. The water uptake values showed an exponential dependence on film thickness:

$$W = 44.621e^{-2.8973t} \quad (2)$$

where W is the water uptake (g of water/g of dry gluten) and t is the film thickness (mm).

The water uptake values seemed to be related to the concentration of sulfate groups in each film. Higher sulfate concentrations resulted in higher water uptake values (see Figs. 2 and 5). The sulfate anions attached to the gluten backbone electrostatically repelled each other. Consequently, this anionic repulsion enabled the gluten chains to expand and retain large amounts of water. The sulfate anions behaved in a similar fashion to the carboxylate anions present in poly(acrylic acid)-based superabsorbent materials.

The temperature of film formation did not have an effect on the water uptake values of the sulfated gluten films. This is shown in Table II. The FTIR results (data not shown) indicate that no differences existed between the spectra of films processed at different temperatures. The TGA and DSC results also show no effect of temperature on the thermal stability and thermal properties of the films, respectively. These results indicate that the temperature range used in this study did not affect the chemical composition and thermal properties of the gluten films before their reaction with sulfuric acid. Consequently, the films could be dried faster at higher temperatures without any effect on their final sulfated gluten properties.

The sulfated gluten films lost some of their water uptake capacity after each soaking. This is shown in Table II. The largest reduction in water uptake occurred after the first soaking. In fact, after the first soaking, the 0.15, 0.3, and 0.6 mm thick films lost approximately 37, 47, and 46% of their water uptake capacities, respectively. For later soakings, the loss in water uptake capacity occurred at a lower rate. This reduced capacity correlated with a decrease in the sulfate concentration after successive soakings. For example, a 0.15-mm film processed at 50°C had normalized sulfate absorbance values of 0.855 ±

TABLE II
Water Uptake of the Sulfated Wheat Gluten Films

Spacer (in.)	Film thickness (mm)	Temperature (°C)	Water uptake (g of water/g of gluten)			
			First soak	Second soak	Third soak	Fourth soak
0.01	0.14 ± 0.01	30[NS]	28.4 ± 8.8[c,D]	17.9 ± 1.2[c,C]	16.0 ± 2.0[c,B]	13.7 ± 2.3[c,A]
	0.15 ± 0.03	50	28.2 ± 9.4[c,D]	19.9 ± 5.2[c,C]	17.7 ± 5.1[c,B]	16.1 ± 5.8[c,A]
	0.16 ± 0.01	70	29.9 ± 4.9[c,D]	16.9 ± 5.1[c,C]	16.1 ± 4.7[c,B]	15.6 ± 4.6[c,A]
0.025	0.33 ± 0.05	30[NS]	15.2 ± 2.5[b,D]	11.3 ± 2.6[b,C]	8.5 ± 4.0[b,B]	9.2 ± 2.7[b,A]
	0.33 ± 0.04	50	18.7 ± 4.3[b,D]	9.9 ± 2.1[b,C]	7.3 ± 1.6[b,B]	7.9 ± 0.6[b,A]
	0.30 ± 0.02	70	19.3 ± 3.0[b,D]	7.2 ± 0.3[b,C]	6.8 ± 2.8[b,B]	6.8 ± 3.2[b,A]
0.050	0.60 ± 0.07	30[NS]	7.7 ± 1.4[a,D]	4.8 ± 0.8[a,C]	2.7 ± 0.3[a,B]	2.2 ± 0.3[a,A]
	0.59 ± 0.03	50	9.2 ± 0.7[a,D]	3.3 ± 0.4[a,C]	2.6 ± 0.6[a,B]	2.0 ± 0.8[a,A]
	0.63 ± 0.06	70	6.5 ± 1.3[a,D]	4.6 ± 0.4[a,C]	3.7 ± 0.2[a,B]	3.5 ± 0.8[a,A]

[a,b,c] Data with different lowercase superscripts in columns are significantly different at $p < 0.05$.
[A,B,C,D] Data with different uppercase superscripts in rows are significantly different at $p < 0.05$.

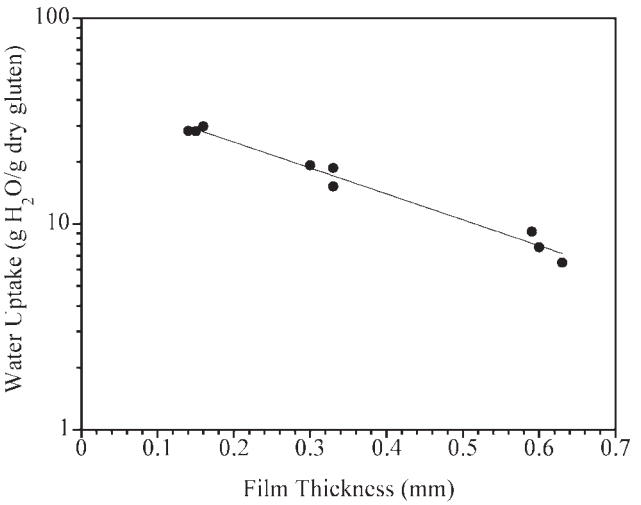

Figure 5 Semilogarithmic plot of the water uptake of the sulfated gluten films as a function of the film thickness. The line is an exponential fit to the data and has an R^2 value of 0.98.

0.31, 0.782 ± 0.039, 0.789 ± 0.023, 0.758 ± 0.036, and 0.745 ± 0.075 after zero, one, two, three, and four soakings, respectively. Such a decrease in the sulfate concentration might have resulted from the progressive removal of water-soluble gluten fractions during the repeated soaking processes. Fewer sulfate anions meant less electrostatic repulsion in the film. Consequently, the gluten film did not expand as much and did not absorb as much water.

The sulfated gluten films exhibited lower water uptake capacity when they were soaked in a saline solution. The 0.15 mm thick film processed at 30°C had a water uptake value of 3.5 ± 0.1 in a 0.9% (w/w) NaCl solution compared to 28.4 in deionized water. This decrease in the water uptake value could be explained by several factors. The first involved the screening effect of Na^+ ions on sulfate anions. The screening of sulfate anions reduced the repulsion between them and resulted in less expansion of the gluten film. In addition, the presence of ionic species in the solution reduced the difference in the osmotic pressure between the solution and the interior of the gluten film. Osmotic pressure is a driving force for water uptake, and a reduction in the pressure difference should result in a lower water uptake. Previous studies have shown that polymers containing sulfate groups should be less sensitive to saline solutions than polymers containing carboxylate groups.[2,20] Sulfate groups ionize more readily than carboxylic acid groups, and consequently, sulfate groups have lower degrees of association with counter ions in saline solution. The lower degree of association should result in a lower screening effect of counter ions on sulfate groups and should lead to higher water uptake values. Several studies have shown that the water uptake in sulfated polymers was less sensitive to added salts than nonsulfated polymers.[2,9,20] However, the sulfated gluten films in this study still showed a large reduction in water uptake when they were soaked in saline solution.

CONCLUSIONS

We produced sulfated wheat gluten films that absorbed up to 30 times their weight in water. The temperatures used to form the films had no effect on the final absorbance properties of the sulfated films. However, thinner films had higher concentrations of sulfate groups than thicker films; this resulted in higher water uptake values. Soaking each film multiple times led to a reduced water uptake capacity. The largest decrease in water uptake values occurred after the first soaking; this was most likely due to a loss of soluble fractions from the film. Also, soaking the sulfated gluten film in a 0.9% (w/w) NaCl solution reduced the water uptake value by approximately one order of magnitude compared to soaking in deionized water.

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