

Mechanical and Moisture Resistance Performance of Silver Nanoparticle Reinforced Fish Skin Gelatin Films

H. Harding^{*}, A. Ludwick^{*}, T. Samuel^{**}, S. Young^{*}, and H. Aglan^{*}

^{*}Department of Mechanical Engineering, Tuskegee University
218 Foster Hall, Tuskegee University, Tuskegee, AL 36088, aglanh@tuskegee.edu

^{**}Department of Pathobiology, Tuskegee University, Tuskegee, AL, USA

ABSTRACT

Films from the biodegradable polymer gelatin have been studied for biomedical applications not only because of the film forming properties of gelatin, but also because of the availability and economical advantages of gelatin. In this work, nanostructured silver reinforced biocompatible films for controlled drug release were developed. Cross-linked and uncross-linked films from cold water fish skin gelatin were prepared. The cross-linking agent was 1,4-butanediol diglycidyl ether (1% (v/v)). Silver nanoparticles at loadings of 0.3% (w/v) were included in both types of films. A plasticizer was used for all neat and silver-loaded films. Cross-linking of the gelatin enhanced the mechanical properties and moisture absorbing properties of the films even though the uncross-linked film is completely soluble in water while the cross-linked is not. However, the strain to failure of the cross-linked films decreased in comparison to the uncross-linked films. Studies of biomedical applications are underway.

Keywords: silver nanoparticles, fish skin gelatin, tearing energy, moisture resistance

1 INTRODUCTION

Gelatin is a heterogeneous mixture of proteins extracted from mainly skin, tendons, ligaments, and bones. The structure of the gelatin contains repeating sequences of glycine-X-Y where X and Y are most frequently proline and hydroxyproline [1]. These sequences are the building blocks for the triple helical structures of the gelatin. Cross-linking of gelatin films is done in order to strengthen the mechanical properties and decrease the solubility of the films for use in biomedical applications. In a recent study, the solubility of fish-skin gelatin films was decreased by increasing the amount of the cross-linking agent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide [2].

In this work, gelatin extracted from cold water fish skin was examined. The objective was to formulate and manufacture fish skin gelatin films containing silver nanoparticles. The microstructure and processing conditions of these films were formulated with silver nanoparticles at a concentration of 0.3% (w/v). The tearing energy and moisture uptake properties of these gelatin films with silver nanoparticles were characterized.

2 MATERIALS AND EXPERIMENTAL

Gelatin (CAS # 9000-70-8) from cod, pollock, and haddock (cold water fish skin), with an average molecular weight of 60 kDa, was purchased from Sigma. This gelatin is a heterogeneous mixture of proteins extracted by a proprietary process, developed by the supplier. It remains unchanged for many years in sealed containers at room temperature, gelling below 35 °C. The isoelectric point of this gelatin is approximately 6. The plasticizer D-sorbitol was purchased from Fisher Scientific (BP439) (CAS# 50-70-4). The gelatin was cross-linked using 1,4-butanediol diglycidyl ether (CAS# 110-63-4) in a standard buffer solution of pH 10, both purchased from Sigma Aldrich.

Silver nano powder (less than or equal to 100 nm in diameter) was purchased from Sigma-Aldrich Inc. (Product #576832). These nanoparticles have an organic coating for dispersion in polar solvents and a high surface area (5.0 m²/g).

2.1 Processing of Neat and Nanoreinforced Films (Neat and 0.3%)

Gelatin powder was dissolved in distilled water while being mixed on a magnetic stirring plate (7.5 grams per 60 ml of water; 12.5%). The ambient temperature was about 20 °C ± 5 °C and it took approximately 15 minutes for the gelatin to dissolve. Sorbitol was added into the gelatin solution (0.3 g sorbitol per 1 g of gelatin) in order to make the films soft [3]. For the nanoreinforced films, the silver nanoparticles were mixed into the 12.5% gelatin solution and the system was mixed overnight. The concentration of the silver nanoparticles was 0.3% (w/v). About 30 mL were poured into Teflon molds lined with Bytac[®] contact paper and dried at 37 °C for 7 h in a convection oven.

After the films cooled to ambient temperature, a thin razor blade was used to carefully lift the outer edges of the film from the mold. All of the films were stored at ambient temperature in plastic sheets with 6 mm holes for ventilation, for at least a week before testing.

2.2 Processing of Cross-linked Neat and Nanoreinforced Films (crNeat and cr0.3%)

A method for cross-linking gelatin films was modified from a recent study involving gelatin-chitosan scaffolds which were cross-linked with 1,4-butanediol diglycidyl ether [4]. First, a 12.5% (w/v) solution of gelatin was fabricated by dissolving gelatin in a standard buffer solution of pH 10. The solution was mixed for about 45 minutes; then 2.625 g of sorbitol was added into the solution and mixed for 10 minutes at room temperature. For the nanoreinforced films, silver nanoparticles were added at 0.3% (w/v) to the solution. The solution was electronically mixed at $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ overnight. The cross-linking agent, 1,4-butanediol diglycidyl ether, was added to form a 1% (v/v) solution and it was electronically mixed at 50°C for 4 hr with an air-cooled condenser. Finally, the solution was poured into Teflon molds and it was dried at 37°C for 7 h in a convection oven.

In this work, the selection of a pH of 10 for the cross-linking reaction was based on convenience. No clear colorless commercial pH 9 buffer was available. Hence a commercial colorless pH 10 buffer was used. Recently, 1,4-butanediol diglycidyl ether was used to cross-link a gelatin, chitosan, and hydroxypropyl hybrid at a pH of 9 for a biomedical application [4]. Cross-linking occurred when the epoxide groups of the agent, 1,4-butanediol diglycidyl ether reacted with the amine groups of gelatin [5]. The extent of cross-linking is unknown since not all of the epoxy groups were cross-linked. However, a study of cross-linking a gelatin film with 1,4-butanediol diglycidyl ether showed at least 50% of the film was cross-linked at a pH of 10 [6]. The result of the current work at pH 10 can be assumed to be similar.

2.3 Mechanical Testing of Gelatin Films

The tensile tests were performed on an electromechanical material testing system with a 25 N load cell. The uncross-linked samples were cut into rectangles with 6.5 mm width and a gauge length of 25.4 mm. The thickness varied from 0.3 - 0.6 mm. The samples were tested at a speed of 5.0 mm/min. The crNeat and cr0.3% films were tested with a 44.5 N load cell. The samples were cut into dog bones with a 4 mm width and a gauge length of 37 mm. At least 5 samples of all films were tested. The tearing energy tests were conducted on notched rectangular samples with 12.7 mm width and a gauge length of 25.4 mm.

2.4 Instrumentation

Fracture surfaces of notched samples from tearing energy experiments were examined with a Hitachi S-2150 scanning electron microscope (SEM). Thermal scans of the films were done on a TA Instruments DSC Q1000.

3 RESULTS AND DISCUSSION

3.1 Mechanical Properties of Neat and Cross-linked Neat Gelatin Films

A comparison of the Neat and crNeat films is shown in Figure 1. The crNeat films had higher ultimate strengths than the Neat films but lower strength to failure [7-8]. The average ultimate strength was increased by 126% and the average strain to failure was considerably reduced by 96% after cross-linking. Strain-hardening is observed in the stress-strain curve of the crNeat film but not in the uncross-linked film. The crNeat film stress increased linearly to about 6 MPa, then decreased to below 5 MPa, and finally rapidly increased until failure near 131% strain. The Neat film stress linearly increased to about 2 MPa and then gradually increased until failure at 300% strain. Cross-linking has caused strain hardening to occur in the crNeat stress-strain behavior. The crNeat films are brittle compared to the Neat film as seen by the lower strain to failure.

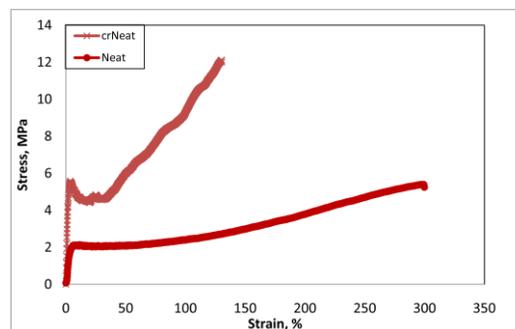


Figure 1: Stress vs. strain behavior of the neat and cross-linked neat films

3.2 Mechanical Properties of Silver-filled Gelatin Films

The stress-strain behavior of 0.3% and cr0.3% films is shown in Figure 2. There was no significant difference between the strain to failure nor the ultimate strength of the 0.3% and cr0.3% films. Strain-hardening was observed in the stress-strain curve of the cr0.3% film. The stress of the cr0.3% film increased linearly to about 3.5 MPa, then decreased to below 3 MPa, and finally rapidly increased until failure near 140% strain. Cross-linking of gelatin films has been observed to increase the tensile strength or have no effect [9]. Interestingly, the strain to failure has been determined to either increase, decrease, or remain constant, after cross-linking [9].

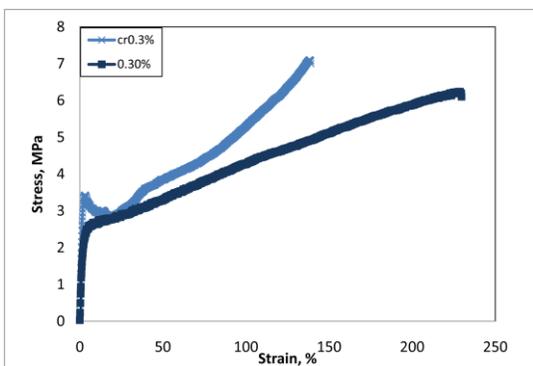


Figure 2: Stress vs. strain behavior of the silver-filled gelatin films

3.3 Tearing Energy of Silver-Filled Gelatin Films

Elastomeric materials have a characteristic property of resistance to tear propagation, known as the tearing energy. A higher tearing energy means the material has a stronger resistance to tearing and is a tougher material. It is important in the mechanical design of materials to calculate the tearing energy to determine if the material has an acceptable tearing energy for a given application. Equations for the tearing energy have been developed and used for many years. In a recent study [10], the following tearing energy equation was used:

$$T = 2KaW_0 \quad (1)$$

where a was the initial cut length, W_0 the strain energy, and $K = \pi/\sqrt{\lambda}$, λ was the extension ratio. The strain energy was calculated by computing the area under the load-displacement curve and dividing it by the volume of the sample. Another factor of performance of polymers is the tear strength [10]. It is the maximum force required to tear a sample with elastic properties. The equation is:

$$T_s = F/d \quad (2)$$

where F is peak load and d is the sample thickness.

The load vs. displacement curves of three typical notched 0.3% samples films are plotted in Figure 3. The load rapidly increased to about 7 N and then more gradually reached an average maximum load of 9.6 ± 0.6 N before the tear propagated to complete fracture. The areas under the curves were approximated by Microcal Origin software since the relationship was non-linear. The cut depth, tearing energy, and tear strength values were calculated for each of the films as seen in Table 1.

In the cross-linked samples, the load vs. displacement curves, plotted in Figure 4, increased more gradually and the average maximum load reached was lower at 7.1 ± 0.8 N.

	Cut Depth (mm)	Tearing Energy (KJ/m ²)	Tear Strength (KN/m)
0.3%	4.5	31.3	2.0
	4	33.8	2.0
	4	34.4	2.6
cr0.3%	5	19.4	1.1
	4.9	18.9	1.1
	5	20.9	1.4

Table 1: Tearing Energy Data for Gelatin Films.

It should be noted that the average tearing energy and tear strength of the uncross-linked films was higher than the cross-linked by 40% and 47%, respectively. This was attributed to the higher resistance to tear propagation observed in the uncross-linked films, thus requiring more tearing energy and tear strength (Table 1). No tearing energy values were found for comparison in the literature. Only the initial tensile strengths to propagate a tear in notched bovine gelatin films has been reported in the literature [11].

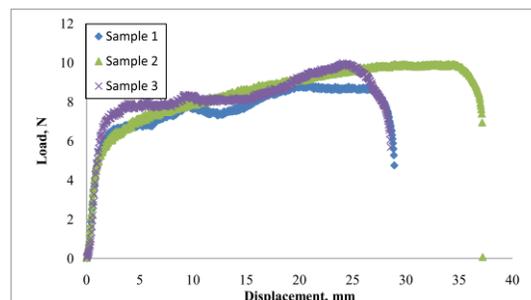


Figure 3: Load - Displacement behavior of notched uncross-linked silver-filled gelatin films.

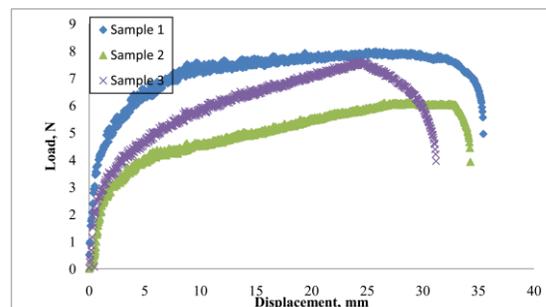


Figure 4: Load - Displacement behavior of notched cross-linked silver-filled gelatin films.

3.4 Moisture Uptake of Gelatin Films

A comparison of the moisture uptake of the cross-linked and the uncross-linked films is shown in Figure 5.

Initially, the moisture uptake properties were not affected by the cross-linking; however, after about 150 hrs, the weight of the cross-linked films was significantly greater than the uncross-linked films. At this point, the cross-linked films absorbed moisture more rapidly than the uncross-linked films. This observation could be related to the differential scanning calorimetry (DSC) patterns noted for the cross-linked and uncross-linked samples. Broader endotherms interpreted as due to water absorption were observed in the DSC scans of the cross-linked films compared to the uncross-linked films.

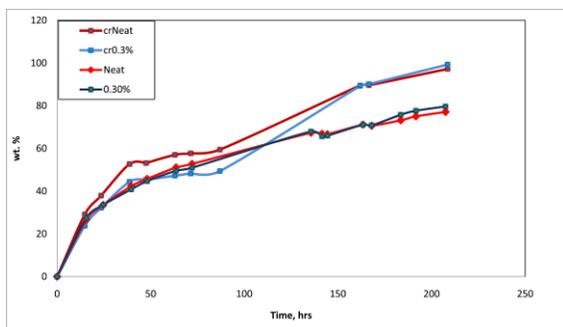


Figure 5: Moisture uptake of cross-linked silver-filled gelatin films compared to uncross-linked films with time.

4 CONCLUSIONS

After cross-linking, the mechanical properties of the uncross-linked films were increased by 126%; the strain to failure was decreased. Strain-hardening was observed in the stress-strain behavior of the cross-linked neat films. The addition of silver nanoparticles slightly increased the ultimate strength of the uncross-linked system and decreased the ultimate strength of the cross-linked system.

The tearing energy was found to be higher for the uncross-linked silver-filled films than for the cross-linked silver-filled films by about 40%. This data was correlated to the SEM observations made from the fracture surface morphologies. It was determined that the mechanism of resistance to tear propagation was tearing ridges. The uncross-linked silver-filled films had coarse tearing ridges and more pull-out material than the cross-linked silver-filled films.

It was found that the moisture uptake properties of the cross-linked films were greater than the uncross-linked after about 150 hrs. At about 208 hrs the percent moisture uptake of the cross-linked silver-filled films increased by about 25% compared to the uncross-linked.

ACKNOWLEDGEMENTS

This work was supported by Alabama A&M Research Institute GRSP contract # AAMURI – 08-C-0016 and NSF- CREST HRD-0317741.

REFERENCES

- Choi, Y., Song, K., Park, M., Nam, Y., *Study on gelatin-containing artificial skin: Preparation and characteristics of novel gelatin-alginate sponge*. *Biomaterials*, 1999. **20**: p. 409-417.
- Piotrowska, B., Sztuka, K., Kolodziejska, I., Dobrosielska, E., *Influence of transglutaminase or 1-ethyl -3-(3-dimethylaminopropyl) carbodiimide (EDC) on the properties of fish-skin gelatin films*. *Food Hydrocolloids*, 2008. **22**: p. 1362-1371.
- Carvalho, R.A., Sobral, P.J.A., Thmazine, M., Habitante, A.M.Q.B., Gimenez, B., Gomez-Guillen, M.C., Montero, P., *Development of edible films based on differently processed Atlantic halibut (*Hippoglossus*) skin gelatin*. *Food Hydrocolloids*, 2008: p. 1117-1123.
- Wang, S., Liu, W., Han, B., Yang, L., *Study on a hydroxypropyl chitosan-gelatin based scaffold for corneal stroma tissue engineering*. *Applied Surface Science*, 2009. **255**: p. 8701-8705.
- Zeeman, R., van Wachem, P., van Luyn, M., Hendriks, M., Cahalan, P., Feijen, J., *Crosslinking and modification of dermal sheep collagen using 1,4-butanediol diglycidyl ether*. *Journal of Biomedical Materials Research* 1999. **46**: p. 424-433.
- Zeeman, R., Dijkstra, P., van Wachem, P., van Luyn, M., Hendriks, M., Cahalan, P., Feijen, J., *The kinetics of 1,4-butanediol diglycidyl ether crosslinking of dermal sheep collagen*. *Journal of Biomedical Materials Research Part A*, 1999. **51**(4): p. 541-548.
- Bigi, A., Panzavolta, S., Rubini, K., *Relationship between triple-helix content and mechanical properties of gelatin films*. *Biomaterials*, 2004. **21**: p. 5675-5680.
- Boanini, E., Rubini, K., Panzavolta S., Bigi, A., *Chemico-physical characterization of gelatin films modified with oxidized alginate*. *Acta Biomaterialia*, 2009. **6**(2): p. 383-388.
- Chiou, B., Avena-Bustillos R., Bechtel, P., Jafri, J., Narayan, R., Imam, S., Glenn, G., Orts, W., *Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties*. *European Polymer Journal*, 2008. **44**(11): p. 3748-3753.
- Aglan, H., Calhoun, M., Allie, L., *Effect of UV and Hygrothermal Aging on the Mechanical Performance of Polyurethane Elastomers*. *Journal of Applied Polymer Science*, 2008. **108**: p. 558-564.
- Rivero, S., Garcia, M.A., Pinotti, A., *Correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films*. *Innovative Food Science and Emerging Technologies*, 2009. **11**(2): p. 369-375.