

## Properties and Structure of Sericin Films: Effect of the Crosslinking Degree

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Sericin is a natural silk protein which is removed from silk in a process called degumming. Thus, finding a use for the extracted sericin as a biopolymer film will create added value product which will benefit both the economy and society. The films were manufactured with silk sericin, using different dimethylolurea (DMU) concentrations as cross-linking agent and glycerol as plasticizer. Sericin films produced by crosslinking method were light yellow, homogeneous, transparent and visually attractive. The average film thickness was  $0.10 \pm 0.02$  mm. The biofilms show low water solubility (up to 30% of total dry mass), good tension strength and high elongation ability. The water vapor permeability is moderate, typical of highly hydrophilic films. Structural transformations in silk sericin films were analyzed using Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectroscopy and X-ray diffraction. This resulted in aggregated  $\beta$ -sheet structure (peak at  $1616\text{ cm}^{-1}$  in the amide I absorption) by FTIR studies and increasing the DMU concentration in film decreased the peak intensity at  $2\theta = 20^\circ$ . Sericin-based film properties are dependent on components used to form film, which can be used to tailor the desired film flexibility and minimize permeability of films.

### 1. Introduction

Sericin is a highly hydrophilic macromolecular protein comprising of 18 amino acids. Molecular weight of sericin protein ranges from 24 to 400 kDa with predominant amino acid groups serine (40%), glycine (16%), glutamic acid, aspartic acid, threonine and tyrosine. Thus it consists of polar side chain made of hydroxyl, carboxyl and amino groups that enable easy cross-linking, copolymerization and blending with other polymers to form improved biodegradable materials (Takasu et al., 2002).

Silk sericin (SS) is one of essential components of cocoon filament comprising granular and high molecular proteins with adhesive and gelatin-like characteristics. The silk fibers are composed of a fibrous core protein fibroin (hydrophobic in nature) with sericin surrounding it (Dash et al., 2007).

Silks have evolved to be some of nature's most impressive composite materials. Silk fibers are not only one of the toughest polymers known, but they have a number of other characteristics that, although less well known, make them an interesting as well as an important object for research in the general areas of biopolymers, protein folding, biomimetics and the coevolution of behavior, morphology and function (Zhang, 2002).

While manufacturing silk, sericin is removed as waste during the degumming process. If sericin can be utilized to make biofilms as a value-added product developed from the

wastes, it will represent a significant source of profit, not to mention the beneficial effect of waste reduction for pollution prevention. This study finds an interesting application.

Sericin with such high potential has been always neglected in the field of tissue engineering due to its weak structural properties and high water solubility. Chemical crosslinking has shown to be an effective approach to suppress excessive swelling of biofilms in order to retain a high selectivity, although this is often accompanied with a reduction in the permeation flux. Films crosslinking will also help improve the thermal, chemical and mechanical stabilities of the films. In the present study, chemically crosslinked sericin films were developed. Dimethylolurea (DMU) appears to be an appropriate crosslinking agent as the crosslinking will occur primarily through the hydroxyl functionality of the polymers (Yao et al., 2005).

The present work establishes a methodology to obtain sericin films with different characteristics. The effect of the crosslinking agent (DMU) concentration on the properties of sericin films was evaluated.

## 2. Materials and Methods

Sericin was extracted from the cocoons of *Bombyx mori* silkworm. The cocoons were cut into small pieces (about 1 cm<sup>2</sup>) and then immersed in distilled water in ratio 3:100 (w/v). In order to obtain a fraction of sericin proteins having relatively large molecular weights, the extraction was carried out in a two-step process. The first step, aqueous SS solutions were extracted with hot water under pressure at 120 °C for 60 min using an autoclave. In the second step, the solution was then deep frozen at -20°C to enhancing precipitation. The frozen solution was later defrosted and filtered under vacuum. The concentration of SS was adjusted to 2 wt % before use. The molecular weight of the sericin obtained was much greater than 200 kDa as measured by gel permeation chromatography. DMU (HPLC grade, Sigma-Aldrich, USA) was used as crosslinking agent and glycerol (Synth, Brazil) as plasticizer.

### Preparation of sericin films

Sericin films were made by casting. Polymer solution with mass fraction of sericin (2 % w/v) was prepared in distilled water already containing glycerol (0.6 g glycerol/g sericin) at room temperature. The solution was mechanically stirred for about 1 h to ensure homogeneity. Afterwards, the temperature of the system was raised to 70 °C and a dilute aqueous DMU solution was added to the biopolymer solution until a total amount of 0.1, 0.2, 0.4, 0.6 and 0.8 g DMU/g sericin. The increase in temperature was necessary to avoid film heterogeneities. A defined amount (70 g) of the crosslinked film forming solution was poured into polystyrene Petri dishes (d = 15 cm) and dried in a convection oven (Marconi – MA035, Brazil) at 40 °C for 18 h. All films were conditioned at room temperature and 52% relative humidity inside desiccators for 3 days before characterization.

### Film characteristics

#### Solubility in water, $S_w$

The solubility in water of the films was measured as proposed by Irissin-Mangata (2001). The moisture weight fraction,  $\omega$ , of the film was gravimetrically determined in a vacuum oven (Lab-Line, Squaroid, USA) at 105 °C for 24 h. Disks cut from the same

film ( $d = 2.5$  cm), were weighed (total mass  $m_o$ ) and immersed in 50 mL of distilled water using a 250 mL beaker maintained under mild agitation (175 rpm) at 25 °C for 24 h (Tecnal, TE-421, Brazil). The final dry matter ( $m_f$ ) of the sample was determined in the same vacuum oven (105 °C/24 h). The fractional solubilized matter ( $S_w$ ) was calculated as a function of the initial dry matter using Eq. (1).

$$S_w = [m_o(1-\omega) - m_f] / m_o(1-\omega) \quad (1)$$

### Mechanical properties

Tensile strength (TS) and percentage tensile elongation at break (E) of the preconditioned films were determined at room temperature using a TA.XT2 (Stable Microsystems SMD, England) according to ASTM standard method D882 (ASTM, 1995b). Films were cut into strips (10 x 2.54 cm) and mounted between the corrugated tensile grips of the instrument. The initial grip spacing and cross-head speed were set at 50 mm and 0.1 cm/s, respectively. The tensile strength was expressed as the maximum force at break divided by the initial cross-sectional area of the film strip and the elongation at break as a percentage of the original length.

### Water vapor permeability coefficient (WVP)

The water vapor permeability coefficient through the films was determined gravimetrically at 25 °C according to method E96-95 (ASTM, 1995a). Circular cuts of the films, 5 cm in diameter, were hermetically fixed using test cells. The effective films free surface area for vapor permeation was 9.6 cm<sup>2</sup>. Granulated calcium chloride (2% RH) was used inside the cells as desiccant. The cells were placed inside desiccators maintained at a constant relative humidity of 75% obtained with saturated NaCl solution. The water vapor permeability coefficient (WVP) was then calculated using Eq. (2). All determinations were made in triplicates.

$$WVP = [(G/t) \cdot L] / [A \cdot P_w \cdot (RH_1 - RH_2)] \quad (2)$$

where  $WVP$  is the water vapor permeability coefficient [g mm/(m<sup>2</sup> day kPa)];  $L$  is the average thickness of the film [mm];  $G/t$  is the apparent steady state rate of weight gain [g/day];  $t$  is the total time [day];  $A$  is the permeation area [m<sup>2</sup>];  $(RH_1 - RH_2)$  is the difference of relative humidities [=0.75] and  $P_w$  is the partial water vapor pressure at test temperature (25 °C) [kPa].

### Fourier transformed infrared spectroscopy

Infrared spectra were obtained using a FTIR spectrometer Varian – series 7000 at a wave-number region of 600-2000 cm<sup>-1</sup>, resolution of 2 cm<sup>-1</sup> and the scan frequency of 16 times.

### X-ray diffraction

X-ray diffraction intensity curves were obtained at a scanning rate of 0.0015° s<sup>-1</sup> for 2θ from 5 to 50° with a diffractometer Shimadzu - D 6000 using Cu radiation.

### Statistical analysis

Analysis of variance (ANOVA) and Tukey Test were used to determine statistically significant differences ( $p < 0.05$ ) among averages using the Software Statistica 7.0.

### 3. Results and Discussion

#### Films physical characterization

Sericin films produced by crosslinking method were light yellow, homogeneous, transparent and visually attractive, whereas glycerol extended film flexibility. The average film thickness was  $0.10 \pm 0.02$  mm. The use of a limited amount of crosslinking agent was necessary to consolidation of the film structure and the addition of DMU reduced film flexibility.

The water solubility of the sericin films with different concentrations of DMU are shown in Table 1. The solubility in water of the films conditioned at 52% RH decreased consistently and significantly by increasing the DMU content present in the crosslinking solution. This is probably caused by the higher crosslinking degree obtained by the sericin films when a determined DMU concentration was used and the better aligned incipient polymeric structure assisted the strengthening action to formation film. Zactiti and Kieckbusch (2006) also observed a decrease in solubility after crosslinking with  $\text{CaCl}_2$  in sodium alginate films.

It was well established that an increase in DMU content decreased film WVP. The WVP of sericin films with more content of DMU was lower than that from low content of DMU sericin films as shown in Table 1. This was because the sericin possesses many hydrophilic groups such as  $-\text{OH}$ ,  $-\text{NH}$  and  $-\text{COOH}$  which regulate water absorption in the monolayer. These results indicated that molecular environment of silk sericin was affected by the addition of DMU not conformation but packing of molecular chains.

The tensile strength (TS) accounts for the film mechanical resistance due to the cohesion between the chains, while the elongation at break (E) measures its plasticity, which is the capacity of the film to extend before breaking. Due to the structural nature of these attributes, usually films with high TS show low E and so that both properties should be analyzed simultaneously. A comparison between the experimental data (Table 1) confirms that variations in the DMU concentration exert an appreciable reducing effect on film elongation but only a moderate positive effect on film tensile strength. According to the classification established by Krochta and De Mulder-Johnston (1997), the water vapor permeability and the mechanical attributes obtained by sericin films can be considered as moderate, compared to synthetic films.

Table 1: Effect of the DMU concentration in physical properties of sericin films.

Film	g DMU/ g sericin	$S_w$ (%)	WVP (g.mm/m <sup>2</sup> .day.kPa)	TS (MPa)	E (%)
DMU 10	0.10	$32.7 \pm 0.33^c$	$9.82 \pm 0.33^d$	$6.3 \pm 0.4^a$	$36,73 \pm 2,31^b$
DMU 20	0.20	$27.1 \pm 0.27^d$	$9.30 \pm 0.28^d$	$6.7 \pm 0.6^{a,b}$	$35,34 \pm 3,07^b$
DMU 40	0.40	$25.6 \pm 0.10^c$	$7.33 \pm 0.16^c$	$7.3 \pm 0.7^{a,b,c}$	$32,89 \pm 2,78^{a,b}$
DMU 60	0.60	$24.9 \pm 0.18^b$	$6.35 \pm 0.12^b$	$8.4 \pm 0.7^{b,c}$	$30,63 \pm 3,55^{a,b}$
DMU 80	0.80	$23.8 \pm 0.12^a$	$0.88 \pm 0.09^a$	$8.8 \pm 0.8^c$	$26,38 \pm 2,13^a$

Average with the same letter in the same column indicates no significant difference ( $p < .05$ ) by Tukey test.

#### Structures of sericin films

Structural transformations in sericin films were analyzed using X-ray diffraction and FTIR studies. The result of wide-angle X-ray diffraction curve intensity is shown in Fig. 1. Miyake et al. (2003) studying high molecular weight sericin films found a diffraction

peak near  $2\theta = 20^\circ$ , and a shoulder peak at near  $2\theta = 12^\circ$ ,  $28^\circ$ , and  $43^\circ$ . In the present study, on further crosslinking with DMU, sericin films showed a decrease in peak intensity at  $2\theta = 20^\circ$ . Earlier studies suggested native sericin containing both random coils and  $\beta$ -sheets representing amorphous and crystalline regions respectively (Dash et al., 2007) and upon crosslinking with DMU caused a transformation of  $\beta$ -sheet and aggregated  $\beta$ -sheet structure to random coil due to intermolecular hydrogen bonding (Teramoto et al., 2007). This resulted in a decrease in the amount of crystallinity of X-ray diffraction curve. FTIR was used to confirm secondary structural transition, when crosslinking with DMU (Fig. 2).

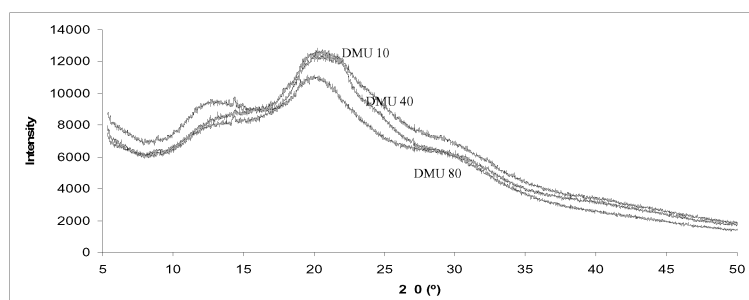


Figure 1: Wide angle X-ray diffraction patterns of sericin films.

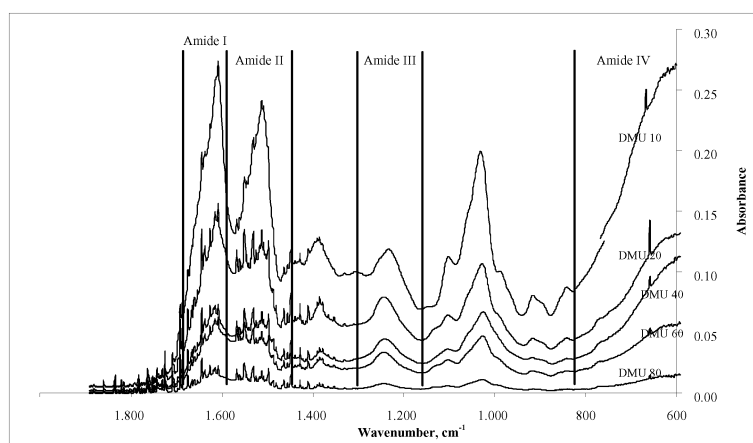


Figure 2: FTIR spectra of sericin films with different DMU concentration.

There are four different types of distinguishable vibration peaks associated with protein amide-amide I ( $1616\text{ cm}^{-1}$ ), amide II ( $1530\text{ cm}^{-1}$ ), amide III ( $1242\text{ cm}^{-1}$ ) and amide IV ( $667\text{ cm}^{-1}$ ). Amide bonds form the polypeptide backbone and due to specific vibrational frequencies result in conformational changes in protein molecules. Crosslinking with DMU caused changes in the secondary structure of sericin films (Fig. 2). The appearance of bands between  $1610$  and  $1625\text{ cm}^{-1}$  is often observed for solvent and

thermally denatured proteins. The stronger the hydrogen bond involving the amide C=O, the lower the electron density in the C=O group and the lower the amide I absorption appears. Hence, the absorptions at lower wavenumber ( $1616\text{ cm}^{-1}$ ) are attributable to aggregated strands having strong intermolecular hydrogen bonds among extended chains (Teramoto and Miyazawa, 2005).

#### 4. Conclusions

Transparent and homogenous sericin films were obtained after a reticulated film manufactured by casting. The increase in DMU concentration in the film increases the tensile strength of sericin films and decreases the solubility in water, moisture content, water vapour permeability and their elongation at break, resulting from a decrease in the amount of crystallinity. As a compromise between film mechanical resistance and flexibility, keeping low solubility, the use of 0.4 g DMU / g sericin is recommended.

#### References

- ASTM. Standard test methods of water vapor transmission of materials. American Society for Testing and Materials, 1995a, Philadelphia, E 96-95.
- ASTM. Tensile properties of thin plastic sheeting. Annual Book of ASTM Standards, American Society for Testing and Materials, 1995b, Philadelphia, D 882.
- Dash, R., Ghosh, S. K., Kaplan, D. L., Kundu, S. C., 2007. Purification and biochemical characterization of a 70-kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. *Comp. Biochem. Physiol. B*, 147, 129-134.
- Irissin-Mangata, J., Bauduin, G., Boutevin, B., Gontard, N., 2001. New plasticizers for wheat gluten films. *European Polymer Journal*, 37, 1533-1541.
- Krochta, J. M., De Mulder-Johnston, C., 1997. Edible and biodegradable polymer films: Challenges and opportunities. *Food Technology*, 51 (2), 61-74.
- Miyake, H., Wakisaka, H., Yamashita, Y., Nagura, M., 2003. Moisture characteristic and structure of high molecular weight sericin films. *Polymer Journal*, 35(8), 683, 687.
- Takasu, Y., Yamada, H., Tsubouchi, K., 2002. Isolation of three main sericin components from the cocoon of the silkworm, *Bombyx mori*. *Biosci. Biotechnol. Biochem.*, 66, 2715-2718.
- Teramoto, H., Kakazu, A., Yamauchi, K., Asakura, T., 2007. Role of hydroxyl side chains in *Bombyx mori* silk sericin in stabilizing its solid structure. *Macromolecules*, 40, 1562-1569.
- Teramoto, J., Miyazawa, M., 2005. Molecular orientation behaviour of silk sericin film as revealed by ATR infrared spectroscopy. *Biomacromolecules*, 6, 2049-2057.
- Yao, W. H., Chen, J. C., Hu, M. S., Teng, M. Y., Huang, P. H., Lin, J. M., Chen, C. C., 2005. Crosslinking of cotton cellulose in the presence of serine and glycine - Physical properties and reaction kinetics. *J. Appl. Polym. Sci.*, 97, 595-603.
- Zactiti, E. M., Kieckbusch, T. G., 2006. Potassium sorbate permeability in biodegradable alginate films: Effect of the antimicrobial agent concentration and crosslinking degree. *Journal of Food Engineering*, 77, 462-467.
- Zhang, Y. Q., 2002. Applications of natural silk protein sericin. *Biomaterials. Biotechnol. Adv.*, 20, 91-100.