Journal of Applied Polymer Science

Novel Cross-Linked Sericin Films: Characterization and Properties

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ABSTRACT: In the present study, an attempt has been made to convert sericin into film form and further make it insoluble by forming sericin–aluminum metal complex using alum salt, which may lead to some extent of cross-linking. After complex formation sericin becomes insoluble in warm water as well as thermal stability and tensile strength improves significantly with increasing alum content. Metal complexed sericin films show good antimicrobial property and both the pure and alum modified sericin (AM-Sericin) films show a very good oil barrier property. But after complex formation moisture content and swelling percentage of sericin film decreases quite significantly with increasing aluminum concentration. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41400.

KEYWORDS: biodegradable; biomaterials; crosslinking; films; properties and characterization

Received 25 October 2013; accepted 15 August 2014
DOI: 10.1002/app.41400

INTRODUCTION

Sericin, a hot water-soluble globular silk protein has been a subject of many recent studies.[1–5] It has some unique properties such as good moisture absorption and releasing propensity and very good compatibility with human skin therefore it finds application in different areas such as cosmetics, pharmaceuticals, textile etc. Its antioxidant nature has been exploited in the production of sericin-coated PET or Nylon indoor air filtration membranes.[6] Its good moisture retention and UV-B light absorption characteristics have been utilized for enhancing the comfort and feel of synthetic and cotton fabrics. It acts as a felt-proofing agent when applied on wool fabric.[7–11] Sericin is also widely used as an additive in soap and shampoo for its hair care and protecting nature. It protects hair by forming a thin protective layer around it. Sericin has remarkable moisturizing property, anti-wrinkle action and it reduces epidermal water loss and also inhibits tyrosinase activity which is responsible for skin melanin synthesis and these properties makes sericin a natural ingredient for cosmetic industry.[12–14] Masahiro and co-worker reported that sericin is a natural ingredient used in food industry as it enhances the bioavailability of iron, magnesium and calcium in rats.[15] It also enhances the wound healing property of rat and it prevents the cellular death and enhances cellular growth after acute serum deprivation and it also accelerates the proliferation of several mammalian cells.[16–18]

Presently sericin is finding new applications in film form both in neat and blended compositions. Sericin is blended with different polymers to modify different properties of sericin film. It is reported that Poly-vinyl alcohol and boric acid blended sericin film enhances the mechanical and thermal stability of sericin film and these blended films find use in skin care and in drug delivery.[19] Glucomanon–sericin composite films reduce the water vapor permeability of the sericin film, which is suitable for food coating.[20] When small amount of sericin is blended with whey proteins, the mechanical strength of whey protein film enhances. It is also reported that sericin improves the surface smoothness of the chitosan–sericin blended film which can be used in food and pharmaceuticals.[21,22]

Thus, it is apparent that sericin and sericin blended films with other polymers has lot of potential applications but at the same time it has some drawbacks like poor tensile strength, poor thermal stability and solubility in warm water, which limits many application of sericin specially in film form.

In our group, earlier work has been done on recovery of sericin from degumming liquor using a combination of microfiltration, ultrafiltration, nanofiltration, and spray drying process. The structural and functional properties of spray dried sericin powder produced from various degumming liquors such as that from industrial high temperature high pressure degumming liquor, and soap-alkali degumming liquor obtained by the membrane filtration technology was compaired.[23] In another study sericin was applied on polyester fabric to improve its comfort property, where sericin was crosslinked to the sodium hydroxide pretreated polyester fabric with glutaraldehyde. Glutaraldehyde make sericin insoluble but it is not eco-friendly and carcinogenic in nature.[9] Chen et.al shows that sericin can absorb gold and other metal via charge interchange or complexation.[24] Thus

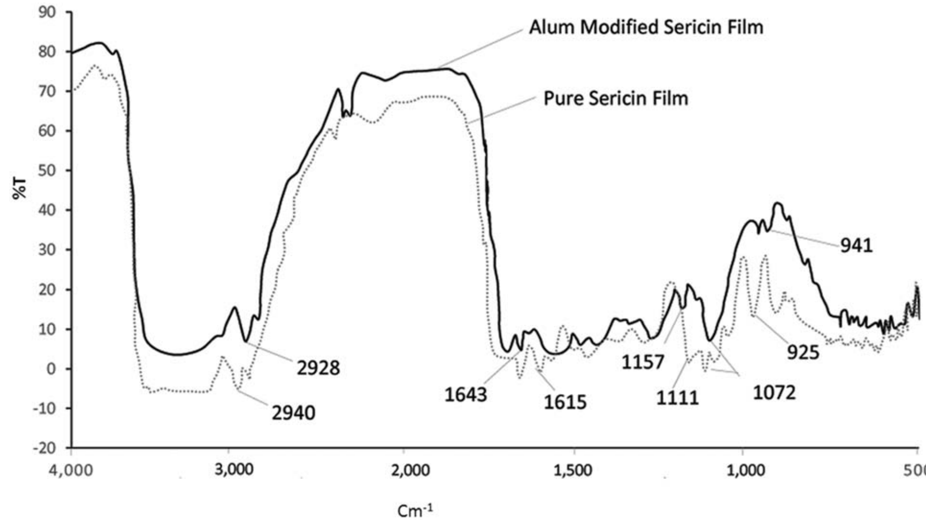


Figure 1. FTIR spectra of pure and alum modified sericin films.

in this present article, a novel ecofriendly approach has been used to enhance the properties of sericin in film form through sericin-metal complex formation using alum salt. It is expected that optimum cross-linking provided by aluminum would enhance the thermal stability and would also make sericin film insoluble in warm water while retaining its other useful properties as discussed above.

EXPERIMENTAL

Materials

Silk flat non-woven cutting waste was supplied by M/s SERICARE, Bangalore. Laboratory grade alum, Sodium sulfate, Ascorbic Acid, Eriochrome Cyanine R (dye), Electrophoresis grade acrylamide, *N*,*N*′-methylenebisacrylamide, (*N*,*N*,*N*′,*N*′-tetra methyl ethylene diamine), ammonium persulfate, Bromophenol Blue, Coomassie Brilliant Blue-250, 2-mercaptoethanol, and Electrophoresis grade glycine, sodium dodecyl sulfate, urea, Tris-HCl, glycerol, methanol, glacial acidic acid, sodium azide and Food grade edible oil has been used for the experiment without further purification.

Sericin Liquor Preparation

Sericin liquor was prepared in the laboratory by aqueous extraction of soluble material from flat non-woven silk fabric at 110°C for 30 min at material-to-liquor ratio of 1:20 in a Glycerine Bath Beaker dyeing machine, made by R. B. Electronics and Engineering, India.

Sericin Film Preparation

Sericin film was prepared by pouring filtered concentrated sericin liquor having 3.5% solid content on a flat Teflon coated surface, followed by drying at 50°C for 20 h.

Alum Treatment

To make sericin film insoluble it was treated with alum to from a sericin-Al complex with aluminum ions of alum. Sericin–Al complex was prepared by two different methods (i) treatment of sericin film with Alum (ii) addition of Alum to the sericin solution before casting the film. In alum treatment after film preparation method, 0.5 g sericin cast film was put into 100 mL of 5% alum solution where on equilibrium, sericin absorbs 0.63 wt % Aluminum or 35 mol. of aluminum per mole of sericin (considering Avg. sericin mol. wt. as 150 kDa). Molecular weight of sericin was checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis method and aluminum concentration was measured by using calibration curve of Eriochrome Cyanine R in UV spectra photometer, and it was cross-checked by ICP (APHA 1998) using metal analysis method. Alum treated sericin films prepared by this method are insoluble in warm water but they are very brittle in nature. In the second method 0.05, 0.1, 0.5, and 1% (w/w) alum was added to the sericin solution just before film preparation.

Solubility Test

To optimize the alum concentration, the water solubility of pure sericin and 0.05, 0.1, 0.5, and 1% (w/w) alum modified

Table I. Effect of Alum Treatment on FTIR Spectra of Sericin and Alum of Different Groups on Sericin Films

	Ratio of peak area			
Bond type	Pure sericin film		Alum modified sericin film	
N—H bending vibration	I_{1615}/I_{2940}	0.36	I_{1643}/I_{2928}	0.19
Carboxylic acid or alkyl hydroxyl containing amino acid side chains	I_{1111}/I_{2940}	0.27	I_{1157}/I_{2928}	0.43
	I_{1072}/I_{2940}	0.31	I_{1072}/I_{2928}	1.47
	I_{925}/I_{2940}	1.21	I_{941}/I_{2928}	0.39

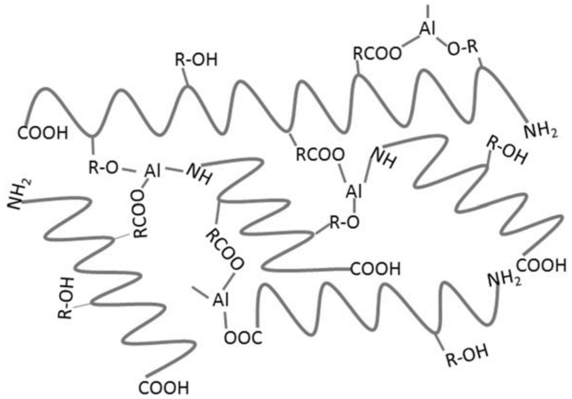


Figure 2. Structure of crosslinked sericin in presence of alum.

sericin (AM-Sericin) were checked at 70°C for 2 h. Solubility test indicated that both pure and 0.05% AM-Sericin film were soluble in warm water while 0.1, 0.5, and 1% AM-Sericin films remained in the film form. Since 1% (w/w) AM-Sericin film was stiff and nonflexible therefore 0.1 and 0.5% AM-Sericin films were selected for further study.

Characterization of Sericin Film

FTIR Analysis. The Fourier Transform Infra-red spectra of pure and AM-Sericin films were recorded on a Perkin–Elmer Spectrum-BX FTIR system from 400 to 4000 cm^{-1} region. The pure and AM-sericin films for FTIR characterization were prepared by casting few drops of dilute pure sericin and alum treated sericin solution on Teflon surface and kept for 20 h at 50°C and finally peeled off from the Teflon surface carefully. Thus very small sized, as much as possible uniform thin sericin films were prepared for FTIR characterization.

Thermo-Gravimetric Analysis. Thermo gravimetric analysis was carried out on a Perkin-Elmer, TGA7 thermal analyzer over a temperature range of 50–600°C at heating rate of 20°C min^{-1} in nitrogen atmosphere.

Moisture Content. Moisture content of pure and AM-Sericin films was calculated as follows

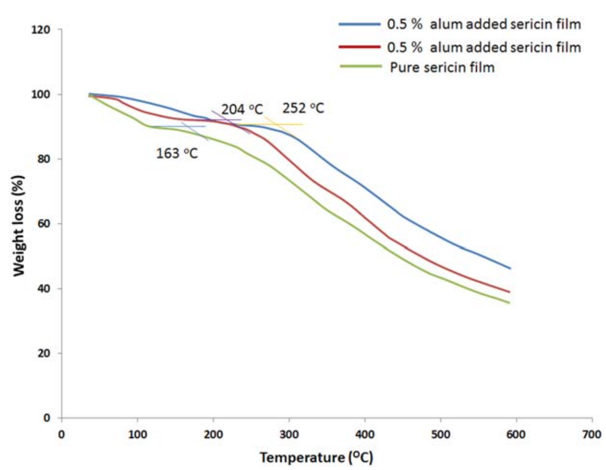


Figure 3. TGA results of pure and alum modified sericin film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

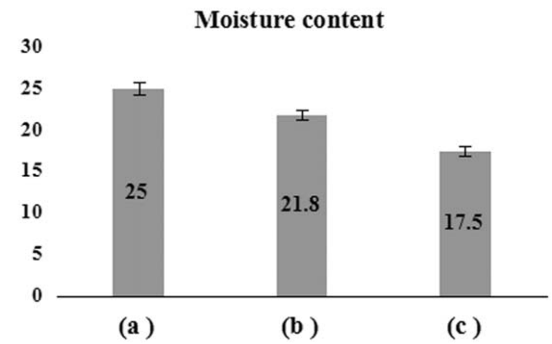


Figure 4. Moisture content (a) pure sericin film (b) 0.1% alum modified sericin film (c) 0.5% alum modified sericin film.

$$\text{Film moisture content}\,(\%) = ((m_1 - m_2)/m_2) \times 100 \quad (1)$$

where m_1 is the weight of sericin film after drying at 105°C for 24 h and m_2 is the weight of sericin film after 12 h conditioning at 27°C and 65% RH.

Film Swelling. Swelling property was measured by putting a small film (0.1 g) in 50 ml 0.02% sodium azide solution for 24 h at 35°C then wet (w_1) and dry (w_2) weight of the film were taken and film swelling percentage was measured using the following equation.[25]

$$\text{Swelling}\,(\%) = ((w_1 - w_2)/w_2) \times 100 \quad (2)$$

The excess solution was carefully wiped out by a soft tissue paper before taking the weight in wet condition and dry weight of sericin film was taken after drying the wet sericin film at 105°C for 24 h till the complete moisture was removed.

Moisture Vapor Permeability (MVP). A sericin film sealed on a porcelain cup of 5 cm diameter containing 5 g of anhydrous calcium chloride was placed in an air permeable desicator at 27°C and 65% RH. After 24 h weight gain by the cup was measured and water vapor permeability was calculated.[21]

$$\text{MVP}\left(\text{g mm/m}^2\text{ day kPa}\right) = WX/ATp \quad (3)$$

where W is the weight gain of the cup in g, X is the film thickness in mm, A is the area of exposed film in square meter, T is the time of gain in hour and p is the vapor pressure across the film in kPa.

Lipid Barrier Property. A tube of 4 cm diameter with 5 mL salad oil was sealed with a sericin film and the tube was placed on the tissue paper in upside down condition and the paper

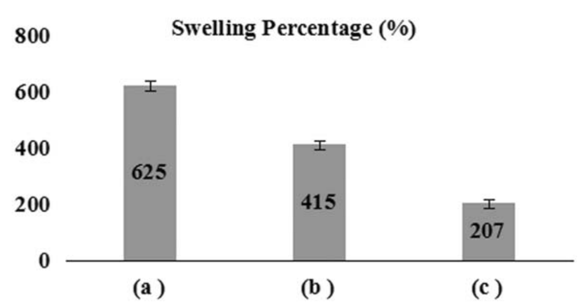


Figure 5. Swelling percentage (a) pure sericin film (b) 0.1% alum modified sericin film (c) 0.5% alum modified sericin film.

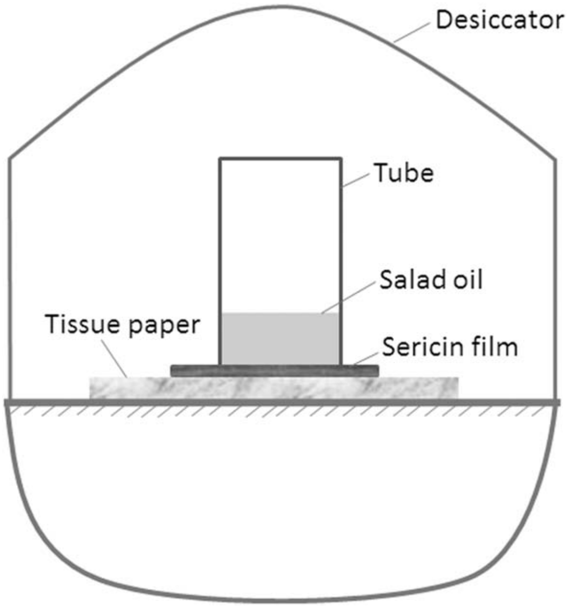


Figure 6. Schematic diagram of set up used for lipid barrier test.

weight was checked every day for a week and lipid permeability (PO) was determined by using following equation.[26]

$$PO = P\Delta W_x/ST \quad (4)$$

where ΔW_x is the weight gain with time in gram, X is the film thickness in mm, S the surface area covered by film in square centimeter and T the time in a day (24 h), and P is the permeability co-efficient of oil used. PO is lipid permeability in gm cm^{-2} day.

Table III. Moisture Vapour Permeability of Pure and Alum-Modified Sericin Films

Sample ID	MVPR (g mm^{-1} m^{-2} day^{-1} kPa^{-1})
Pure sericin film	0.034 ± 0.00037
0.1% Alum modified film	0.038 ± 0.00055
0.5% Alum modified film	0.045 ± 0.00077

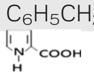
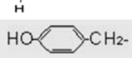
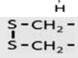
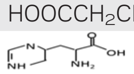
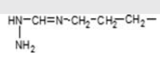
Surface Morphology. The surface characteristics of pure and AM-sericin film samples were probed by placing them on a double sided adhesive tape, coating with gold and by examination on a scanning electron microscope (SEM ZEISS EVO 50) at an accelerating 5–10 kV AC voltage.

Antimicrobial Test. The antimicrobial property of the sericin films was tested using parallel streak (AATCC 147) method, where *Escherichia coli* (*E. coli*) was used as a test organism and (2 inch × 1 inch) sample dimension was used.[27]

Film Thickness. Film thickness was measured by using Essdiel Thickness Gauge made by Shirley Development at ten random locations of the film and an average value taken.

Tensile Properties. The tensile properties of the sericin films were measured on Instron universal testing machine, model 4202 at 20 mm min^{-1} testing speed using 1 kg load cell and 10-cm gauge length. Before testing samples of (5 × 120) mm^2

Table II. Different Amino Acids Present in Sericin[30]

Name of amino acids	Chemical structure (—R—)	% of total sericin weight	Nature
Glycine	H—	8.15	Hydrophobic
Alanine	CH_3—	3.06	Hydrophobic
Valine	$(CH_3)_2$ CH—	3.08	Hydrophobic
Leucine	$(CH_3)_2CHCH_2$—	1.37	Hydrophobic
Isoleucine	$CH_3CH_2CH(CH_3)$ —	0.67	Hydrophobic
Methionine	$CH_3SCH_2CH_2$—	0.08	Hydrophobic
Phenylalanine	$C_6H_5CH_2$—	0.43	Hydrophobic
Proline		0.44	Hydrophilic
Tyrosine		4.85	Hydrophilic
Tryptophane		0.56	Hydrophilic
Cystine		0.39	Hydrophobic
Serine	$HOCH_2$—	30.14	Hydrophilic
Threonine	$CH_3CH(OH)$—	8.75	Hydrophilic
Aspartic acid	$HOOCCH_2$—	17.35	Hydrophilic
Glutamic acid	$HOOCCH_2CH_2$—	5.55	Hydrophilic
Arginine		1.54	Hydrophilic
Lysine	$H_2NCH_2CH_2CH_2CH_2$—	3.29	Hydrophilic
Arginine		1.54	Hydrophilic

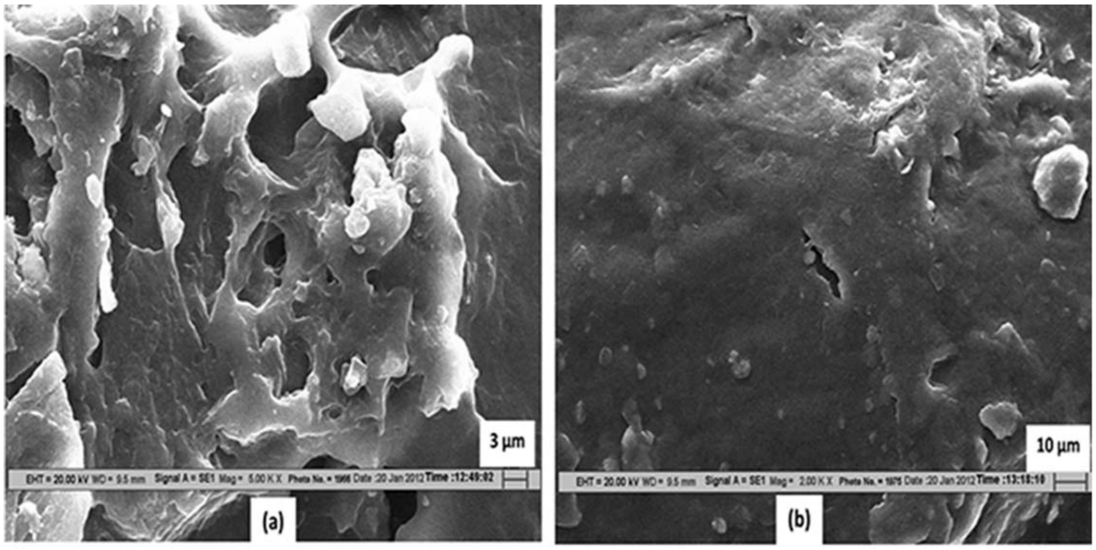


Figure 7. SEM picture of (a) alum-modified sericin (b) pure sericin film.

dimension were conditioned under a standard condition of 27°C, RH 65% for 24 h. Finally five readings of breaking load, breaking elongation and Young's modulus were taken for each sample and average value taken.

RESULTS AND DISCUSSION

IR-Spectroscopy

Figure 1 shows the IR spectra of pure and AM-Sericin film between 4000 to 400 cm^{-1} region. After comparing both the spectra it was observed that the bands in 4000 to 2800 cm^{-1} region that are responsible for O—H stretching and N—H stretching (around 3200 to 3600 cm^{-1}) and for C—H stretching (around 2900 cm^{-1}) are similar in nature. It seems that the absorption bands in this region are not affected by alum modification. Because the C—H stretching band at 2928 cm^{-1} in case of AM-Sericin film and at 2940 cm^{-1} in case of pure sericin film are not affected by alum treatment the area under these can be taken as internal standard for quantitative assessment of change in spectra. However, the peaks at 1643 cm^{-1} in AM-Sericin film spectra and at 1615 cm^{-1} in case of pure sericin film spectra, for N—H bending vibration and peaks at 1111, 1072, and 925 cm^{-1} in case of untreated sericin film and peaks at 1157, 1072, and 941 cm^{-1} in case of AM-Sericin film for carboxylic acid and alkyl hydroxyl containing amino acid side chains are affected by alum treatment.

To comprehend the effect of alum treatment on sericin, the area under the non-affected peaks and affected peaks was calculated and is reported in Table I. The values indicate that the ratios of affected area to nonaffected area of corresponding peaks are not same in pure sericin and AM-Sericin film. This may be due to attachment of alum (aluminum ion) with different groups of sericin such as amide, carboxylic acid and alkyl hydroxyl groups of amino acid side chains etc. It is known that protein remain in α-helix form in solvent thus when alum is added to sericin solution, it is expected to react with the aluminum ion of alum and it may form a cross-linked structure as shown in Figure 2.

Thermo-Gravimetric Analysis (TGA)

TGA results of pure sericin, 0.1 and 0.5% (w/w) alum treated films in Figure 3 indicate that the initial degradation temperature increases as alum is added to sericin: it is 165°C for pure sericin, 205°C for 0.1% alum added and 252°C for 0.5% alum added film. These results indicate that the thermal stability of AM-Sericin films is superior to that of pure sericin film. It has been observed that thermal degradation of silk protein molecule transpires due to breakdown of side chain groups of amino acid residues as well as the cleavage of peptide bonds.[28,29] Improved thermal stability of AM-Sericin film may be due to the formation of crosslinked structure as indicated in Figure 2. The side chain groups of amino acid residues like carboxyl, hydroxyl, or amine are bonded to aluminum ions forming a chelated structure in alum modified sericin is definitely stronger than the secondary bonds such as hydrogen bonding etc between these groups present in neat sericin and hence need more energy to break resulting in higher thermal stability. The swelling data also support this argument as discussed later.

Table IV. Tensile Property of Pure Sericin and Alum-Modified Sericin Films

Sample ID	Breaking strength (kg mm^{-2})	Elongation at break (%)	Modulus (kg mm^{-2})
Pure sericin film	0.1401 ± 0.013	94.6 ± 16.47	0.2501 ± 0.035
0.1% Alum modified sericin film	0.1937 ± 0.009	52.18 ± 7.63	0.4230 ± 0.025
0.5% Alum modified sericin film	0.3217 ± 0.011	31.59 ± 8.25	1.1531 ± 0.031

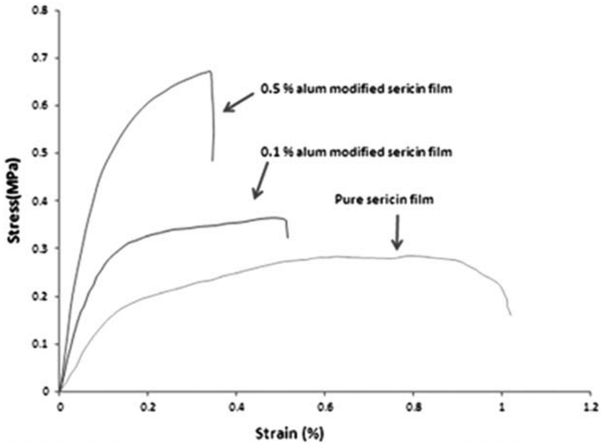


Figure 8. Stress–strain curve of pure and alum modified sericin film.

Moisture Content

Moisture content of pure and AM-Sericin film is shown in Figure 4. The test results show that moisture regain decreases with increasing alum content. This may be due to attachment of aluminum ions with the hydrophilic groups of sericin which reduces the available hydrophilic groups for water attachment resulting in lower moisture regain of AM-Sericin films.

Swelling Test

The swelling properties of pure and AM-Sericin films is shown in Figure 5. The results show that swelling percentage decreases with increasing alum content. Swelling in neat sericin is mainly because of hydrophilic groups such as carboxyl, hydroxyl and amine present as side groups of amino acids which readily take up water resulting in swelling. However in alum modified sericin these groups enter into bonding with aluminum forming a crosslinked chelated structure. This cross linking of sericin molecules in these alum modified films makes the structure such that water absorption is inhibited due to its rigid structure that is not able to swell as much as the original sericin which is a more open structure with groups free for water absorption. These are in agreement with TGA and moisture content results.

Lipid Barrier Property

Figure 6 shows the setup for lipid barrier test, the observations of lipid barrier test shows that there was no weight gain on the tissue paper even after keeping for 7 days for all the pure and AM-Sericin film samples. This implies that both the pure and AM-Sericin films have a very good lipid barrier property.

Table II shows the nature and percentage of different types of amino acids present in sericin.[30] It was observed that sericin contains high amount of hydrophobic or neutral amino acids in its structure for which sericin film shows very good oil barrier properties.

Moisture Vapor Permeability (MVP) and SEM Analysis

Table III shows the moisture vapor permeability of pure and AM-Sericin films. MVP test results show that with increasing alum content the moisture vapor permeability through the sericin film increases.

When alum is added to sericin solution, a kind of phase separation takes place; as the alum concentration increases the degree of phase separation becomes more prominent. Figure 7 shows the SEM picture of pure and AM-Sericin film. The surface view as seen in SEM pictures show the presence of number of pores on the surface of the AM-Sericin film but in case of pure sericin film much less pores are visible and the surface is also smoother than AM-Sericin film. This rough porous surface structure of AM-Sericin film may be due to phase separation of sericin by addition of alum. This porous and rough structure of AM-Sericin film makes it to be more permeable to moisture vapor.

Tensile Property

The pure and AM-Sericin films have an average thickness of 0.1 ± 0.02 mm. The tensile properties and stress–strain behavior of these films are tabulated in Table IV and Figure 8, respectively. The tensile testing result shows that the tensile strength and modulus both increases significantly with increasing alum content but the elongation at break decreases with increasing alum content.

The macromolecular chains are randomly arranged in amorphous sericin film and when a tensile load is applied on it, the molecular chains may get stretched in the direction of force applied but in case of AM-Sericin film, aluminum forms a crosslinked structure with the randomly oriented sericin molecules which restrict the mobility of polymer chains. At the same time, after cross-linking the interaction between sericin chains increases due to intermolecular sericin–alum linkages which

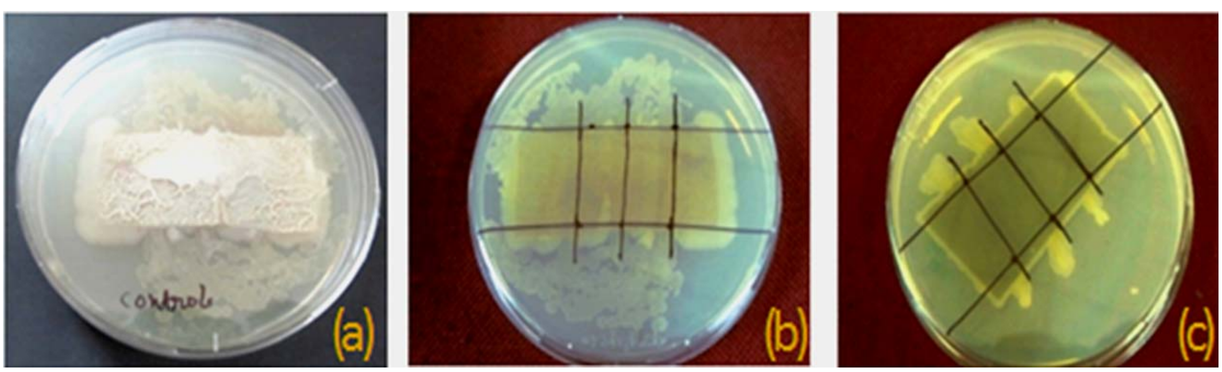


Figure 9. Antimicrobial test results of (parallel streak method) (a) pure sericin film (b) 0.1% alum modified sericin film (c) 0.5% alum modified sericin film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

results in higher tensile strength and Young's modulus. As the alum concentration increases, the degree of crosslinking also increases as a result of which the tensile strength and Young's modulus of the sericin film further increases. But the alum modified films show lower elongation at break as compared to pure sericin film because of restricted mobility of chains due to dense crosslinking at higher alum concentration.

Antimicrobial Activity

Figure 9 shows the pictures of parallel streak antimicrobial tests results of pure and AM-Sericin film using *E. coli* bacteria. It is clearly seen that there is microbial growth on the surface of pure sericin and 0.1% alum added sericin films, and thus they do not show any effective antimicrobial activity. But in case of 0.5% AM-Sericin film there is no microbial growth at all below the film, indicating its anti-microbial nature. This may be due to crosslinking of aluminum ion with sericin molecule and therefore there is no release of aluminum in the agar media which results in no zone of inhibition. Thus the antimicrobial property is mainly due to a barrier mechanism, where aluminum crosslinked with sericin at the surface does not allow microbial growth.

CONCLUSION

This study clearly demonstrates that addition of 0.5% w/w alum to sericin results into novel cross-linked sericin films which are insoluble in water, show higher thermal stability, have good antimicrobial property as well as improved water vapor permeability and good lipid barrier property. These novel AM-Sericin films can find application in wound dressing, skin care or in food packaging areas.

ACKNOWLEDGMENTS

The authors thank M/s Sericare, Bangalore for supplying silk flat non-woven wastes. They acknowledge the funding received from Department of Biotechnology, Govt. of India, under which a project on Extraction, Purification and characterization of sericin from degumming liquor was carried out.

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