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Original Article

Preparation and Characterization of Mulbery Silk Fibroin Films

Mahadevaiah[1], Thejas Urs G[1], K Byrappa[2] and R Somashekar*[1]

[1]Department of Studies in Physics,
University of Mysore, Manasagangotri, Mysore – 570006

[2]Department of Studies in Earth Science,
University of Mysore, Manasagangotri, Mysore – 570006

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Corresponding author: *Department of Studies in Physics, University of Mysore, Manasagangotri, Mysore – 570006*, India

E-mail address:
rs@physics.uni-mysore.ac.in

ABSTRACT

The silk films were prepared by dissolving degummed Bombyx mori raw silk fibers in LiBr salt solution using double distilled water as a solvent and subjecting the solution to dialysis using cellulose tube. These films may be of some use in treating burn injuries. We have characterized these films using X-rays and other physical methods to obtain information about the structure-property relation. We have also compared the parameters of silk films with that of silk fibers.

INTRODUCTION

The silk fibroin is used to prepare fibers, films, nano particles, thin strips and patches. Our earlier research works is on the silk fibers,[1,2] silk is a natural protein produced by the domestic as well as wild silk worms. The amino acid composition of silk fiber of Bombyx mori mainly consists of glycine, alanine and serine. These three simple amino acids form the crystalline regions of silk fiber, while the amino acids with polar side chains form the amorphous regions. The silk fiber is partly crystalline and partly amorphous. Bombyx more fibers are composed of fibrous proteins with fibroin core (72–81%) and a surrounding glue protein Sericine (19–28%). The silk protein has a molecular mass of around 300 kDa and it is a linear polypeptide.[3,4]

Silk fiber is used on daily basis as surgical suture for several centuries due to its good biological properties including bio-compatibility and low inflammatory reaction[5]. Further silk fibers have characteristic properties like good water vapour and oxygen permeability[6,7], blood compatibility[8], accelerated collagen formation and proliferation of cultured human skin fibroblasts[9,10]. It also has an easy control of structural conformation[11]. Silk has been applied in a wide variety of biomedical applications such as drug delivery systems[12] and enzyme immobilization[13]. For drug delivery, especially silk protein drugs, silk materials exhibits high encapsulation efficiency and controllable drug release kinetics due to control of crystalline beta-sheet formation[14].

In this work we have prepared pure silk film from raw fibers using simple solution casting method and its characterization has been carried out using X-ray diffraction and FTIR analysis. We have also carried out the anti-microbial activity test for the obtained silk fibroin in its solution form.

EXPERIMENTAL PART

Materials and Method

Bombyx mori silkworm fresh silk reel were collected from local Central Silk Board at Mysore. All other chemicals used here were purchased from Merk India.

Preparation of Silk solution

Silk solution was prepared according to the protocol described in similar studies. Briefly, a fresh Silk fiber (8g/100ml) of Bombyx mori was degummed by boiling in 0.2 M Na_2CO_3 solution for 30 min. The degummed silk fibers were washed with water to remove residual Sericine and then air dried. The dried silk was then immersed in 9.3M LiBr solution at 80^{o}C to produce silk solution. This solution was dialyzed against deionized water for 48 h using dialysis cellulose tube (MWCO 12 kDa, Sigma) to remove LiBr salt. The silk solutions (approximately 5%) obtained after filtration was stored at 4^{o}C. This solution was casted on glass plates at room temperature for 5-6days. After drying, these films were carefully removed from glass plates, and were stored in a desiccator for further use[15]. Figure **1** shows the photographs of silk fiber and the silk films including the dialysis setup. (Figure **1**).

X-ray Diffraction Studies

The X-ray diffraction patterns for the silk films and silk fibers were recorded using Rigaku Denki Miniflex II Desktop Diffractometer, with Cu-Kα radiation for the 2θ range of 6^0 -70^0 with scanning speed of 5^0/min and step size of 0.02^0. These measurements were done at room temperature and care was taken to avoid mechanical distortions. Figure **2** show recorded X-ray patterns.

FT- IR analysis

Secondary structure and conformation of the silk films and silk fibers were analyzed by Fourier Transform Infrared (FTIR) Spectroscopy (Perking - Elmer Spectra GX USA) keeping air as reference. In this analysis resolution of 4cm^{-1} was chosen and data collection was in the range 1900 - 700 cm^{-1}. Figure **4** shows the recorded FTIR spectrum for film and fibers.

Antimicrobial activity by Well-Diffusion Method

The Silk fibroin solution was tested for antimicrobial activity by well-diffusion method against *E. coli* and *Bacillus subtilis*. The pure cultures of organisms were sub cultured in nutrient bath at 35°C on a rotary shaker at 200 rpm. Wells of 6-mm diameter were made on nutrient agar plates using gel puncture. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette10 µL, 20 µL and 30 µL of the sample of silk fibroin solution was added onto each wells on all plates with the positive control of Chloramphenicol[16,17]. Figure **5** Shows.

RESULT AND DISCUSSION

The X-Ray Diffraction profile obtained for the Silk fibers (a), degummed silk fibers (b) and the silk film is shown in Figure **2**. The profile obtained shows that there is no such changes in the diffraction peaks obtained for silk fibers and silk film, but it is seen that the silk film is more amorphous as compared to the silk fibers. We have determined the average crystallite size and the lattice strain in these samples by employing W-H plot method[18] and also the percentage crystallinity was calculated. The obtained results from these calculations do support that the silk fiber is more crystalline than that of the silk film. The calculated results are tabulated in Table **1**. Pair correlation study was also carried out to quantify the changes in the radial arrangement of molecules in the silk film with respect to that of the fibers using XRD data obtained. Figure **3** shows the pair correlation function for these samples. Values of pair correlation function decreases with the formation of film and the disorder sets in the region of distances 5 to 6 Angstroms. This confirms the mere crystallinity in film to that of the Silk fibers. (Table **1**, Figure **2** and **3** should be placed as possible after this paragraph).

The FTIR spectra obtained for silk fibers and silk film show the same absorption spectrum and functional groups confirming the purity of the silk film prepared here. The spectral scan between 1900 cm^{-1} and 700 cm^{-1} is considered for the prediction of amide groups in silk protein. The silk is usually characterized by the β sheet absorption peaks which are found around 1630, 1530 and 1240 cm^{-1} and an α-helix absorption peaks around 1655 cm^{-1}. From the obtained spectra shown in Figure 4, it is seen that the absorption peaks lies in the range of 1625-1630cm^{1}(amide I),1520-1530 cm^{-1} (amide II) and 1265-1270 cm^{-1} (amide III),which are the characteristic absorption peaks of β-sheet which confirms the Bombyx Mori silk fibers and silk film. (Figure **4** should be placed below this).

From the test for its antimicrobial activity, after incubation at 35°C for 24 hours, there was no zone of inhibition was observed. This result indicates that the obtained silk fibroin solution did not show any antimicrobial activity against tested organisms. Since silk is a chain of proteins, it does not possess any property of resisting the growth of microbes on its own. But further it can be made antimicrobial by adding some antimicrobial agents to it, with the view of using these silk films in medical applications. As we have discussed before, these silk fibroin films can be used as patches for treating burn injuries by adding desired drug and antimicrobial agents before drawing films.

CONCLUSION

We are successful in preparing silk fibroin films in the laboratory using solution casting method. It is evident from the studies carried out here that crystallinity and crystallite size decreases with the formation of silk films. This is supported by the pair correlation function of these samples which decreases with the formation of film and sets disorder in the region of distances 5 to 6 Angstroms. In FTIR studies, the observed absorption peaks do support the beta-pleated arrangements of the chains for both silk film and fibers. The Antimicrobial activity test shows that the silk fibroin solution prepared does not show any opposition to the growth of microbes, but further it can be used for treating in desired applications by adding and drawing these silk fibroin films with the drug to fulfill our requirements. Further work is in progress.

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Table 1. The Lattice parameters obtained for the silk fibers and the Silk film

Sample	% Crystallinity	Average Crystallite Size in Å	Average Lattice Strain in %
Silk fibre (a)	7.7	84.85	0.048
Silk fibre (b)	6.51	57.06	0.21
Silk film	2.15	19.83	1.8

Figure 1. Shows (a) photograph of the silk fibers, (b) photograph of the silk fibers after the removal of sericine, (c) photograph of 5% silk solution dissolved in LiBr, (d) photograph of the dialysis set up against distilled water (e) silk films obtained

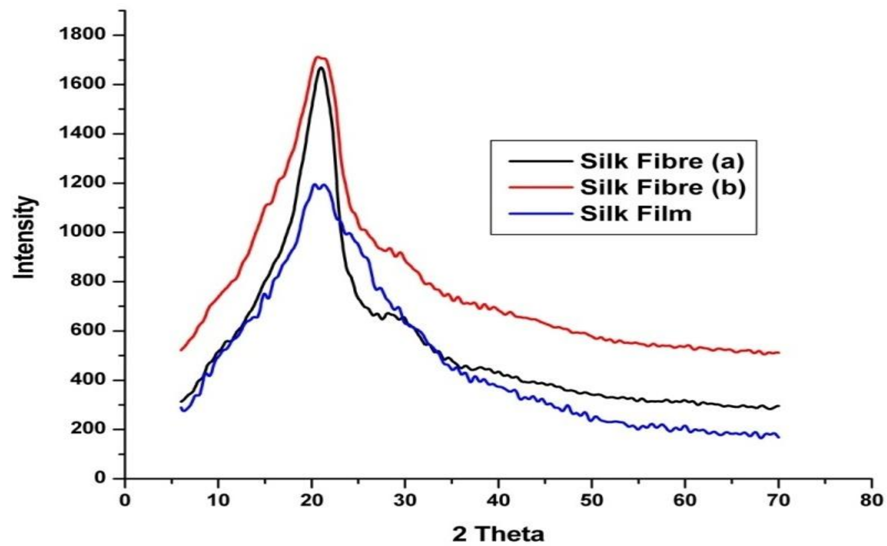

Figure 2. X-ray diffraction pattern obtained for degummed silk and silk film

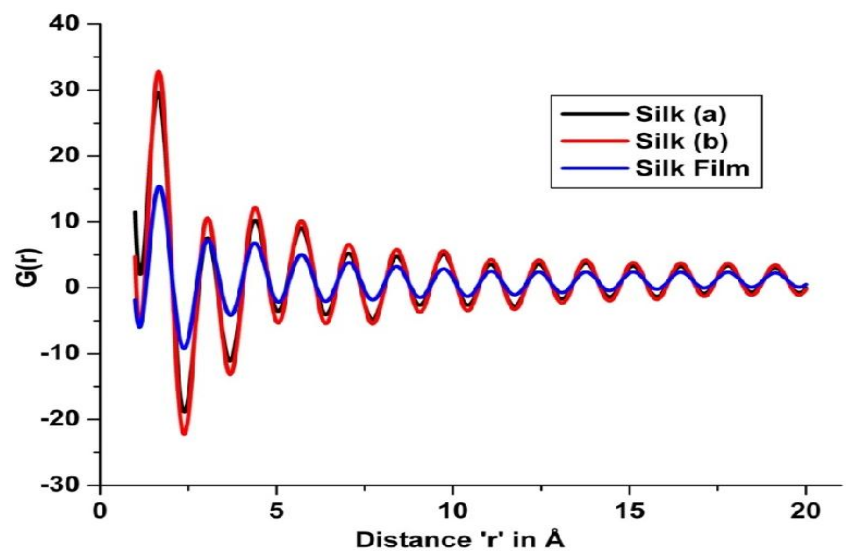

Figure 3. The pair correlation function obtained

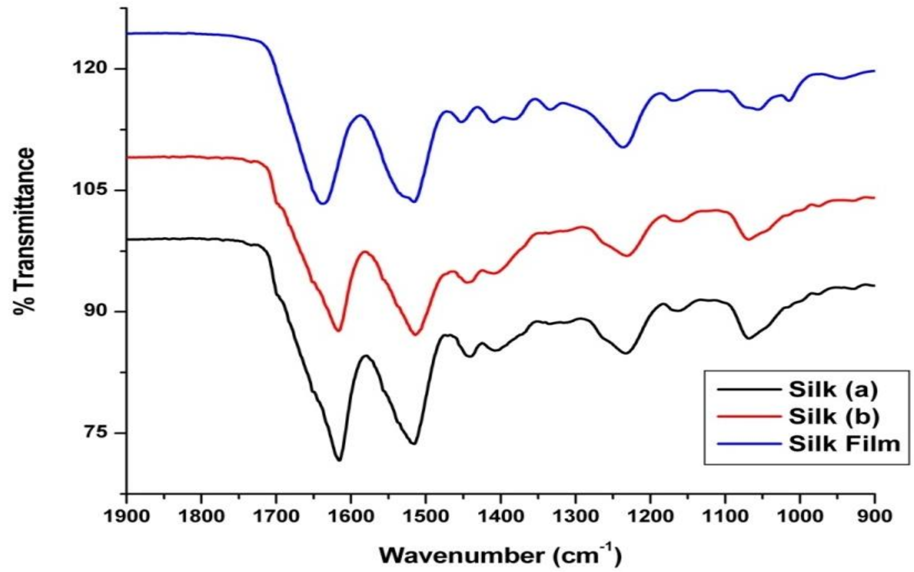

Figure 4. FTIR spectra of silk fiber (a), degummed silk fiber (b) and silk film

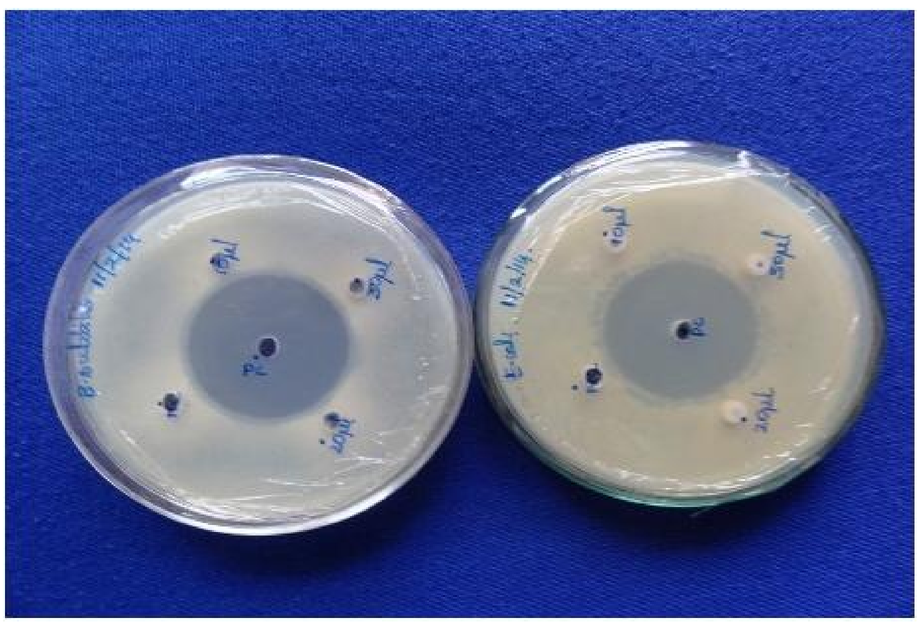

Figure 5. Photographs of the antibacterial test of the silk film