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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (23): 1526-1530, 2009
ISSN 1028-8880

Morphology, Secondary Structure and Thermal Properties of Silk Fibroin/Gelatin Blend Film

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Abstract: This study aimed to prepare Silk Fibroin (SF) and Gelatin (G) blend film and study its morphology, secondary structure and thermal properties compared to native SF and G films. The films were prepared from the SF solution by casting on the polystyrene plates. They were investigated their secondary structure by fourier transform-infrared (FTIR) spectroscopy, morphology using Scanning Electron Microscope (SEM). In addition, Thermogravimetric Analysis (TG) and Differential TG (DTG) were used for thermal properties investigation. The results found that the SF/G blend film composed of both α-helix and β-sheet structures which were similar characteristics of the native SF and G. This result was similar to the TG and DTG analysis according to blending between SF and G is not enhancing thermal stability of the film. However, changes in some absorption bands and temperatures were also observed from the blend film. The result suggested that chemical interaction and hydrogen bonding between SF and G could be formed. The formation could be affected to the uniform of the surface throughout the film under SEM.

Key words: Biomaterial, silk fibroin, gelatin, film, properties

INTRODUCTION

Biodegradable biomaterials have been wildly considered and used in current for medical and related application (Piskin, 1995). Generally, it divided into synthetic and natural polymers. The development of biodegradable biomaterials specifically developed for novel biomedical technologies including tissue engineering, regenerative medicine, gene therapy, controlled drug delivery and bionanotechnology (Nair and Laurencin, 2007).

Advantages of polymeric materials such as biocompatibility, biomimetic structures, mechanical and degradation properties are requires. Synthetic biodegradable polymers have been commercially developed including poly (α-ester), polyurethanes, poly (ester amide), poly (ortho esters), polyanhydrides, poly (anhydride-co-imide), cross-linked polyanhydrides, poly (propylene fumarate), pseudopoly (amino acid), poly (alkyl cyanoacrylates), polyphosphazenes and polyphosphoester. On the other hand, natural polymers have been reported including protein and poly (amino acids) and polysaccharides (Nair and Laurencin, 2007).

Silk, a natural protein polymer has numerous studied as technical and biomedical applications (Min *et al*., 2004). It is produced by Lepidopteran insects of the family Bombycidae and Saturniidae (Mandal *et al*., 2009). SF can be used in various forms including gel, powder, fiber or membrane, depending on application (Park *et al*., 2004). Each silk fiber is composed of fibroin core protein coated with sericin (Servoli *et al*., 2005). Silk Fibroin (SF) has been used as biotechnological and biomedical resources since their unique properties including nontoxicity, biocompatibility and biodegradability were reported (Foo and Kaplan, 2002; Altman *et al*., 2003).

Gelatin (G) is a derivative collagen. It is suitable as a biomaterial for tissue engineering. Gelatin shows excellent properties including low cost, good biocompatibility, biodegradability, low immunogenicity, increased cell adhesion, migration, differentiation and proliferation (Han *et al*., 2009). In the two past decades, gelatin has been wildly used as sealants for vascular prostheses, carriers for drug delivery, wound dressings, health caring devices and tissue engineering scaffolds (Huang *et al*., 2004; Gil *et al*., 2007; Lee *et al*., 2008). Moreover, it can be applied alone or as a blend polymer (Huang *et al*., 2004).

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In this study, SF/G blend film was prepared and investigated for their morphology, secondary structures as well as thermal properties. The goal of this work is extended to know about two biomaterials characteristics for material design and applications.

MATERIALS AND METHODS

This study was done from July 1, 2009 to November 10, 2009. Most of the experiment was carried out at Department of Chemistry and the Central Instrument Center, Faculty of Science, Mahasarakham University.

Materials: *Bombyx mori* (*B. mori*, locally called Nang-Lai) silk cocoons were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand. The cocoons were kept in air-dried room until use. Gelatin powder was purchased from Fluka. All used chemicals were analytical grade obtained commercially.

Methods

Preparation of SF solution: The *B. mori* cocoons were firstly striped into small pieces and then washed with distilled water to exclude those of impurity, dried in oven at 40°C. The sericin protein was extracted from the cocoons by boiling twice with 0.5% (w/v) Na_2CO_3 solution. Each time, the cocoons were thoroughly rinsed in distilled water. They were then air-dried at room temperature. The dried cocoons were dissolved with the tertiary solvent system of $CaCl_2$-Ethanol-H_2O (1:2:8 by mole ratio). The mixture of solvent and striped cocoons was boiled at 90-95°C for 1 h and stirring. The SF solution was obtained and then dialyzed in dialysis bag (Mw. cut off 7500 kDa) against distilled water for 3 days. SF concentration was calculated by evaporation method and adjusted to 1% (w/v).

Preparation of gelatin solution: 1% (w/v) of gelatin solution was prepared by dissolving 1 g of gelatin powder using distilled water. The mixture was stirred and stand at room temperature until absolutely dissolved.

SF/G blend films: Various ratios of SF/G blend films were prepared by casting on the 5 cm polystyrene plates at room temperature. The plates were dried at 40°C in oven for 3 days.

Morphology observation: The blend films were cut and sputter coated with gold to enhance their surfaces conductivity before observation under Scanning Electron Microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan).

Secondary structure analysis: Secondary structure of the blend films were analyzed using Fourier transform infrared (FTIR) spectrometer (Perkin Elmer-Spectrum Gx, USA) with resolution of 2 cm^{-1} and 32 scans. The intensity at wave numbers from 500 to 4000 cm^{-1} was recorded.

Thermal behavior measurement: The SF/G blend films were loaded in a platinum crucible. The Thermo Gravimetric Analysis (TGA) was performed using TA instruments, SDT Q600 (Luken's drive, New Castle, DE). The samples were non-isothermal heated from 50°C to 700°C at a heating rate of 20°C min^{-1}. The TGA was carried out in nitrogen with the flow rate of 100 mL min^{-1}. The differential TG (DTG) was recorded with TA instrument's Q series explorer software. The analyses of the data were done using TA Instrument's Universal Analysis 2000 software (version 3.3B).

RESULTS

Fourier transform-infrared (FT-IR) spectroscopy: SF film showed many absorption bands at 1690, 1654 and 1636 cm^{-1} (amide I), 1558 and 1522 cm^{-1} (amide II) and 1242 cm^{-1} (amide III) (Fig. 1a). The FT-IR spectrum of gelatin (G) film showed absorption bands at 1621, 1567, 1546 and 1242 cm^{-1} (Fig. 1c). In SF/G blend film, the spectrum indicated similar characteristic bands of the native polymers (Fig. 1b). Blend with G led to shifting of amide I bands (1678, 1653 and 1634 cm^{-1}), amide II (1559 and 1526 cm^{-1}), except amide III (1242 cm^{-1}) in the spectrum of SF.

Scanning Electron Microscopy (SEM): The morphology of SF, G and SF/G films were observed by SEM. It was found that the surface of the blend film (Fig. 2b) has uniform without phase separation, compared to both SF (Fig. 2a) and G (Fig. 2c). The surface area of SF film is highly rough in comparison with other. In addition, SF/G blend film showed smoother surface than that of SF film.

Thermal analysis by TG and DTG: The function of %wt loss vs. temperature of SF, G and SF/G were studied by TG. In case of SF, two-stage decomposition was occurred in the temperature range 225-310°C and 310-340°C (Fig. 3). In addition, minor peak was also found in the temperature range 340-400°C. In case of G film, also two-stage decomposition was found in the temperature range 225-330°C and 330-360°C, with the shoulder peak in temperature range 360-440°C. However, In case of SF/G blend film, at least four-stage decomposition was found in temperature range 225-300°C, 300-338°C, 338-355°C and

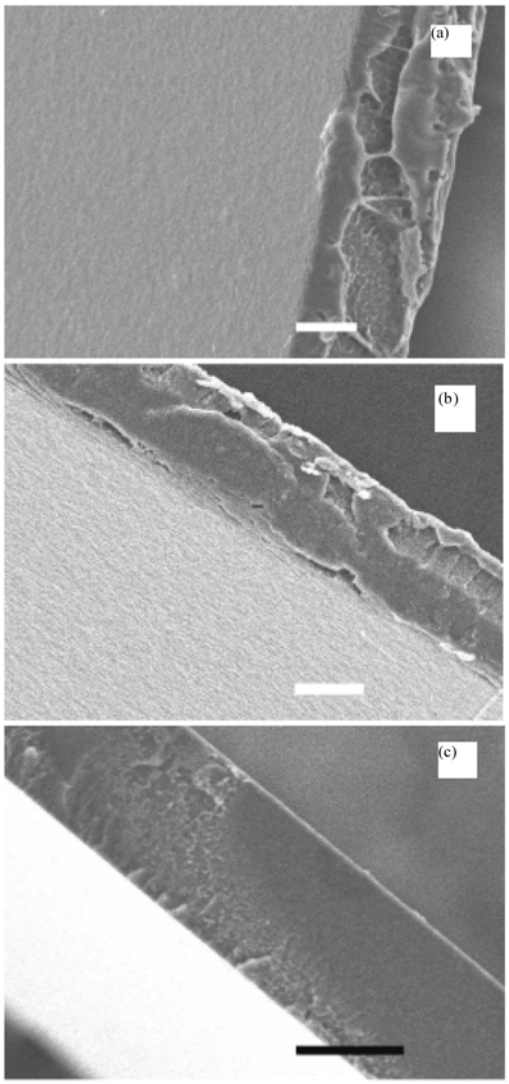

Fig. 1: SEM micrographs of SF/G blend films; (a) 3/0, (b) 1/1 and (c) 0/3 (w/w). Bars = 10 μm

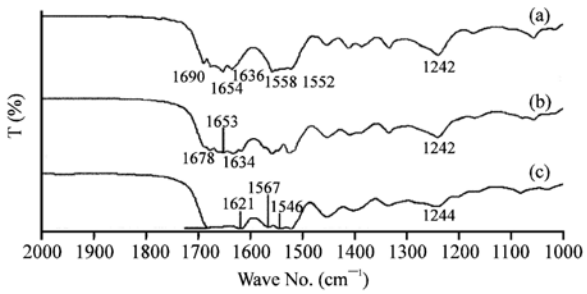

Fig. 2: FT-IR spectra of SF/G blend films; (a) 3/0, (b) 1/1 and (c) 0/3 (w/w)

355-440°C, respectively (Fig. 3). DTG analysis of all films was also studied as a function of rate of weight loss vs

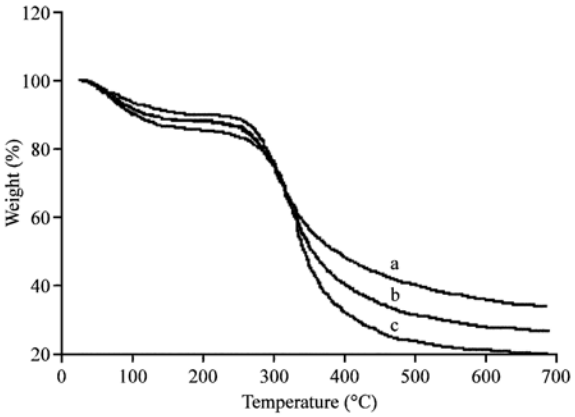

Fig. 3: TG curves of SF/G blend films; (a) 3/0, (b) 1/1 and (c) 0/3 (w/w)

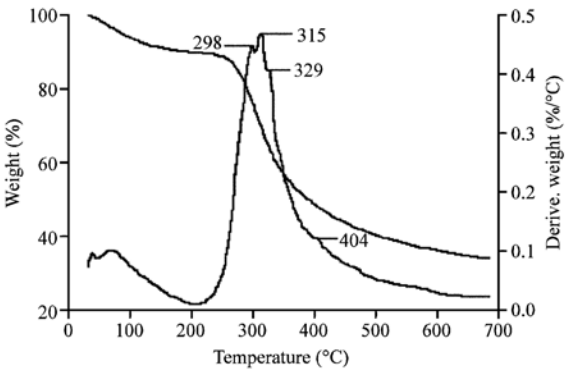

Fig. 4: TG and DTG curves of SF film

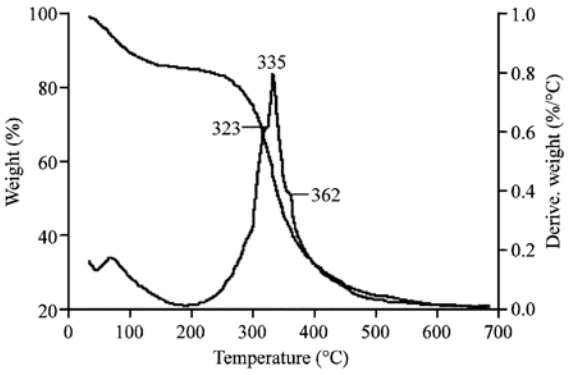

Fig. 5: TG and DTG curves of G film

temperature. The maximum decomposition temperature ($T_{d,\ max}$) of SF film was found at 298, 315 and 392°C, respectively (Fig. 4), whereas G film was found at 323, 335 and 362°C, respectively (Fig. 5). Mixture characteristic between SF and G was found in SF/G blend film since the $T_{d,\ max}$ was indicated at 294, 309, 334 and 346°C, respectively (Fig. 6).

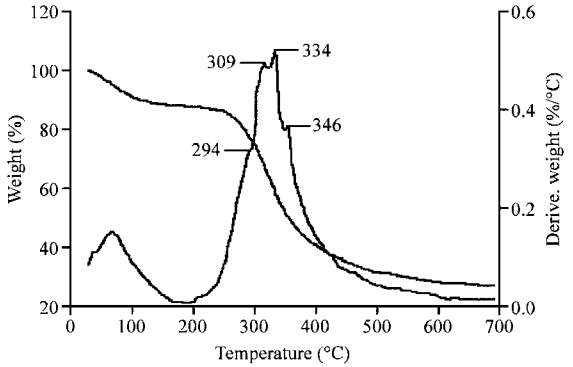

Fig. 6: TG and DTG curves of SF/G blend film

DISCUSSION

In this study, SF of *B. mori* from Thai strain (locally called Nang Lai) solution was blended with G in order to study their morphology, secondary structure and thermal properties. The films were successfully prepared by using evaporation technique. Both SF and G have been reported as an excellent biomaterials used in various fields (Han *et al*., 2009; Patel *et al*., 2008; Fan *et al*., 2008; Gil *et al*., 2007). SF indicated various excellent properties, especially mechanically robust. It was found that the amino compositions and their structures are main factor on this property. If the structures were changed the SF properties were changed too. Generally, the secondary structures of protein are indicated by the amide groups of the silk composition (Kweon *et al*., 2000; Hino *et al*., 2003). In this work, FT-IR data indicated that the secondary structures of SF film showed 1690, 1655 and 1636 cm^{-1} (amide I, C=O stretching), 1558 and 1522 cm^{-1} (amide II, N-H deformation), 1242 cm^{-1} (amide III, C-N stretching), assigned to mixture of α-helix and β-sheet forms (Li *et al*., 2003; Mandal *et al*., 2009). The gelatin film was characterized by those carbonyl and amino peaks at 1631, 1545 and 1244 cm^{-1} which are due to C = O stretching, amide II and amide V, respectively (Han *et al*., 2009). In SF/G blend, FT-IR spectrum depicts similar characteristic bands of the native materials. The intensity of the absorption bands concerned the C = O and amide II at about 1678, 1653, 1634 cm^{-1} and 1559, 1526 cm^{-1}. This result suggested a chemical interaction between SF and G molecular chains and intermolecular bonding of hydrogen of SF and G formation. It is might be suggested that SF provides as area for G in the binding and structure in the blend film (Han *et al*., 2009).

SEM micrographs revealed uniform throughout the surface area. The micrographs reflected that SF and G form a continuous phase in the blend film. Thermal decomposition of SF film took place in a similar step with G film whereas SF/G blend film has different points. The blend film took maximum decomposition temperature characteristics of both SF and G indicated that the interactions between two materials are formed. However, DTG studies are evident that SF/G blend film is not thermally more stable than the native films.

CONCLUSION

The morphology, secondary structure and thermal properties of SF, G and SF/G blend films were reported in this study. The results showed that all of films showed uniform throughout surface area under SEM. The secondary structure of SF slightly changed after blending with G which was observed by the FT-IR. It was suggested that SF and G could be formed interaction together. TG and DTG showed that SF/G blend is not enhancing thermal stability of the film since it has decomposition temperature pattern as similar as native materials.

ACKNOWLEDGMENT

We appreciate to thank Division of Support and Development, Mahasarakham University for financial support of this study.

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