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Characterization of Tetracycline-loaded Thai Silk Fibroin/Gelatin Blend Films

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Abstract: This study was aimed to prepare SF/G blend films loaded tetracycline by a solvent evaporation method. Firstly silk cocoons were degummed and then dissolved by $CaCl_2$:Ethanol:H_2O (1:2:8 by mole). On another way, Gelatin (G) was prepared from gelatin powder. The mixture solution of SF/G ratios with 3:0, 2:1, 1:1, 1:2 and 0:3 were prepared before casting on the 5 cm polystyrene plates. All of films were investigated their morphology, chemical structures, thermal properties and percent transparency by using Scanning Electron Microscope (SEM), Fourier transform infrared (FTIR) spectroscopy, Thermogravimetric Analyzer (TA) and UV-Vis spectrometer, respectively. The results found that the surfaces of blend films were gradually smooth without phase separation when the SF component decreased. The FTIR results of SF/G films showed strong regions for amide I, amide II and amide III which were the mixture characteristics of SF and G. The blend films rapidly decomposed in maximum rate after 300°C. The rate of weight lost depending on the content G meanwhile rapidly increased of weight lost when the G content was increased. The heat flow curves indicated that the blend films composed of multiple peaks of maximum decomposition temperatures as well as endo/exo-thermic. Finally, tetracycline could be interacted with G in excellent profile affected to the lowest of percent transparency.

Key words: Chemical structures, gelatin, morphology, silk fibroin, tetracycline, thermal properties

INTRODUCTION

The blending method is a great advantage of materials since it can be tailored those of their properties by combining component and changing composition ratios of polymers. Generally, the blending process must be done in a fluid condition. Therefore, the dissolution of the polymers is also considered as an important step (Marsano *et al*., 2008). Moreover, natural homopolymer demands are not sufficient for biomaterial products (Fan *et al*., 2008). Recently, biopolymers such as: chitosan (She *et al*., 2008), gelatin (Martucci and Ruseckaite, 2009), cellulose and silk fibroin (Marsano *et al*., 2008) have been extensively studied due to their acted as potential environmentally friendly and biodegradability (Martucci and Ruseckaite, 2009).

Silk is a fibrous polymer that is produced by some Lepidoptera larvae including *Bombyx mori* (*B. mori*) silkworm (Altman *et al*., 2003). Each silk fiber consists of two types of proteins; fibroin and sericin. The silk fibroin (SF) is one of the candidate materials for biomedical applications. Recently, SF has been applied in various fields including cosmetics, medical materials and food additives (Min *et al*., 2004). Moreover, SF can be performed in many types depend on the applications. The SF film is one kind of regenerated form which was widely used in many fields. However, it often requires chemical treatments in order to enhance their stability and mechanical properties (Tasukada *et al*., 1995).

Gelatin (G), derivatives of collagen, is an edible and biodegradable polymer (Martucci and Ruseckaite, 2009). It shows excellent oxygen barrier as well as relative humidity and good mechanical properties (Jongjareonrak *et al*., 2006). All most reports, gelatin was performed in film form. The gelatin films tend to be brittle and moisture sensitive (Hernandez-Munoz *et al*., 2004). It has been used in pharmaceutical and medical fields (Rhim *et al*., 1998), drug delivery (Mandal *et al*., 2009) and wound dressings (Choi *et al*., 1999). The gelatin can be used alone or as a blend forms (Huang *et al*., 2004).

Tetracyclines are broad-spectrum antibiotics. They have been used for curing of bacterial infections over 50 years (Fritz and Zuo, 2007; Kolesnikov *et al*., 1996). This study was aimed to prepare the Thai silk fibroin/gelatin blend films for containing tetracycline which used as hydrophilic model drug. The some properties of the films such as morphology, chemical structure and percent transparency were investigated.

MATERIALS AND METHODS

This study was performed for 4 months from March 1, 2010 to July 1, 2010. The experiments were carried out on Department of Chemistry, Faculty of Science. The

characterization of all films properties were done at the Central Instrument, Faculty of Science, Mahasarakham University, Thailand

Materials: The *Bombyx mori* (locally called Nangnoi) silk cocoons were kindly supplied from Silk Innovation Center (SIC) Mahasarakham University, Thailand. All experimental reagents were analytical grade. Tetracycline hydro chloride was purchased from Sigma-Aldrich (USA).

Methods

Silk fibroin preparation: The silk cocoons were cut into small pieces and then firstly with boiled 0.5% (w/v) Na_2CO_3 to exclude sericin or gum. The silk fibroin (SF) was obtained and then used as subjected for preparation of SF solution.

Silk Fibroin (SF) solution: The pure SF was dissolved using tertiary solvent system of $CaCl_2$-Ethanol-H_2O (1:2:8 by mole), with magnetic stirred at 90-95°C for 2-3 h to obtain SF solution. The SF solution was then dialyzed using dialysis bag (MC = 7 kDa) against distilled water for 3 days. The obtained solution was calculated percent weight and kept for preparing SF films.

Gelatin (G) solution: The G solution was prepared at concentration of 1% wt. by weighing G powder for 1 g and then added distilled water up to 100 mL. The mixture was stirred for 30 min at room temperature until the G powder completely dissolved.

SF/G blend films preparation: The several of SF/G blend films were prepared by mixing SF/G solution at various ratios of 1:0, 3:1, 1:1, 1:3 and 0:1 by volume, respectively. The total volume of 20 mL in each composition was mixed with 0.04% w/v tetracycline and then poured on the 5 cm polystyrene plates. The plates were left at 40°C in an oven for 3 days to obtain films.

Characterization of blend films: The morphology of SF/G blend films with tetracycline were observed under scanning electron microscopy (SEM) (JEOL JSM-6460LV, Japan). The films were firstly cut into small pieces and then mounted on the stub with double side carbon tapes. The stubs were then sputter coated with gold to enhance surface conductivity.

The secondary structures of the films were analyzed by using Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer-Spectrum Gx, USA) in the spectral region of ~4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans.

Thermal properties were measured using TA instruments, SDT Q600 (Luken's drive, New Castle, DE). The films weight of 8-10 mg were prepared and loaded in a platinum crucible. The samples were non-isothermal heated from 50 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 TG and heat flow were recorded with TA Instrument's Q series explorer software. The analysis of the data were done using TA Instrument's Universal Analysis 2000 software (version 3.3B).

Percent transparency of the films was studied by UV-Vis spectrophotometer (Geneq-Genesys 20) with wavelength at 660 nm.

RESULTS

Morphology: As shown in Fig. 1a-e, the native SF showed rougher of surface area than those of other blend films. The smooth surfaces of blend films gradually increased when the SF component decreased. With cross section, the native SF showed smooth surface with homogeneous phase through out the film. SF/G at 2:1 ratio, separate phase of the film surface was found. However, the separate phase was decreased when the SF content decreased. In addition, smooth surfaces can be obtained when blended SF and G at 1:2 ratio.

Chemical structure: The chemical structures of all SF/G blend films were analyzed using FTIR. The results showed strong region for 3 zones: amide I (1700-1600 cm^{-1}), amide II (1600-1500 cm^{-1}) and amide III (1250-1150 cm^{-1}) (Fig. 2). FTIR results indicated that SF/G blend films composed of the mixture characteristics both SF and G, compared to native films. At amide I region, native G film showed the absorption peak at 1632 cm^{-1} with shoulder peak at 1661 cm^{-1} whereas, SF showed the absorption peaks at 1632, 1661 and 1697 cm^{-1} in similar intensity. The absorption peaks from 1500-1300 cm^{-1} occurred slightly different profile depended on the components of SF and G. In contrast, the amide III regions showed similar peaks in all of blend films.

Thermal properties: Thermogravimetric (TG) curves of SF/G blend films were shown in Fig. 3. G native films decomposed its weight in lower than other films until 300°C. After that, the blend films rapidly decomposed in maximum rate in range from 300-350°C. The native G films remain its weight the lowest at 400°C about 33% as well as 600°C about 22%. The weight of blend films gradually decreased when the content of G increased. The results of differential thermogravimetric (DTG) curve showed slight difference depended on the components of SF and G (Fig. 4). The maximum decomposition temperatures of each blend films were 316°C (3:0 ratio), 321°C (2:1 ratio),

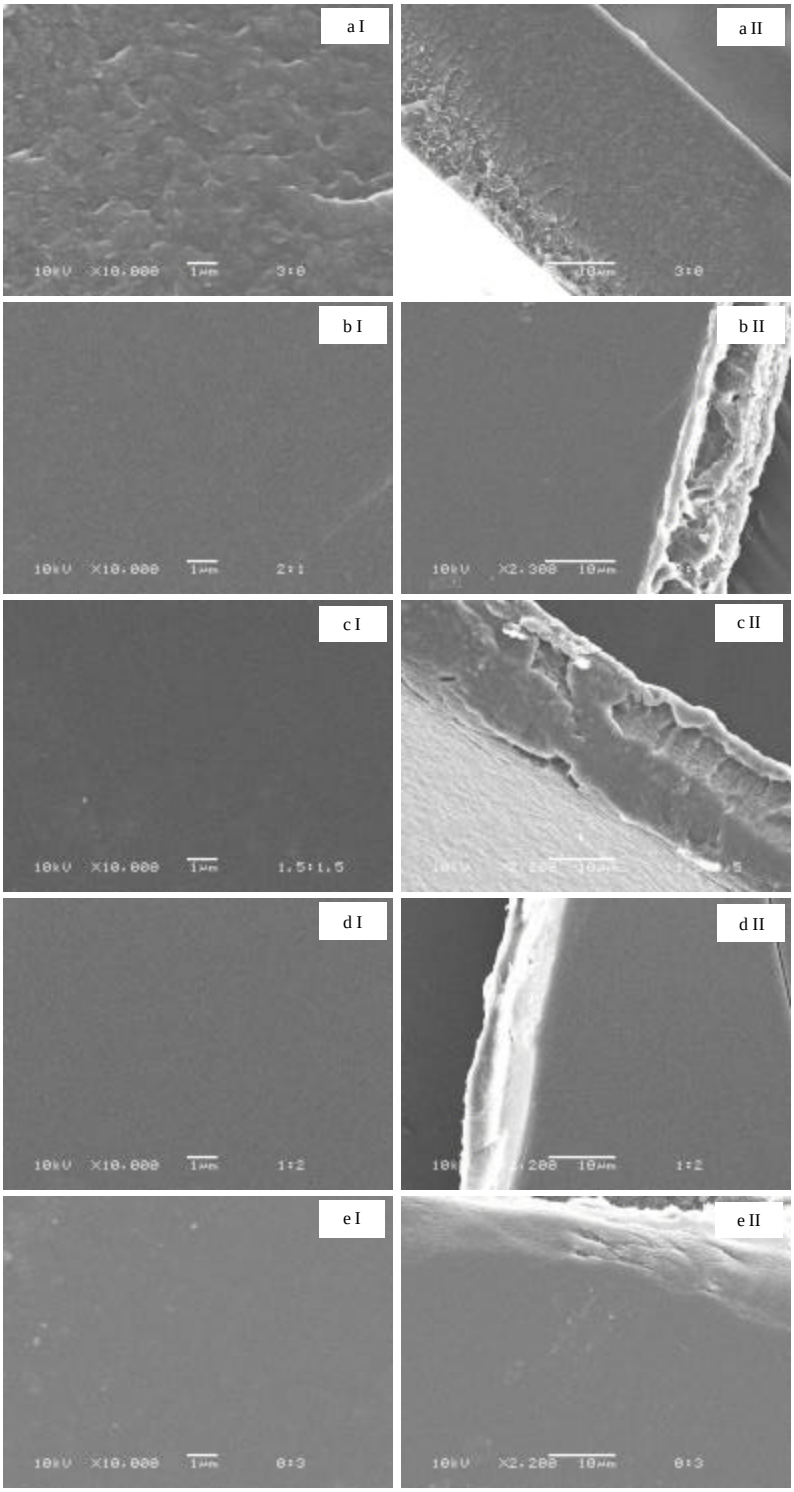

Fig. 1: SEM micrographs of SF/G blend films with different ratios: (a) 3:0, (b) 2:1, (c) 1:1, (d) 1:2 and (e) 0:3. Note surfaces and cross section were presented in column I and column II. Most contained 0.04% tetracycline

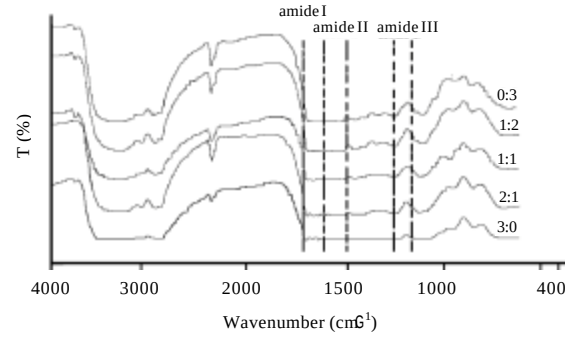

Fig. 2: FTIR spectra showed amide regions of SF/G blend films with different ratios

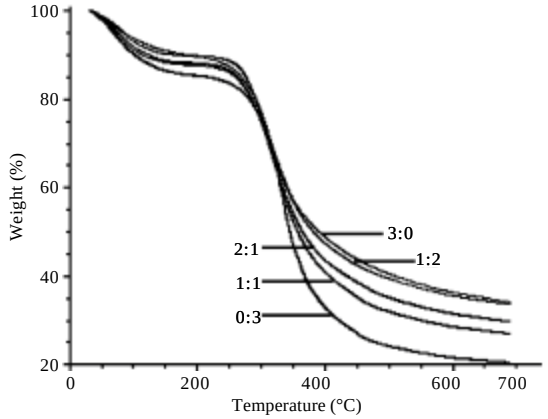

Fig. 3: TG curves of SF/G blend films with different ratios

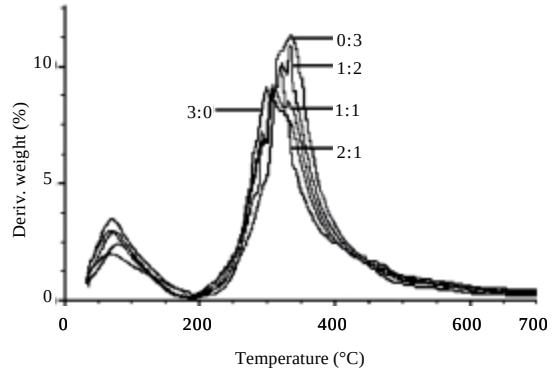

Fig. 4: DTG curves of SF/G blend films with different ratios

316 and 334°C (1:1 ratio), 318°C (1:2 ratio) and 334°C (2:1 ratio) (Table 1). The native SF film showed one peak of endothermic at about 300°C while native G film showed endothermic peaks at least 3 points at about 227, 330 and 360°C. The exothermic peaks of both native films showed

Table 1: Maximum decomposition temperatures of SF/G blend films

Film types (SF/G)	Decomposition temperature (°C)
3:0	66 315
2:1	75 290 310 321
1:1	69 292 316 334
1:2	81 293 312 321
0:3	69 334

Table 2: Multiple Exo/Endo-thermic peaks of SF/G blend films with different ratios

Film types (SF/G)	Exothermic peaks (°C)	Endothermic peaks (°C)
3:0	223 313 380	80 296 328
2:1	193 243 330 391	84 221 265 341
1:1	195 248 334 394	79 227 281 360
1:2	188 253 340 394	90 227 283 360
0:3	206 253 340 405	80 227 333 362

Table 3: Percent transparency of SF/G blend films with different ratios. (n = 4)

Film types (SF/G ratio)	Transparency (%)
SF without drug	88.55±0.47
3:0	85.55±0.51
2:1	83.30±0.67
1:1	67.30±1.92
1:2	54.18±1.44
0:3	37.50±1.80
G without drug	89.88±0.15

broad peaks at about 200 and 400°C. Moreover, all of blend films showed slightly different of endo/exo-thermic peaks depending on the components of SF and G which were listed in Table 2.

Percent transparency: Table 3 showed the native SF and G films without tetracycline have higher percent transparency than all of films that composed of tetracycline. The percent transparency gradually decreased when the content of G increased. The lowest of percent transparency obtained in the native G film contained tetracycline at 37.50±1.80%.

DISCUSSION

The SF and G are biomaterials which were wildly used especially biomedical applications according to their suitable properties including biocompatibility, high and mechanical strength (Jin *et al*., 2004). Both are protein, however, they are different types of amino acids composition. This point might be influenced to differences of morphology, chemical structures, percent transparency or even interaction with drug. On the other hand, drug loading on the material used for control release was highly increased. Tetracycline is an antibacterial which was applied in many fields (Enomfon-Akpan and Umoh, 2004).

With SEM micrographs, the native SF showed rougher surface than other films. It is suggested that SF composed of large or various molecules which affected on

the interaction together. The films all display homogeneous structure, indicated a high level of miscibility of the blend (Yang *et al*., 2000). However, the obviously homogeneous surface was adjusted by decreasing of SF contents. This result indicated that G helps to improve both charge and bond formation of the blend films (Okhawilai *et al*., 2010). Conformational structure is significantly influenced on protein properties (Lee *et al*., 2003). The chemical structures of protein were sensitively analyzed by FTIR in the regions of amide I (1700-1600 cm^{-1}), amide II (1600-1500 cm^{-1}) and amide III (1300-1200 cm^{-1}) (Hino *et al*., 2003). Generally, the structures of SF and G are arranged in random coil and α-helix structures, respectively (Songchotikunpun *et al*., 2008). The FTIR results indicated that SF/G blend films showed individually characteristics of both SF and G with the co-existed of α-helix and β-sheet structures (Mandal *et al*., 2009; Tao *et al*., 2007). The results might be suggested that high number of hydrogen bonds have been formed between carbonyl groups and amino groups of protein. The obtained result showed as same as previously reported by our group (Wilaiwan *et al*., 2010). In different ratios of SF/G blend films, percent transparency was differed. The decreasing of percent transparency was obtained when the G content was increased. The results indicated that tetracycline (hydrophilic drug) could be interacted very well with G (polar material) and affected on the film transparency. This result concluded that percent transparency of the films should be influenced by material charge (Remunan-Lopez and Bodmeier, 1997).

Thermal properties analysis showed that native G film has higher strength than other films. However, it was rapidly decomposed after maximum decomposition temperatures. The blend films showed higher decomposition steps than that of native films. This might be from the blending between the characteristics of SF and G (Kweon *et al*., 2001).

CONCLUSIONS

Those of SF/G blend films loaded-tetracycline were prepared and investigated for their morphology, chemical structures, thermal properties and percent transparency. All of SF/G blend and G films have smooth surfaces, except native SF film. The surfaces of the blend films depended on the content of both SF and G components. Chemical structures indicated that SF and G could be interacted by hydrogen bonds formation. The component ratio of SF and G was a key point for thermal properties of the blend films. In conclusion, SF/G blend films properties could be adjusted and applied for loading hydrophilic substances.

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