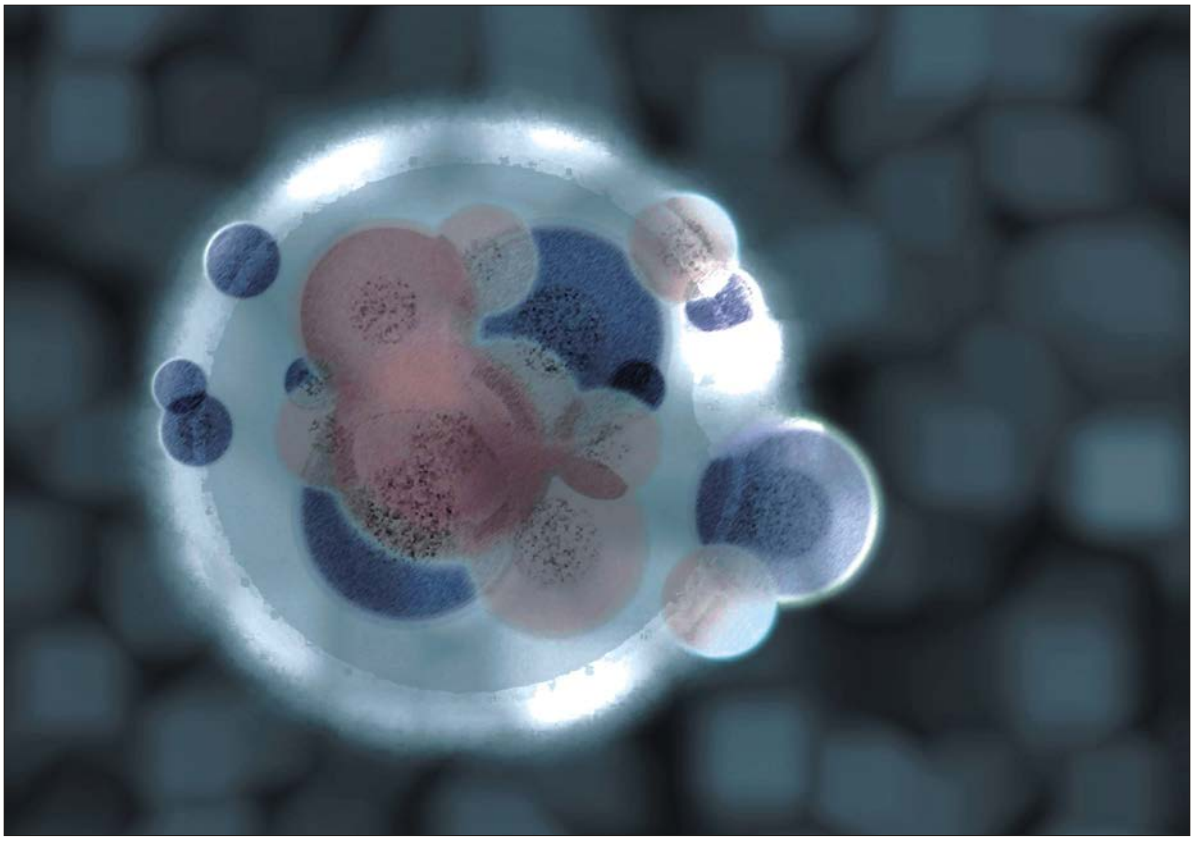

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Silk Fibroin/Gelatin Hybrid Films for Medical Applications: Study on Chlorhexidine Diacetate

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Abstract: This study aimed to prepare silk fibroin (SF)/gelatin (G) hybrid films by a solvent evaporation method for loading chlorhexidine diacetate (CHX). The SF and G solution in different ratios were mixed with CHX and placed on the 5 cm polystyrene plates before drying to obtain hybrid films. The films were determined their secondary structures and thermal properties by using Fourier transform infrared (FT-IR) spectrometer and thermogravimetric analysis (TGA), respectively. The results found that all of film composed of α-helix and β-sheet structures. However, differences of the α-helix and β-sheet structures were differed according to each component. The hybrid films showed soft texture and decreased of brittle compared to SF film only when the G content increased. Thermal properties of the films indicated that decomposition temperature profiles of all films did not differ dramatically, however, combination of characteristics both SF and G were appeared in hybrid films. The releasing rate of CHX-loaded in the films was found that the CHX has released from the SF film in higher rate than hybrid and G films, respectively. It is a promising that polarity, flexibility as well as component ratio of each polymer play important role on the releasing of CHX.

Key words: Gelatin, hybrid films, releasing rate, silk, thermal properties

INTRODUCTION

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).

In the recent year, natural polymers have highly interested for study according to their properties such as non-toxicity, biodegradability and biocompatibility. Collagen, gelatin, chitosan, silk and hyaluronic acid are natural polymers which have been studied and applied in various applications. However, their high cost and questionable purity have limited their uses (Cheung *et al*., 2008). While natural homopolymer demands are not sufficient for biomaterial products, therefore, blend polymers have been studied to improve the performance of the individual natural polymer (Fan *et al*., 2008).

Silks are fibrous protein that is produced by some Lepidoptera larvae. Their compositions and properties differ widely by the specific sources (Altman *et al*., 2003). Each silk fiber consists of two types of proteins; fibroin and sericin. Silk fibroin (SF) is an insoluble fibrous component, whereas sericin is a glue-like protein that is well soluble in hot water or some organic solvents. The 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).

Gelatin (G) is a derivative collagen. It shows excellent properties including low cost, good biocompatibility, biodegradability, low immunogenicity, increased cell adhesion, migration, differentiation and proliferation (Thein-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 (Gil *et al*., 2007; Lee *et al*., 2008). Moreover, it can be applied alone or as a blend polymer (Huang *et al*., 2004).

The research about SF/G hybrid film loaded chlorhexidine diacetate has limited available information. In this research, we prepared the hybrid film of SF/G and then studied the secondary structure and thermal behavior of the film as well as releasing rate of chlorhexidine diacetate. The goal of this study is to evaluate the SF/G film as a biomaterial for loading some medical drugs to apply in delivery system.

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MATERIALS AND METHODS

This study was constructed for 3 months from January 10, 2010 to April 10, 2010. All of experiments were carried out on Department of Chemistry, Faculty of Science. The characterization of secondary structure was done at the Central Instrument, Faculty of Science, Mahasarakham University, Thailand.

Materials: *Bombyx 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. Chlorhexidine diacetate (CHX) was kindly supplied by Osoth Inter Laboratories Co., Ltd., Thailand. All used chemicals were analytical grade obtained commercially.

Methods

Silk Fibroin (SF) solution preparation: The *B. mori* cocoons were firstly striped into small pieces. The sericin 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 silk strips 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 1h and stirring until SF solution was obtained. The solution was then dialyzed in dialysis bag (Mw. cut off 6500 kDa) against distilled water for 3 days. SF concentration was calculated by evaporation method and adjusted to 1% (w/v) by distilled water.

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

SF/G hybrid films preparation: The mixture of SF/G solution at various compositions (1:0, 3:1, 1:3 and 0:1 (by volume)) was prepared. Chlorhexidine diacetate (CHX) (2% w/v), a drug model was loaded in the solution. The 20 mL of each mixture solution was cast on the 5cm diameter polystyrene plates. The plates were left at 40°C in an oven for 3 days.

Structure characterization: The native SF, G and SF/G hybrid films were analyzed for their secondary structure by using FT-IR spectrometer (Perkin Elmer-Spectrum Gx, USA) in the spectral region of 2000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans.

Thermal properties determination: The films (8-10 mg) were loaded in a platinum crucible. The thermogravimetric analysis (TGA) was then performed using a TA instruments, SDT Q600 (Luken's drive, New Castle, DE). The samples were non-isothermal heated from 50 to 1000°C at a heating rate of 20 °C min^{-1}. The TGA was carried out under 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 analyses of the data were done using TA Instrument's Universal Analysis 2000 software (version 3.3B).

***In vitro* drug release test:** All films (1×1 cm^2) were immersed in a 20 mL of phosphate buffer saline (PBS, pH 7.4) solution at 37 °C for 48 h with continuously stirred at 150 rpm. The supernatant of PBS (5 mL) was replaced with a fresh buffer solution at a time interval. The absorbance at 254 nm of the aqueous solution was measured. The amount of CHX was calculated by the absorbance according to the standard curve.

RESULTS

FT-IR analysis: FTIR spectra of G film showed absorption bands at 1689, 1680, 1624, 1573, 1522, 1450, 1245, 1167 and 692 cm^{-1} while absorption peaks of SF films occurred at 1678, 1654 and 1615 cm^{-1} (amide I) 1559 and 1522 cm^{-1} (amide II) 1240 cm^{-1} (amide III) 1076 cm^{-1} (amide IV) and 655 cm^{-1}(amide V) (Fig. 1). The spectra of the SF/G hybrid films appeared the characteristic absorption bands of both SF and G films. In addition, the tendency of absorption bands depended on the component of SF or G.

Thermal properties: Thermal decomposition of native SF, G and SF/G blend films was analyzed from the thermogravimetric (TG) curves as shown in Fig. 2. All of films did not completely decompose even at 1000°C. The

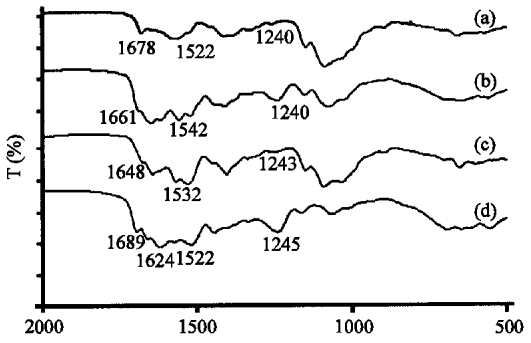

Fig. 1: FT-IR spectra of SF (a), SF/G (3:1) (b), SF/G (1:3) (c) and G (d) films

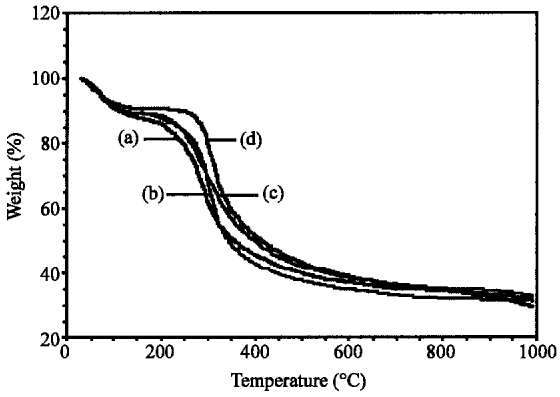

Fig. 2: TG curves of SF (a), SF/G (3:1) (b), SF/G (1:3) (c) and G (d) films

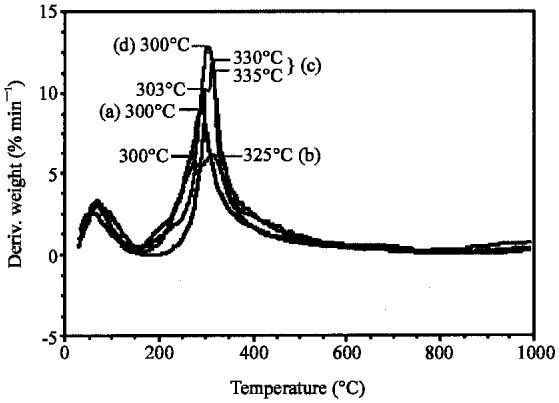

Fig. 3: DTG curves of SF (a), SF/G (3:1) (b), SF/G (1:3) (c) and G (d) films

rapid weight loss was observed at around 300°C. Clearly evidence of thermal decomposition was supported by differential TG (DTG) curves as shown in Fig. 3. The G and SF films took place in single step decomposition at the maximum of 300°C and 308°C, respectively. On the other hand, SF/G blend films appeared at least two decomposition steps. The SF/G hybrid film at 1: 3 ratios took placed for three steps; at 303, 330 and 335°C. Moreover, SF/G hybrid film at 3:1 ratios took place for two steps; at 300 and 325°C, respectively. Figure 4 showed heat flow thermograms of the films measured at the temperature range of 50 to 1000°C. Those of all films showed sharp endothermic peaks at less than 100°C. The characteristics of each component were found and different endo/exo peaks. SF films showed endothermic peaks at 280°C, while G film has only one exothermic peak at 320°C. The SF/G hybrid films showed characteristic of SF or G exothermic peaks at 315°C with small shoulder at 250°C (ratio SF/G; 1:3) and at 300 and 322°C (ratio SF/G; 3:1).

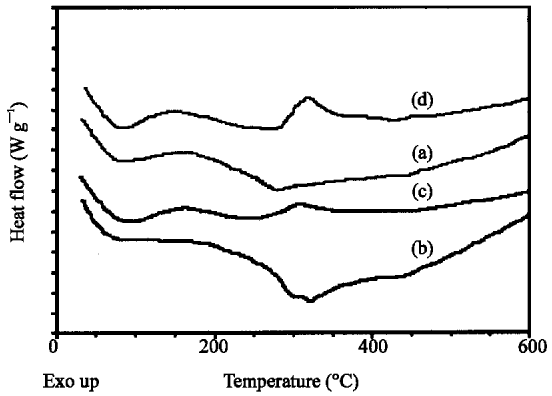

Fig. 4: Heat flow curves of SF (a), SF/G (3:1) (b), SF/G (1:3) (c) and G (d) films

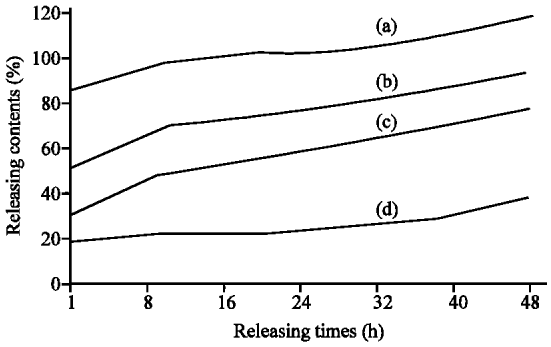

Fig. 5: Releasing curves of CHX from SF (a), SF/G (3:1) (b), SF/G (1:3) (c) and G (d) films

Releasing of chlorhexidine diacetate: The results found that CHX was rapidly released from SF film in first 30 min, contrast with G film which was slowly released of the drug (Fig. 5). In case of SF/G hybrid films, the releasing of CHX was affected by the component. The result indicated that CHX from hybrid film was rapidly released following the high ratio of SF.

DISCUSSION

Both SF and G have been reported as an excellent biomaterials used in various fields (Gil *et al*., 2007; Fan *et al*., 2008; Patel *et al*., 2008; Thein-Han *et al*., 2009). Generally, secondary structures of the film were analyzed using FTIR, especially for SF film (Kweon *et al*., 2000; Hino *et al*., 2003). This was due to the peptide bonds (amide group) in protein were sensitively absorbed to the light energy. This region composed of amide I (1700-1600 cm^{-1}), amide II (1600-1500 cm^{-1}) and amide III (1300-1200 cm^{-1}). FT-IR data indicated that the secondary structures of SF film showed 1678, 1655 and 1615 cm^{-1}

(amide I, C = O stretching), 1559 and 1522 cm^{-1} (amide II, N-H deformation), 1240 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). Moreover, these regions were the characteristics of SF film with CHX (Noi *et al*., 2009). The G film showed those of carbonyl and amino peaks at 1689, 1680, 1624, 1573, 1522 and 1245 cm^{-1} which are due to C = O stretching, amide II and amide V, respectively (Thein-Han *et al*., 2009). For hybrid films, the absorption peaks showed specifically bands of both SF and G. This result suggested a chemical interaction between SF and G molecular chains and intermolecular bonding of hydrogen of SF and G formation (Watcharin *et al*., 2009; Thein-Han *et al*., 2009).

From thermal analysis, native film took place single decomposition step. However, hybrid films showed more decomposition steps than the native films. This was due to the different characteristics of each hybrid components (Kweon *et al*., 2001). The maximum decomposition temperature were arranged from SF<G<hybrid SF/G (3:1) <G/SF (3:1) films. This result indicated that higher flexibility film (G) acted as plasticizer to improve the flexibility and strength of the film.

The CHX was rapidly released from the SF film, but was lowest from G film. The results indicated that polarity of the drug and flexibility of material would be affected to the releasing profile. Since CHX is a polar drug whilst SF is a hydrophobic polymer, the interaction might be hard to form results to rapidly release of CHX from the films (Thakur *et al*., 2008; Zeng *et al*., 2005). G is a polar material and should be interacted very well with CHX which affected to the slowest released rate (Remuñán-López and Bodmeier, 1997).

CONCLUSION

SF, G and SF/G hybrid films loaded CHX could be prepared using casting and evaporation technique. The mixture of SF and G enhanced bonding formations which were affected to the changes of secondary structure as well as thermal characteristics of the films. The hybrid films showed higher flexibility than SF native. Moreover, G also decreased hydrophobic part of the SF which be led to sustain releasing of CHX from the film. In conclusion, releasing of CHX was affected by polarity, flexibility and content ratio of materials

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