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Silk Fibroin Film Loaded Chlorhexidine Diacetate: Interaction and Characteristics

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Abstract: This study aimed to prepare Silk Fibroin (SF) films with different weight (0.5, 1 and 1.5%) for loading chlorhexidine diacetate as substrate for study their characteristics including morphology, structure and thermal properties. The morphological observation under scanning electron spectroscopy found that all of films have a smooth surface. With cross-section micrographs, the lowest SF content was smoother texture than other. The secondary structures of various films were determined by Fourier transform infrared (FTIR) spectrometer. The results showed that SF film composed of β-sheet structures in different ratio depending on the SF content. The result suggested that high SF content formed crystalline by interaction between amino acids molecules in higher ratio than other. However, thermal properties of the SF films did not dramatically differ compared between low and high SF content. It was also found that CHX did not affect on the SF characteristics.

Key words: Chlorhexidine, film, silk fibroin, secondary structure, thermal property

INTRODUCTION

Chlorhexidine (CHX) is an antibacterial and has been used in endodontic therapy, in treating root canal infections (Nerurkar *et al*., 1995). It is used to a large extent in various formulations due to its wide spectrum of bactericidal and antiviral activity (Musteata and Pawliszyn, 2005; Giannelli *et al*., 2008). However, some limitations when used this drug has also observed such as precipitate in the oral cavity, rapidly released rate, low solubility (Zeng *et al*., 2009) or adverse effects on oral tissues and cells (Giannelli *et al*., 2008). Therefore, drug control-released has been developed for delivery system. This system composed of various advantaged including preservation integrity and function, control release kinetic and potentially targeted delivery to specific position (Whitaker *et al*., 2001). The critical point is a suitable material that can be composed of excellent properties for delivery system including high strength, biocompatibility, biodegradability, especially easy use.

Both natural and synthetic polymers have been focused as biomedical applications including drug control-released system. Silk Fibroin (SF), a natural fibrous protein, has numerous studied as biomedical applications (Min *et al*., 2004). The SF can be used in various forms including gel, powder, fiber or membrane, depending on application (Park *et al*., 2004). The SF has wildly used since their unique properties including nontoxicity, biocompatibility and biodegradability were reported (Foo and Kaplan, 2002). In addition, it showed very excellent mechanical properties. Therefore, SF is considered an ideal biomaterial and promoted studied on its structure and properties (Kweon *et al*., 2000).

So far, the study about SF film loaded CHX has limited available information. In this research, we prepared the SF with different SF %weight. The goal of the study is to explore the characteristics of SF films before and after loading CHX. Moreover, their interactions were investigated to assess the possibility for using SF film as delivery system of CHX.

MATERIALS AND METHODS

This study was carried out for 3 months from May 1, 2009 to August 30, 2009. All of the experiment was carried out at Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Materials: *Bombyx mori* (*B. mori*) silk cocoons were kindly supplied by Silk Innovation Center (SIC), Mahasarakham University, Thailand. Chlorhexidine diacetate (CHX) was kindly supplied by Osoth Inter Laboratories Co., Ltd., Thailand. All used chemicals were analytical grade obtained commercially.

Methods

Preparation of SF solution: The *B. mori* cocoons were firstly striped into small pieces and 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. They were

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hen air-dried at room temperature. The dried cocoons were dissolved with the tertiary solvents system of calcium $CaCl_2$-Ethanol-H_2O (1:2:8 by mole). The mixture of solvent and striped cocoons was boiled at 90-95°C for 1 h and stirring. The SF solution was dialyzed in dialysis bag against distilled water for 3 days. Finally, SF concentration was calculated by evaporation method and adjusted to 1% (w/v).

SF films-loaded chlorhexidine preparation: The SF films were prepared by casting of 15 mL of mixture between SF solution and CHX (0.004 g) on the 5 cm polystyrene plates at room temperature. In this study, %weight of SF was varied from 0.5, 1 and 1.5% to find out suitable protein content for film construction. The plates were dried at 40°C in oven for 3 days to obtain SF films.

Morphological observation: The SF films loaded CHX were sputter coated with gold (for enhanced surface conductivity) and observed under the scanning electron microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). A voltage of 15 kV was used. Moreover, KBr disc method was used for CHX analysis.

Structure analysis: The secondary structures of all of the films were analyzed using a Fourier transform infrared (FTIR) spectrometer (Perkin Elmer-Spectrum Gx, USA). Analysis was performed in the spectral region of 4000-500 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans.

Thermal behavior measurement: Eight to ten milligrams of each film was loaded in a platinum crucible. The thermogravimetric analysis (TGA) was performed using 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 10°C min^{-1}. The TGA was carried out in nitrogen with a flow rate of 100 mL min^{-1}. The TG and heat flow data were recorded with TA instrument's Q series explorer software. The analyses of the data were performed using TA Instrument's Universal Analysis 2000 software (version 3.3B).

RESULTS

SEM observation: The SEM micrographs of SF-loaded CHX showed smooth and took uniform throughout their surfaces area (Fig. 1). The SF film prepared form low % wt. showed smoother than other. The surfaces of the SF film was gradually rough when increased SF content. More detail can be observed by cross-section images. It was found that SF film was also smoother and well packed at low SF content compared to other. Phase separation was appeared when the SF content increased.

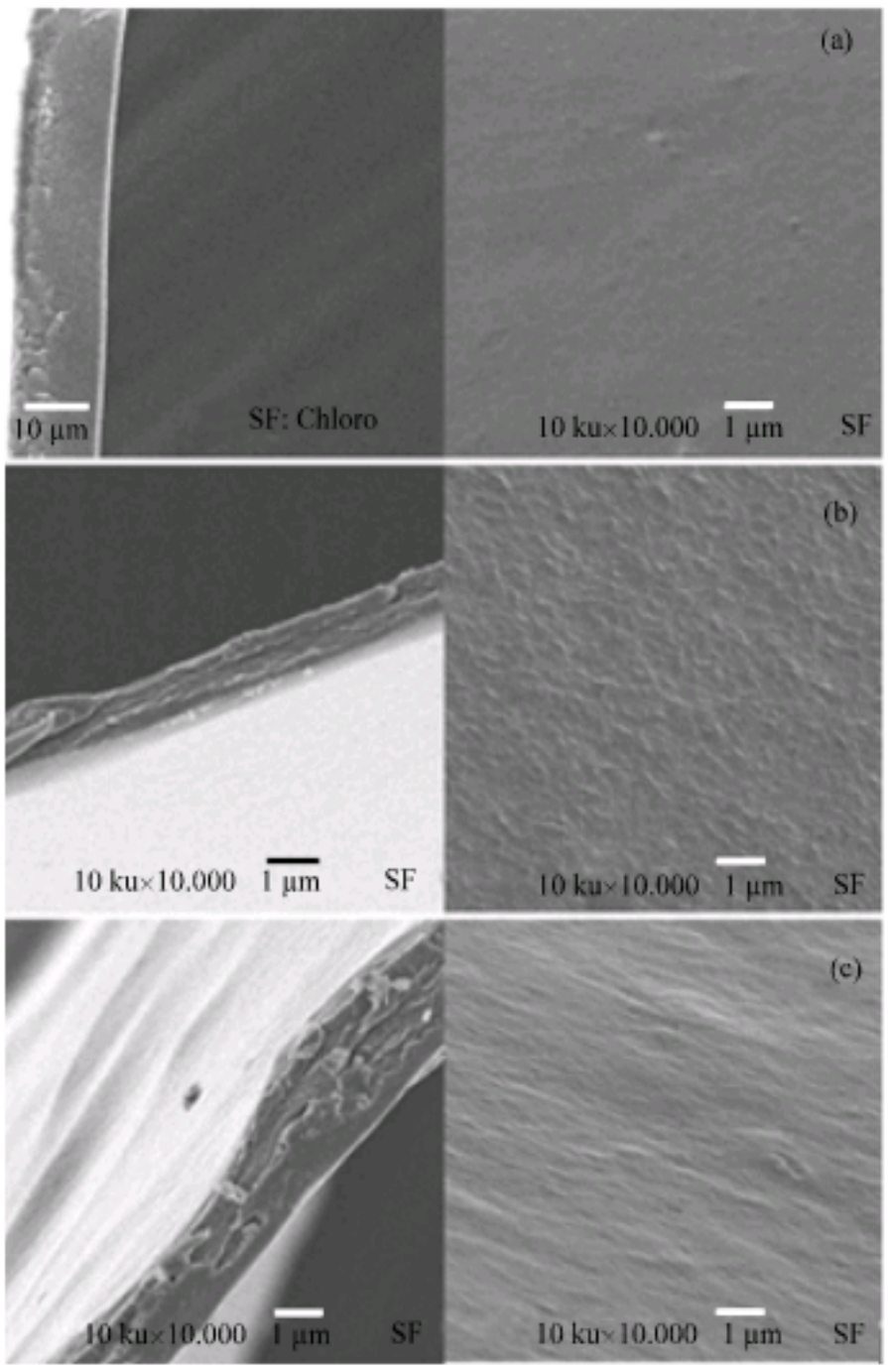

Fig. 1: SEM micrographs of SF films-loaded chlorhexidine surfaces (right column) and cross-section (left column); (a) 0.5% SF-loaded CHX, (b) 1% SF-loaded CHX and (c) 1.5% SF-loaded CHX

Structural changes of SF films: As shown in Fig. 2, amide II peak of native SF film appeared at 1521 cm^{-1} while CHX showed strongly peak at 1571 cm^{-1}. It was found that SF films prepared form different SF contents have similar spectra. However, they showed slightly difference absorption peak after loading CHX. The 0.5% wt. SF film showed amide II peak at 1559 cm^{-1}, 1% wt at 1534 cm^{-1} and 1.5% wt. at 1522 cm^{-1}, respectively. In addition, CHX showed dominantly peak at 2884 cm^{-1} which differed from most of SF.

Thermal behavior: The SF films loaded CHX did not completely decompose even at 1000°C. From the thermogravimetric (TG) curves, rapid in weight loss was shown at the temperature around 300°C (Fig. 3). All of films did not differ much in their spectra. The decreasing of the thermal stability of the SF films was clearly indicated by DTG thermograms (Fig. 4). From the results, SF films showed maximum decomposition temperature

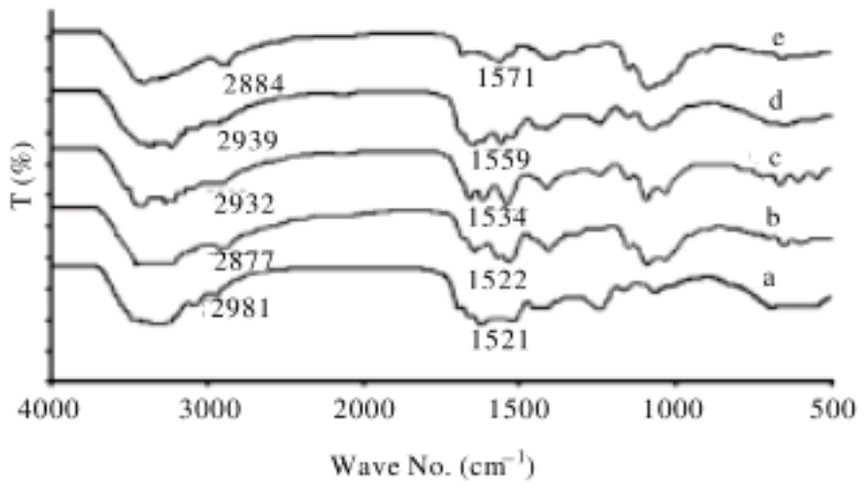

Fig. 2: FT-IR spectra of SF films-loaded chlorhexidine; (a) SF, (b) 0.5% SF-loaded CHX, (c) 1% SF-loaded CHX, (d) 1.5% SF-loaded CHX and (e) CHX

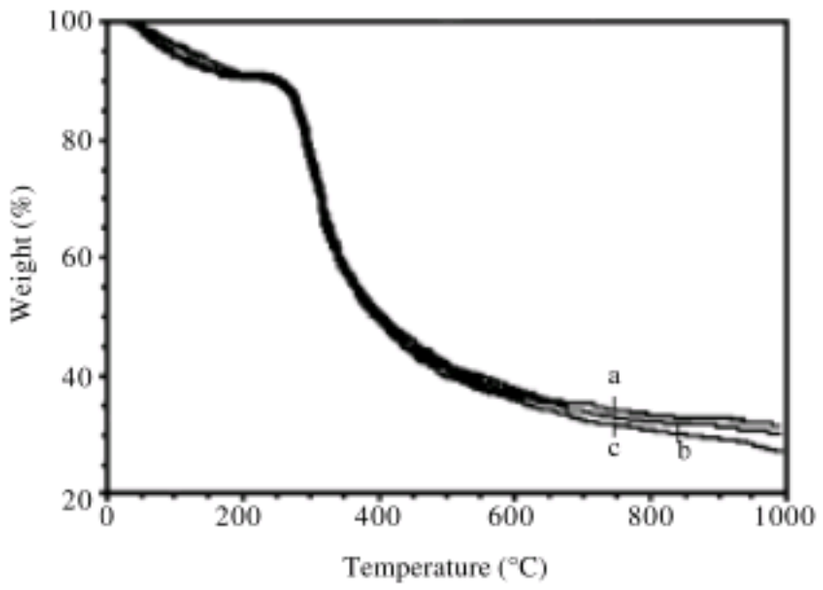

Fig. 3: TG curves of different % weight SF films loaded CHX; (a) 1.5%, (b) 1% and (c) 0.5%

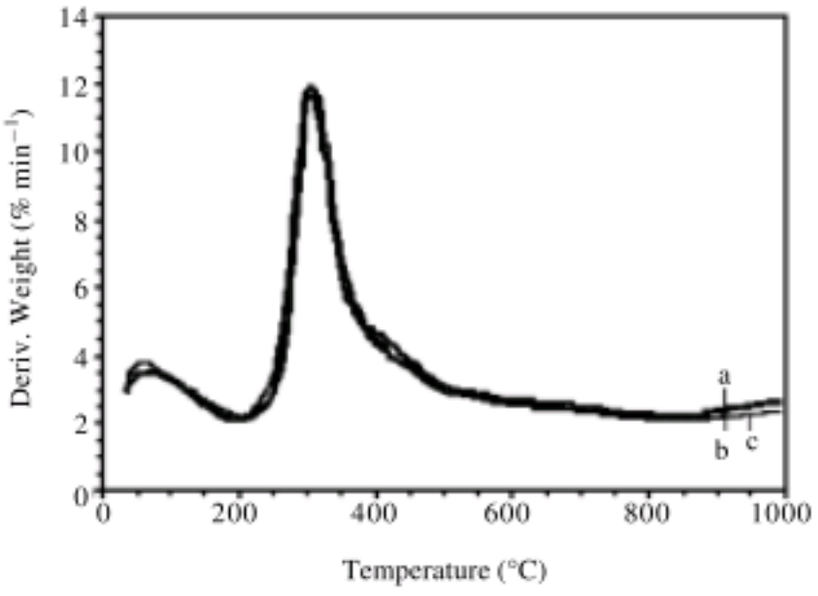

Fig. 4: DTG curves of different %weight SF films loaded CHX; (a) 1.5%, (b) 1% and (c) 0.5%

with the same point at approximately 308°C (Fig. 5). It was showed that SF films took place in single step. With heat flow thermograms, SF films showed strong endothermic peak at around 300°C. Moreover, endothermic peaks at below 100°C were appeared in all of SF films.

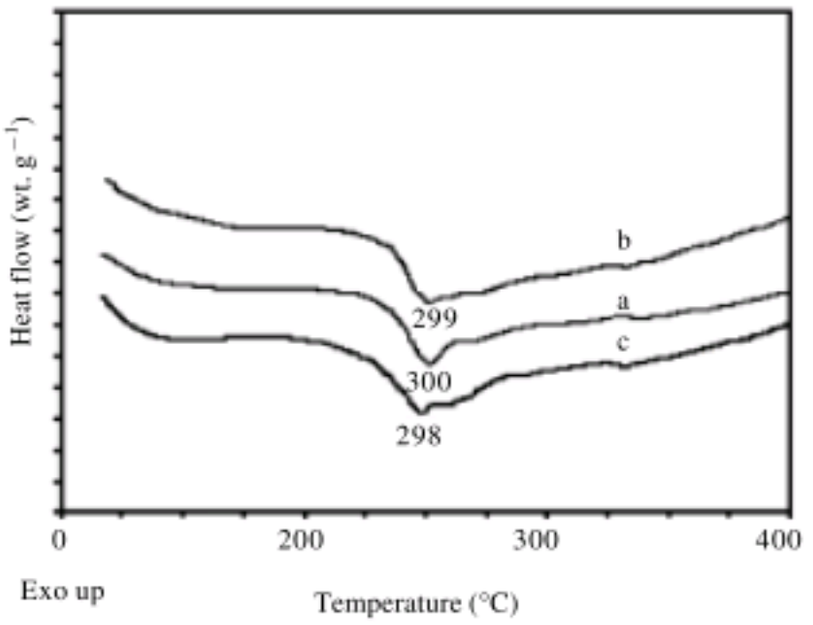

Fig. 5: Heat flow thermograms of different %weight SF films loaded CHX; (a) 1.5%, (b) 1% and (c), 0.5%

DISCUSSION

The SF films loaded CHX could be prepared. The obtained films were thin, smooth and appeared uniform throughout their surfaces area. The results of SEM micrographs indicated that SF content was an important parameter in the film texture. It is revealed that SF surfaces was smoother by using low SF content and gradually rough was obtained by increasing the content of SF. This might be caused from the crystalline formation of the amino acid composed in the SF protein. However, FTIR spectrum showed strong absorption peak of amide II at 1521 cm^{-1}, attributed to β-sheet structure (Kweon *et al*., 2001). The β-sheet was gradually decreased from high to low SF content used. At high SF content, β-sheet structure was higher formation than that of low SF content which was reflected to the crystalline formation in the film (Chen *et al*., 1997).

This might be suggested the interaction between amino acids component were formed (Baimark *et al*., 2009). On the other hand, the β-sheet structure of SF films were not increased by adding CHX. Nevertheless, some characteristic peaks of the SF were shifted when contained of the CHX. This might has participated in some interaction between SF and CHX, the reason is not yet clear by now.

The maximum decomposition temperature of SF-loaded CHX occurred at around 298°C, indicated to a depolymerization of amino acids chain of SF (Peniche-Covas *et al*., 1993). Most of SF films-loaded CHX appeared single maximum peaks, indications that the thermal decomposition of only SF characteristic. It could be confirmed that CHX did not affect on the SF stability. However, it was also observed that the maximum decomposition temperature shifted slightly with different SF content.

The other results from DTG and heat flow thermograms showed similar trend with TG curves. It is suggested that SF content was an important factor to the film appearance and characteristics without affect from CHX (Kweon *et al*., 2000), However, more details about the interaction between SF and CHX should be further investigated.

CONCLUSION

The SF films with different SF contents were prepared and loaded with CHX to study their characteristics. With SEM micrographs, SF films with low SF content showed smoother than other but less of β-sheet structure. This was due to the interaction between amino acids was the lowest which was indicated by FTIR spectra. It was also suggested that high SF contents enhanced the crystalline part to make the strength of the SF film. The results are related to the thermal behavior, indicated by TG, DTG and heat flow curves. The CHX characteristic did not affect on the SF films. It might promise that the SF could be used as materials for encapsulation of the CHX for delivery system.

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