

# Chitosan/Silk Sericin Blend Microparticles Prepared by Water-in-Oil Emulsification-Diffusion for Controlled Release of Silk Sericin Antioxidant

Theeraphol Phromsopha, Yodthong Baimark\*

*Biodegradable Polymers Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand*

*Received: 7<sup>th</sup> Jun, 17; Revised 25<sup>th</sup> Oct, 17, Accepted: 24<sup>th</sup> Nov, 17; Available Online: 25<sup>th</sup> Dec, 2017*

## ABSTRACT

This study aimed to develop the novel drug delivery carriers to control the release of silk sericin antioxidant. The chitosan/silk sericin blend microparticles were successfully prepared by the water-in-oil emulsification-diffusion method. Influence of silk sericin ratio on characteristics and *in vitro* silk sericin release behaviors of the blend microparticles was investigated. All the spherical-shaped blend microparticles had an average size of 41 – 48  $\mu\text{m}$  and could load the silk sericin in high loading efficiency (69 – 75%). The FTIR and thermogravimetric results indicated the blend microparticles with different silk sericin contents can be prepared by varying the silk sericin feed ratio. The blend microparticles exhibited sustained release profiles of the silk sericin. The *in vitro* silk sericin release content increased as the silk sericin ratio increased. Antioxidant activities of the blend microparticles assessed by FRAP assay were more than the neat chitosan microparticles and steadily increased with the silk sericin ratio. The results demonstrated that the chitosan/silk sericin blend microparticles prepared by the water-in-oil emulsification-diffusion method should be useful drug delivery carriers for the controlled release silk sericin antioxidant.

**Keywords:** Chitosan, silk sericin, emulsification-diffusion, blend microparticles, controlled release.

## INTRODUCTION

Controlled release drug delivery carriers made from biodegradable polymers provided several benefits over traditional formulations<sup>1</sup>. Prior to release, the drug is protected from degradation or premature metabolism by the polymeric matrix. The drug release is sustained over days to months, thereby keeping the drug concentration in the plasma at an effective level for longer periods of time and reducing toxic side-effects. This decreases the frequency of drug dosing and increases patient compliance. The removal of these biodegradable polymer-based devices at the end of therapy is not required. Drug delivery carriers have been widely made from a variety of both natural and synthetic biodegradable polymers. The natural polymers such as chitosan, silk and starch etc. are cheaper and easier to find.

Chitosan is a natural biocompatible and biodegradable polysaccharide that has been prolifically investigated in a variety of applications including drug delivery, wound dressing and food packaging<sup>2,3</sup>. Chitosan particles have been widely investigated for use in controlled release drug delivery applications<sup>4-6</sup>. For this purpose, various methods have been reported for fabrication the chitosan microparticles entrapped model drug<sup>7</sup>. Our previous works has shown that the water-in-oil emulsion solvent diffusion method was a potential method for preparing the microparticles of both the chitosan<sup>8</sup> and silk sericin<sup>9</sup>. The

polymer aqueous solution and ethyl acetate were used as water and oil phases, respectively. The main advantages of this method are fast and low-cost. Although, the blend microparticles of chitosan/silk fibroin<sup>10</sup> and silk fibroin/silk sericin<sup>9</sup> have been prepared by the water-in-oil emulsification-diffusion method, however, investigations focusing on the preparation of chitosan/silk sericin blend microparticles have yet to be published.

Silk sericin is a hydrophilic natural biodegradable polymer that extracted from silk cocoons. The silk sericin has been widely investigated as a biomaterial for use in biomedical applications due to its biocompatibility and biodegradability<sup>11,12</sup>. The silk sericin has excellent antioxidant activity, moisture properties and resistant to UV radiation<sup>13,14</sup>. The silk sericin showed then potential for use as an antioxidant agent. However, controlled release of the silk sericin from biodegradable microparticles has been scarcely published. Electrospraying technique has been applied to produce alginate/silk sericin blend microparticles for controlled releasing the entrapped silk sericin<sup>15</sup>. In the present work, chitosan/silk sericin blend microparticles were prepared by the water-in-oil emulsification-diffusion method. The influence of the chitosan/silk sericin blend ratio on microparticle characteristics and drug release behaviors were evaluated.

\* Author for Correspondence: [yodthong.b@msu.ac.th](mailto:yodthong.b@msu.ac.th)

## MATERIALS AND METHODS

### Materials

A chitosan powder with de-acetylation degree and molecular weight of 90% and 15,000 Da, respectively, was purchased from Seafresh Chitosan Lab Co., Ltd., (Thailand). A silk sericin aqueous solution was extracted from the silk cocoons of *Bombyx mori* silkworms. The silk cocoons (1 g) were boiled in de-ionized water (100 mL) in an autoclave oven at 120 °C for 30 min to extract the silk sericin. This process is referred to as de-gumming of the silk fibers. The silk sericin aqueous solution was collected by filtration. The silk sericin concentration was adjusted to 0.5 %w/v against de-ionized water before use. The 1.0% acetic acid aqueous solution was used as a solvent to prepare the 1.0 %w/v chitosan aqueous solution. All chemicals and solvents in analytical grade were used without further purification.

### Preparation of chitosan/silk sericin blend microparticles

The blend microparticles were prepared by the water-in-oil emulsion solvent diffusion method. 2.0 mL of a chitosan/silk sericin blend solution (water phase) was slowly added drop-wise to 400 mL ethyl acetate (oil phase) under magnetic stirring at 900 rpm to prepare the microparticles. The emulsification-diffusion process took 1 h. The beaker was tightly covered with aluminum foil to prevent ethyl acetate evaporation during the emulsification-diffusion step. The obtained microparticles were collected and rinsed with fresh ethyl acetate before drying in a vacuum oven at room temperature overnight. The dried microparticles were kept in a desiccator before characterisation of their properties and silk sericin release testing. The microparticles with chitosan/silk sericin feed ratios of 99/1, 95/5 and 90/10 (w/w) were investigated. The neat chitosan and silk sericin microparticles were also prepared by the same method for comparison.

### Characterisation of chitosan/silk sericin blend microparticles

The morphology of the microparticles was analysed by scanning electron microscopy using a JEOL JSM-6460LV scanning electron microscope (SEM). The microparticles were sputter-coated with gold to enhance the surface conductivity before scan. The average particle size of the microparticles was determined from several SEM images by counting a minimum of 100 particles using the smile view software (version 1.02).

The chemical functional groups of the microparticle samples were determined by Fourier transform infrared (FTIR) spectroscopy using a Perkin-Elmer Spectrum GX FTIR spectrometer. A resolution of 4 cm<sup>-1</sup> and 32 scans were employed.

The thermal decomposition behavior of the microparticles was determined using non-isothermal thermogravimetric analysis (TGA, TA-Instrument SDT Q600). For the TGA analysis, 5 – 10 mg of microparticles was heated at 20 °C/min under a nitrogen atmosphere over the temperature range 50 to 1,000 °C.

The silk sericin loading content of the microparticles was measured by complete dissolving the ~10.0 mg of microparticles in 5.0 mL of 1.0% acetic acid solution for 24 h. The silk sericin content was determined by the Lowry

protein assay. Subsequently, the absorbance at 600 nm was recorded. Bovine serum albumin (BSA) was used as a standard protein to obtain the following linear regression equation: Y (absorbance) = 0.008X (silk sericin concentration) + 0.006 (r<sup>2</sup> = 0.9980).

According to a predetermined silk sericin concentration-UV-vis absorbance standard curve, the silk sericin concentration of the solution was obtained. Theoretical loading content (LC<sub>theoretical</sub>) and actual loading content (LC<sub>actual</sub>) of silk sericin were calculated from equations (1) and (2), and were used to measure the loading efficiency (LE) of silk sericin as in equation (3).

$$LC_{\text{theoretical}} (\%) = \frac{\text{feed silk sericin (mg)}}{\text{feed chitosan} + \text{feed silk sericin (mg)}} \times 100 \quad (1)$$

$$LC_{\text{actual}} (\%) = \frac{\text{loaded silk sericin (mg)}}{\text{blend microparticles (mg)}} \times 100 \quad (2)$$

$$LE (\%) = \frac{LC_{\text{actual}}}{LC_{\text{theoretical}}} \times 100 \quad (3)$$

### In vitro silk sericin release test

An *in vitro* silk sericin-release test was performed in a phosphate buffer solution (PBS, 0.1 M, pH 7.4) at 37 °C under shaking. The blend microparticles (~5-10 mg) were suspended in 1.0 mL of buffer. At predetermined time intervals, release medium was collected after centrifugation at 5,000 rpm for 5 min. Then 1.0 mL of fresh buffer was replaced. The silk sericin content in the release medium was monitored the absorption value at 600 nm using the Lowry protein assay. The BSA protein was introduced as the standard, and the calibration curve was as follows: Y (absorbance) = 0.008X (silk sericin concentration) + 0.0120 (r<sup>2</sup> = 0.9961). Cumulative release of silk sericin was calculated in terms of the ratio of the cumulative mass of the released silk sericin at a given time against the initial silk sericin loading in the microparticle sample. *In vitro* silk sericin release tests were performed in triplicate (n = 3).

### Antioxidant activity

The ferric reducing ability of plasma (FRAP) assay was used to assess the antioxidant activity of the blend microparticles. The FRAP assay was performed based on the method described by Feng *et al.*,<sup>16</sup> with minor modifications. The FRAP reagent contained 1.0 mL of 10 mM TPTZ solution (dissolved in 40 mM hydrochloric acid), 1.0 mL of 20 mM ferric chloride solution, and 100 mL of the 300 mM acetate buffer solution (pH 3.6). The 60 µL of sample solution obtained by dissolving the blend microparticles in 1.0 %w/v acetic acid solution was combined with 180 µL of the de-ionized water and 1,800 µL of the FRAP reagent, and redox reaction performed at 37°C for 10 min without light before the absorbance at 593 nm was recorded. Ferrous sulfate (FeSO<sub>4</sub>) was introduced as the standard, and the calibration curve was as follows:

Table 1: Average particle size, drug loading and antioxidant capacity of chitosan/silk sericin blend microparticles.

Chitosan/silk sericin (w/w)	Average particle size <sup>a</sup> (μm)	LC <sub>theoretical</sub> <sup>b</sup> (%)	LC <sub>actual</sub> <sup>c</sup> (%)	LE <sup>d</sup> (%)	FRAP (mg Fe <sup>2+</sup> /g)
100/0	47 ± 7	-	-	-	354 ± 24
99/1	41 ± 7	1.0	0.75 ± 0.09	75.0	1,207 ± 85
95/5	48 ± 9	5.0	3.60 ± 0.13	72.0	2,328 ± 112
90/10	48 ± 8	10.0	6.90 ± 0.52	69.0	3,119 ± 157

<sup>a</sup> Determined from several SEM images.

<sup>b</sup> Calculated from equation (1).

<sup>c</sup> Calculated from equation (2).

<sup>d</sup> Calculated from equation (3).

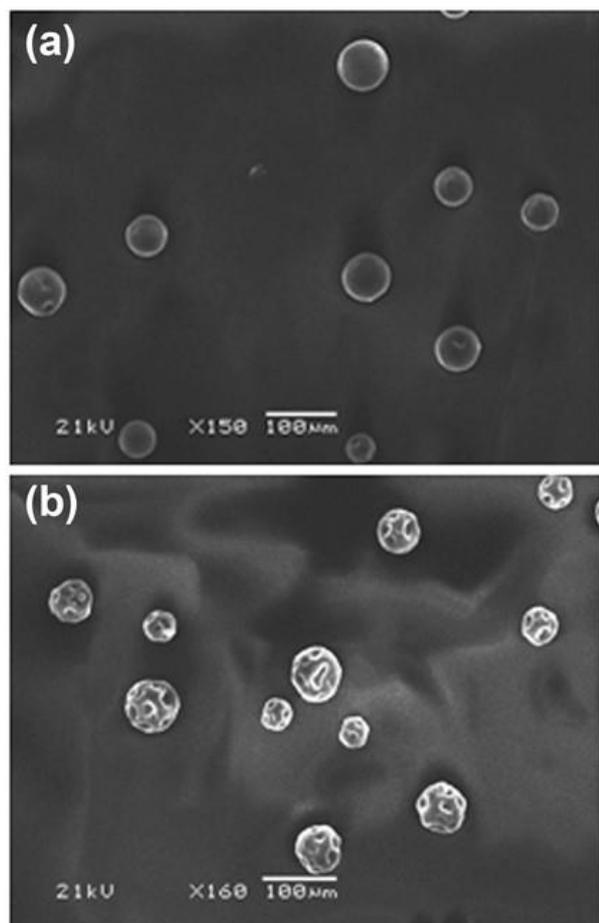


Figure 1: SEM images of (a) neat chitosan and (b) neat silk sericin microparticles (all bar scales = 100 μm).

$Y$  (absorbance) = 0.475 $X$  (FeSO<sub>4</sub> concentration) + 0.002  
 $r^2 = 0.998$ . Results were presented as milligrams of FeSO<sub>4</sub> per gram of microparticle sample.

#### Statistical analysis

The data were expressed as mean ± SD. Statistical analysis was performed using a one-way Analysis of Variance (ANOVA).

## RESULTS AND DISCUSSION

### Morphology and size of blend microparticles

The morphology of the microparticles was determined from SEM images. Figure 1 shows SEM images of the neat chitosan and sericin microparticles. The neat chitosan

microparticles were nearly spherical in shape. Meanwhile the neat silk sericin microparticles were irregular in shape. The deflated surfaces of the silk sericin microparticles may be explained by faster solidification of the silk sericin than the chitosan during the emulsion-diffusion process. Therefore, more shrinkage of the sericin particle surfaces was obtained<sup>9</sup>. The SEM images of the blend microparticles were illustrated in Figure 2. They were nearly spherical in shape and had a smooth surface.

The average particle sizes of the neat chitosan and blend microparticles obtained from the SEM images are summarized in Table 1. It was found that their average sizes were similar in range 41-48 μm. The results indicate that the silk sericin blending did not affect the morphology and average size of the blend microparticles.

### FTIR of blend microparticles

The chemical functional groups of the chitosan and silk sericin of the microparticles were determined from FTIR spectra as shown in Figure 3. The FTIR spectrum of neat chitosan microparticles [Figure 3(a)] shows absorption bands at 1654 cm<sup>-1</sup> (amide groups of residue chitin units) and 1570 cm<sup>-1</sup> (free amino groups of chitosan units). The band at 1103 cm<sup>-1</sup> is attributed to the saccharide structure of chitosan. The amide I (C=O stretching vibration of the amide group), II (N-H bending and C-N stretching vibrations) and III (CN stretching vibration coupled to the N-H in-plane bending vibration) bands in ranges 1710-1590, 1570-1480 and 1270-1200 cm<sup>-1</sup>, respectively, are usually used to indicate the structure of the silk sericin<sup>17</sup>. A broad band around 3350 cm<sup>-1</sup> was assigned to the OH groups on the hydrophilic side chain residues of silk sericin<sup>11</sup>.

As would be expected, band intensities of the silk sericin characteristics including OH and amide I bands of the blend microparticles increased as the silk sericin blend ratio increased as shown in Figures 3(b) – 3(d). The FTIR results supported the fact that blend microparticles with various silk sericin blend ratios can be prepared. In addition, the bands of free amino groups of chitosan and amide III of silk sericin slightly shifted for the blend microparticles suggested molecular interactions between chitosan and silk sericin had occurred.

### Thermal decomposition behaviors of blend microparticles

The thermal decomposition behaviors of the blend microparticles were determined from thermogravimetric (TG) and derivative TG (DTG) thermograms as shown in Figures 4(above) and 4(below), respectively. From the TG

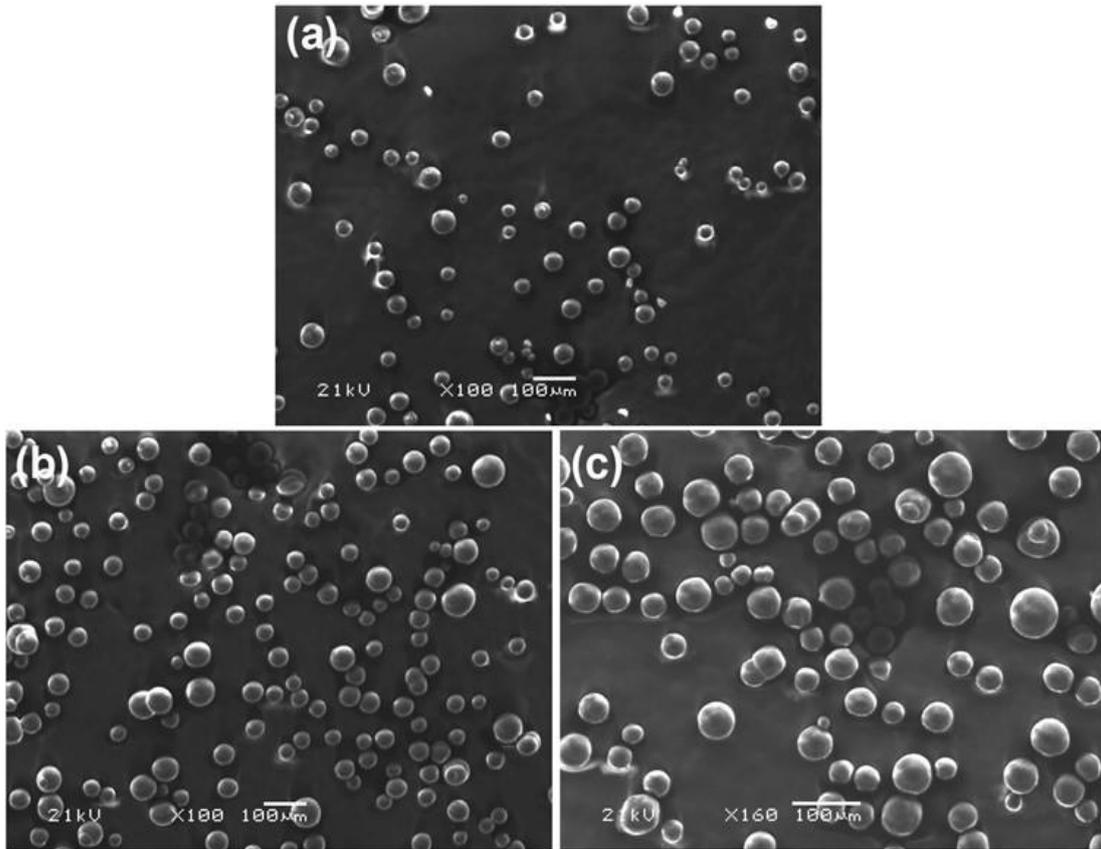


Figure 2: SEM images of blend microparticles prepared with chitosan/silk sericin ratios of (a) 99/1, (b) 95/5 and (c) 90/10 (w/w) (all bar scales = 100  $\mu\text{m}$ ).

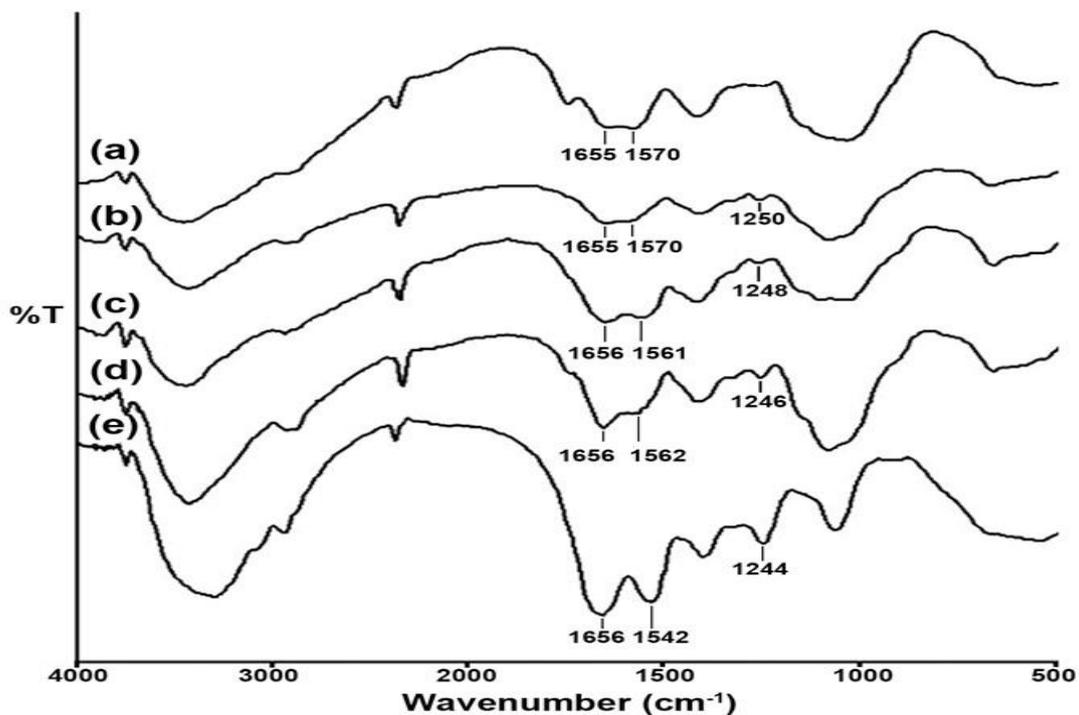


Figure 3: FTIR spectra of (a) neat chitosan microparticles and blend microparticles prepared with chitosan/silk sericin ratios of (b) 99/1, (c) 95/5 and (d) 90/10 (w/w), and as well as (e) neat silk sericin microparticles.

curves, weight losses of the neat and blend microparticles in range 50 – 100  $^{\circ}\text{C}$  were due to residue moisture evaporation. The neat silk sericin microparticles were

faster thermal decomposed in range 200 – 300  $^{\circ}\text{C}$  than the neat chitosan microparticles. The TG curve of the 99/1

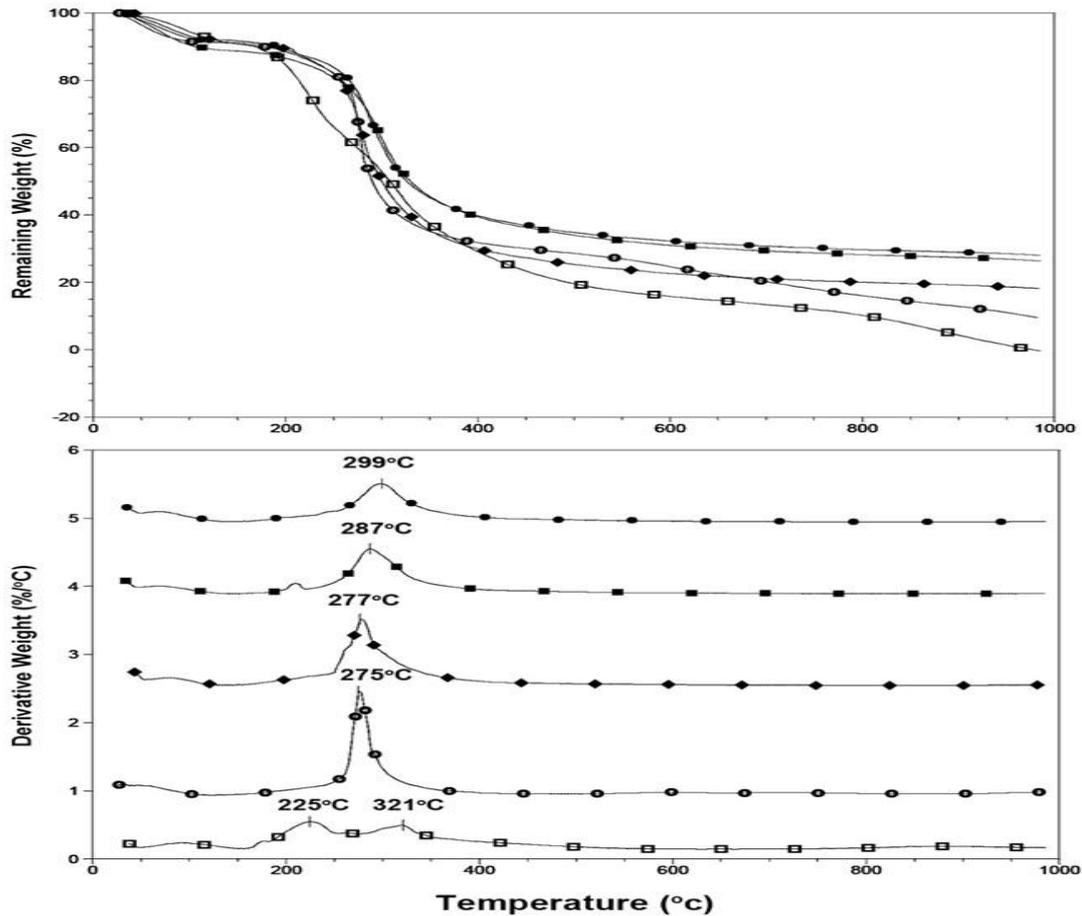


Figure 4: TG (above) and DTG (below) thermograms of (●) neat chitosan microparticles and blend microparticles prepared with chitosan/silk sericin ratios of (■) 99/1, (◆) 95/5 and (O) 90/10 (w/w), and as well as (□) neat silk sericin microparticles.

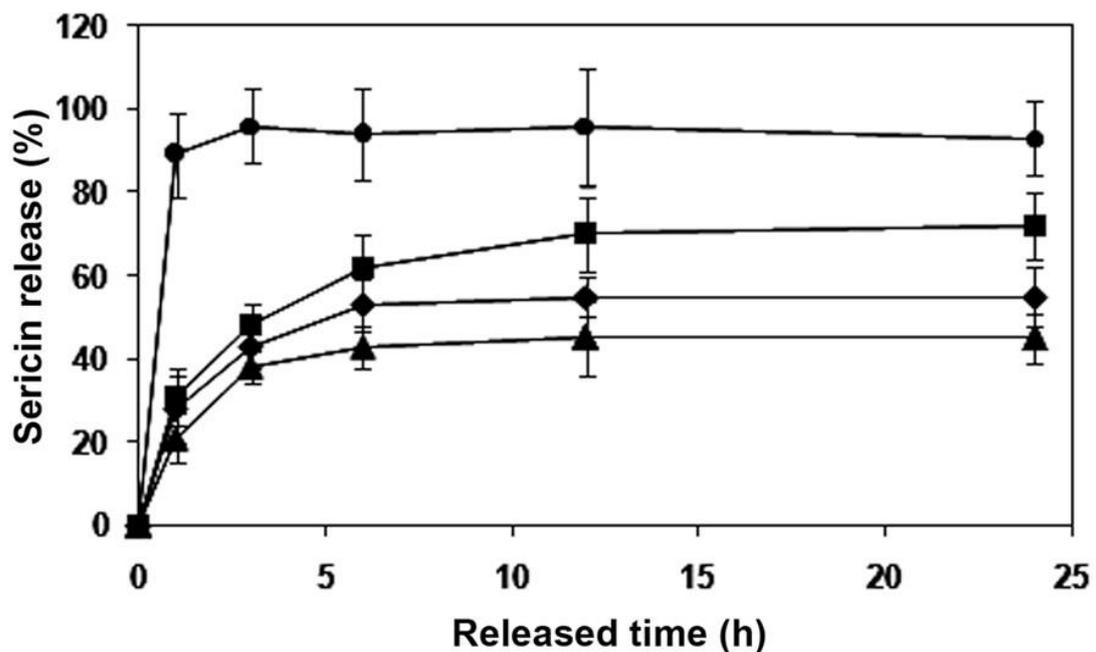


Figure 5: *In vitro* silk sericin release profiles of blend microparticles prepared with chitosan/silk sericin ratios of (▲) 99/1, (◆) 95/5 and (■) 90/10 (w/w) as well as (●) neat silk sericin microparticles.

(w/w) chitosan/silk sericin microparticles was similar to the neat chitosan microparticles. However the 95/5 and 90/10 (w/w) chitosan/silk sericin microparticles showed largely weight losses in the range 300 – 400 °C. The remaining weights at 1,000 °C of the blend microparticles decreased significantly as the silk sericin ratio increased. This confirms that the blend microparticles with different silk sericin ratios can be prepared.

The thermal decomposition behaviors can be clearly investigated from the DTG curves [see Figure 4(below)]. The DTG curves exhibited peaks for the temperature of maximum decomposition rate ( $T_{d, \max}$ ). The neat chitosan microparticles showed only a single  $T_{d, \max}$  at 299 °C. While the two  $T_{d, \max}$  values at 225 °C and 321 °C were detected for the neat silk sericin microparticles. All the blend microparticles exhibited a single  $T_{d, \max}$  similar to the neat chitosan microparticles. This may be due to the chitosan acted as a main component. The  $T_{d, \max}$  values of the blend microparticles shifted to a lower temperature by blending with the silk sericin, which suggested that interactions between chitosan and silk sericin chains had occurred. Hydrogen bonds can be formed between amino groups of both the chitosan and silk sericin molecules.

#### *Silk sericin loading and releasing of blend microparticles*

The theoretical loading content ( $LC_{\text{theoretical}}$ ), actual loading content ( $LC_{\text{actual}}$ ) and loading efficiency (LE) of the silk sericin on the blend microparticles are also reported in Table 1. The  $LC_{\text{actual}}$  values were in the range 0.75 – 6.90% that increased as the feed silk sericin ratio increased. These microparticles with different  $LC_{\text{actual}}$  values were then used to investigate the influence of silk sericin loading content on drug release behaviors. The LE values of the blend microparticles were in the range 69.0 – 75.0% that slightly decreased as the silk sericin ratios increased. This may be due to the higher silk sericin feed ratio induced more diffusion out of silk sericin in the ethyl acetate medium during microparticle solidification.

The *in vitro* silk sericin release from the blend microparticles was investigated in a phosphate buffer pH 7.4 at 37°C for 24 h compared with the neat silk sericin microparticles. Figure 5 shows the silk sericin release profiles from the neat silk sericin and blend microparticles as a function of the silk sericin ratio. The neat silk sericin microparticles showed complete release (~96% sericin release) in the release medium within the first 6 h of release time. This is due to the fast dissolution of the silk sericin microparticle matrix. The initial burst release within the first 6 h of release time was followed by a further sustained release that can be observed for the blend microparticles. The results suggested that the blend microparticles showed potential for use as controlled release devices of silk sericin.

The initial burst release of the drug model from the microparticles is due to the releasing of drug on or near the microparticle surfaces<sup>18</sup>. At the 6 h of release time, the silk sericin release values were 38%, 43% and 48% for the silk sericin ratios of 1, 5 and 10 wt%, respectively. The initial burst release effects increased as the silk sericin ratio increased. For silk sericin ratios of 1, 5 and 10 wt%, the silk sericin release levels at 24 h were 45%, 55%, and 72%,

respectively. The level of silk sericin release also steadily increased as the silk sericin ratio increased. This may be explained by the higher silk sericin loading content that could affect the properties of the polymeric network in the microparticle matrix, thus affecting the diffusion barrier. The results of the silk sericin release demonstrated that the silk sericin release from the blend microparticles was controlled by the silk sericin loading content.

#### *Antioxidant activity*

The FRAP results were expressed as mg  $Fe^{2+}$ /g of microparticle sample as also reported in Table 1. Both the chitosan and silk sericin have given antioxidant activities<sup>19,20</sup>. The FRAP values of the neat chitosan microparticles was 354 mg  $Fe^{2+}$ /g. The FRAP values of the blend microparticles were more than the neat chitosan microparticles that increased as the silk sericin ratios increased. This can explain by the FRAP value of the neat silk sericin microparticles (4,929 mg  $Fe^{2+}$ /g) was more than the neat chitosan microparticles. The FRAP results also confirm that the blend microparticles with different silk sericin contents can be prepared by the water-in-oil emulsification method. The FRAP values strongly depended upon the loaded silk sericin of the blend microparticles.

## CONCLUSIONS

This study is the first report on the preparation of chitosan/silk sericin blend microparticles by the water-in-oil emulsification method for use as controlled release devices of silk sericin antioxidant. The SEM images indicate that the blend microparticles were spherical in shape. The FTIR and TGA analyses as well as FRAP measurement of the blend microparticles confirm the different chitosan/silk sericin ratios in the blend microparticles. The silk sericin loading efficiencies of blend microparticles were in range 69 – 75% that depended on the silk sericin feed ratio. The blend microparticles prepared in this work show sustained release of the silk sericin. The blend microparticles containing higher silk sericin ratio exhibited more silk sericin release content. In conclusion, the water-in-oil emulsification-diffusion method was an effective method for production the chitosan/silk sericin blend microparticles. The silk sericin release contents from the blend microparticles depended on the silk sericin ratio. These chitosan/silk sericin blend microparticles have potential for use as controlled-release delivery carriers for silk sericin antioxidant.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Mahasarakham University (fiscal year 2016) for financial support. The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education, Thailand is also acknowledged.

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