Morphology and thermal stability of silk fibroin/starch blended microparticles

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Abstract. In the present study biodegradable microparticles of silk fibroin (SF)/starch blends were prepared by a simple water-in-oil emulsion solvent diffusion technique. SF/starch blended solution and ethyl acetate were used as water and oil phases, respectively. The influence of SF/starch ratios on characteristics of the blended microparticles was investigated. The SF conformation of microparticle matrices from FTIR analysis was changed from random coil to β -sheet form by blending with starch. The blended microparticles had lower dissolution in water than those of SF and starch microparticles. The 1/3 (w/w) SF/starch blended microparticles exhibited the lowest dissolution. The SF and starch microparticles showed irregular and deflated shapes, respectively. The blended microparticles were nearly spherical in shapes and smaller sizes. Thermal stability of the blended microparticles slightly increased with the starch blended ratio. The results suggested that SF conformational transition, thermal stability, morphology and dissolution of the blended microparticles can be adjusted by varying the blended ratio.

Keywords: biopolymers, silk fibroin, starch, conformational transition, morphology

1. Introduction

Silk fibroin (SF) is a biodegradable and biocompatible natural protein polymer created by the *Bombyx mori* silkworm [1, 2] and has recently been extensively investigated as a biomaterial suitable for uses such as a matrix for cell culture substrates [3] and drug delivery systems [4, 5]. The minimal inflammatory reactions *in vitro* and *in vivo* of SF have been reported [6]. The SF has been processed into fibers, films and particles. Most research work has been focused on the widely studied SF films. However, the applications of SF films are limited due to their being very brittle in the dry state. The poor mechanical properties of the SF film could be improved by blending it with other polymers [7, 8, 9]. Other properties, such as conformational transition and thermal properties are dependant on the

blended ratios. Preparation of SF microparticles by various techniques has been reported for drug delivery applications [4, 5, 10–13]. The SF/chitosan blended microparticles have been prepared by the coacervation/cross-linking method [14]. Starch is a non-toxic, biodegradable, biocompatible, edible and relative inexpensive material that has been widely studied in the entrapment of food ingredients [15, 16] and drugs [17–19]. Almost all starch microparticles have been prepared by an emulsification-cross-linking reaction [17–20]. The preparation of alginate/starch blended microparticles has been reported by Roy *et al.* [21]. However, no information on SF/starch blended microparticles has been published.

In the present work, we report the conformational transition, morphology and thermal stability of

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SF/starch blended microparticles as a function of blended ratios. The dissolution in water of SF/starch blended microparticles was also determined.

2. Materials and methods

2.1. Materials

Silk cocoons from *B. mori* were kindly supplied by the Silk Innovation Center, Mahasarakham University, Mahasarakham, Thailand. Na_2CO_3 (99.5%, Carlo Erba, code no. 479306, MI, USA), CaCl_2 (94%, Unilab, code no. 0701013, Auckland, New Zealand), ethanol (99.8%, Carlo Erba, code no. 414608, France) and ethyl acetate (99.8%, Lab Scan, code no. A3511, Ireland) were used without further purification. Cassava starch (food grade, 96% starch, amylase content 16.8%) was purchased from Bangkok Inter Food Co., Ltd. (Bangkok, Thailand) and used without pretreatment.

Aqueous silk fibroin (SF) solution was prepared as follows. Silk cocoons were de-gummed by boiling in 0.5% Na_2CO_3 solution at 95°C for 30 min to remove sericin and then rinsed with distilled water before drying at room temperature. De-gummed SF fibers were dissolved in the ternary solvent system, CaCl_2 -ethanol-water (mole ratio = 1:2:8), by stirring at 90°C. The SF solution was then dialyzed in cellulose tubular membranes (molecular weight cut-off 7000 Da, Lot# KC132124, Thermo Scientific, IL, USA) against distilled water for 3 days. After filtration, the SF concentration was adjusted to 1% w/v with distilled water. The 1% w/v starch solution was prepared by dispersing starch in distilled water at 80°C and stirring until gelatinization.

2.2. Preparation of blended microparticles

SF/starch blended solutions were firstly prepared by mixing SF and starch solutions with a magnetic stirrer for 1 hr before microparticle formation. The blended microparticles were fabricated in a one-step process by the water-in-oil (W/O) emulsion solvent diffusion method. Briefly, about 1.0 ml of blended solution was slowly added drop-wise into 200 ml of ethyl acetate with magnetic stirring at 900 rpm for 1 hr. The beaker was closed with aluminum foil to prevent organic solvent evaporation during the emulsification-diffusion process. The

blended microparticles were recovered by centrifugation before being dried in a vacuum oven at room temperature overnight to remove residue organic solvent. Microparticles with SF/starch blended ratios of 4/0, 3/1, 2/2, 1/3 and 0/4 (w/w) were prepared and investigated.

2.3. Characterization of blended microparticles

The chemical structures and conformational transition of the microparticles were measured with Fourier transform infrared (FTIR) spectroscopy using a Spectrum GX FTIR spectrophotometer (Perkin-Elmer Co. Ltd., Massachusetts, USA) with air as the reference. The resolution of 4 cm^{-1} and 32 scans were used. FTIR spectra were obtained using a KBr disk method. Thermal stability of the microparticles was analyzed by thermogravimetry (TG) using a SDT Q600 thermogravimetric analyzer (TA-Instrument Co. Ltd., New Castle, DE, USA). For TG analysis, 5–10 mg sample was heated from 50 to 1000°C at a heating rate of 20°C/min under a nitrogen atmosphere. Morphologies of the microparticles were determined by scanning electron microscopy (SEM) using a JSM-6460LV SEM (JEOL, Tokyo, Japan). The microparticles were coated with gold to enhance the surface conductivity before scanning. Particle size distribution of the microparticles was measured by the sieving method. For this purpose, the microparticles were sieved into three particle size ranges of <80, 80–150 and >150 μm before weighing. A dissolution test of microparticles in distilled water was performed. For this test, the microparticles were incubated in 20 ml of distilled water at 30°C and shaken at 100 rpm. The incubation times of 3 and 6 hrs were used. At the appropriate time, residual microparticles were separated by centrifugation at 10 000 rpm for 20 min before drying at 50°C in a vacuum oven for 24 hrs. The remaining SF microspheres were weighted after being stored in a desiccator for 6 hrs. Percentage of dissolution was calculated from Equation (1). The mean % dissolution values are the average of three parallel measurements. The differences between data sets are expressed by analysis of variance (ANOVA) and multiple *t* tests. ≤ 0.05 was considered the statistically significant.

$$\% \text{ dissolution} = \frac{\text{initial microparticles [mg]} - \text{remaining microparticles [mg]}}{\text{initial microparticles [mg]}} \cdot 100 \quad (1)$$

where initial and remaining microparticles are the weights of microparticles before and after dissolution tests, respectively.

3. Results and discussion

3.1. FT-IR analysis

The functional groups of SF and starch components, and SF conformational transition were determined from the FTIR spectra of pure and blended microparticles as shown in Figure 1. The positions of absorption bands, especially amide I, II and III bands indicate the conformational transition of SF. The FTIR spectrum of SF microparticles (Figure 1a) showed absorption bands at 1654 cm⁻¹ (amide I), 1545 cm⁻¹ (amide II) and 1240 cm⁻¹ (amide III), assigned to the random coil SF conformation. The FTIR results indicated that the SF microparticle matrices were predominantly in random coil form. Whereas FTIR spectrum of starch microparticles in Figure 1e showed broad absorption bands at 3385–3550 and 2929 cm⁻¹ due to O–H and C–H stretching. The band at 1415 cm⁻¹ is assigned to C–H bending vibration. While the absorption band at 1000–1155 cm⁻¹ can be attributed to the saccharide structure of starch [20, 21]. As would be expected, band characteristics of both SF and starch were detected in the FTIR spectra of the blended microparticles. Intensities of starch characteristic bands at 2929, 1415 and 1000–1155 cm⁻¹ increased with the starch blended ratio (Figures 1b–1d). It should be noted that the FTIR spectra of 2/2 and 1/3 SF/starch blended micropar-

ticles showed O–H stretching bands of starch in the range of 3385–3550 cm⁻¹. While the FTIR spectrum of 3/1 SF/starch blended microparticles exhibited N–H stretching bands of SF at 3400 cm⁻¹.

It can be seen that the amide I and II bands of SF shifted slightly to a lower wave number after blending with starch. This shifting increased as the starch in the blended ratio increased. Whereas the amide III bands of SF shifted to a higher wave number after blending with starch. The results indicated that the SF microsphere matrices changed from random coil to β -sheet form. The fraction of β -sheet SF form of the blended microparticles increased as the starch blended ratio increased, suggesting interaction and miscibility between SF and starch. The shifting of SF amide bands in the FTIR spectra of its blends has been used to detect interactions and miscibility between SF and blended molecules [8, 22, 23]. For SF/hydroxyl propyl methyl cellulose (HPMC) blends, it has been proposed that the hydrogen bonding between NH in the amide groups of SF and OH of HPMC had occurred [22]. Therefore, the intermolecular bonds in the SF/starch blends in this work may be expected to be hydrogen bonding between NH in the amide groups of SF and OH of starch.

3.2. Morphology

The microparticles were formed and solidified after diffusion out of water from dispersed emulsion droplets to the continuous ethyl acetate phase. The microparticles suspended in ethyl acetate were obtained before being collecting by centrifugation. Morphology of the microparticles was characterized from the SEM images, as shown in Figure 2. It was found that the SF microparticles had an irregular-shape (Figure 2a). The microparticles of other proteins such as insulin have been prepared by the spray-drying method [24]. They were erythrocyte-like in shape. Solid shells formed because the drying started at the outside of the droplets. Almost all starch microparticles showed deflated-like shapes (Figure 2e). So, it was surprising that the blended microparticles were nearly spherical in shape and had a smooth surface (Figures 2b–2d). It should be

Figure 1. FTIR spectra of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles

Figure 2. SEM micrographs of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles. All bars = 100 μm .

noted that the blended microparticles with SF/starch blended ratio of 3/1 (w/w) exhibited a uniform spherical shape compared to the other blended ratios. Some deflated microparticles can be found when the starch blended ratio was increased. This suggests that in the blending of SF with starch at appropriate ratios, uniformly spherical-shaped microparticles with smooth surfaces could be fabricated by the W/O emulsion solvent diffusion method.

The internal morphology of microparticles was determined from the SEM images of broken surfaces, as illustrated in Figure 3. This indicates that the microparticles contained voids or porous structures. The starch microparticle matrix was denser

than that of the SF microparticle matrix. The numbers of voids decreased as the starch in the blended ratio increased. However, these porous forms were completely covered with a smooth surface for all blended ratios. Porous microparticles of a water-insoluble polymer have been prepared by the water₁-in-oil-in-water₂ (W₁/O/W₂) double emulsion method [25, 26]. The void structures were formed and solidified because of the diffusion out of W₁ to W₂ phase. It would be expected that the porous structures of SF, starch and their blended microparticles occurred due to diffusion out of water from emulsion droplets to the external ethyl acetate phase.

Figure 3. SEM micrographs of broken surfaces of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles. All bars = 5 μm .

3.3. Thermal stability

Decomposition and thermal stability of SF, starch and blended microparticles was determined from thermogravimetric (TG) thermograms as shown in Figure 4. Both the SF and starch microparticles did not completely decompose at a temperature of 1000°C. The TG thermograms of SF, starch and blended microparticles showed a single decomposition stage. The remaining weights at 1000°C were approximate 30 and 8% for SF and starch microparticles, respectively. The remaining weights at 1000°C of all blended microparticles were similar to the SF microparticles. The remaining weights at 1000°C of blended microparticles slightly decreased as the SF in the blended ratio decreased (Figures 4b–4d).

Figure 4. TG thermograms of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles

For SF microparticles (Figure 4a), it can be seen that they exhibited two major weight loss phases.

The initial weight loss at around 100°C is due to loss of moisture. The weight loss in this phase depends on the moisture content of the SF microparticles. The sharp weight loss in the second phase took place in the temperature range of 200–400°C associated with the breakdown of side chain groups of amino acid residues as well as the cleavage of peptide bonds. For starch microparticles (Figure 4e), the first phase shows the evaporation/dehydration that begins immediately after the temperature is increased and finishes at around 100°C. The moisture content of starch microparticles was higher than that of the SF microparticles. The thermal decomposition in the second phase of starch microparticles started at approximately 300°C. The TG thermograms of blended microparticles also present two phases of weight losses. The initial weight losses occurred due to moisture content exhibit between SF and starch microparticles. However, the second weight loss phase of blended microparticles did not separate.

The thermal stability of the samples can be clearly observed from differential TG (DTG) thermograms as shown in Figure 5. The small peaks in the range of 50–150°C occurred from moisture evaporation. The large peaks in the range of 150–500°C were due to decomposition of SF and starch microparticle matrices. From DTG thermograms, the temperature of maximum decomposition rate ($T_{d,max}$) can be determined and has been summarized in Table 1. The blended microparticles had a single $T_{d,max}$ between the SF and starch components. The $T_{d,max}$ values of the blended microparticles slightly increased as the starch in the blended ratio increased. This supports the intermolecular interac-

Table 1. $T_{d,max}$ of SF/starch blended microparticles

SF/starch ratio (w/w)	$T_{d,max}$ [°C]
4/0	306
3/1	319
2/2	321
1/3	325
0/4	326

tions between SF and starch as according to the FTIR analysis.

3.4. Particle sizes

Particle size distributions of blended microparticles were measured by the sieving method instead of the scattering method (suspension in water) because of the partial swelling and dissolution of microparticles. The particle sizes are presented in Figure 6. It can be seen that both SF and starch microparticles showed the largest weight fraction in the range of >150 μm in size. This may be due to the rapid solidification of microparticle matrices during the emulsification-diffusion process which resulted in a large particle size. For the blended microparticles, the particle sizes slightly decreased as the SF blended ratio increased. Both the 3/1 and 2/2 blended microparticles showed the largest weight fraction in the range of <80 μm in size. These results corresponded to their SEM images in Figure 2.

3.5. Dissolution test

Figure 7 shows percentage of dissolution in water of the microparticles with different incubation times. The dissolutions of all the microparticles after 3 hrs incubation showed no differences. The % dissolution values were in range of 44–64%. While for the incubation time of 6 hrs, the dissolutions of both SF and starch microparticles were nearly complete (91 and 95%, respectively). All blended microparticles had lower % dissolution values than those of SF and starch microparticles. The interactions between SF and starch in the blended microparticles may decrease microparticle dissolution. The 1/3 (w/w) SF/starch blended microparticles show the lowest % dissolution. This can be explained from the FTIR analysis, the 1/3 (w/w) SF/starch blended microparticles contained the largest water-insoluble or β -sheet form of SF

Figure 5. DTG thermograms of SF, starch and blended microparticles

Figure 6. Particle size distributions of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles. Error bars are standard deviations ($n = 3$).

Figure 7. Dissolutions in distilled water of (a) SF, (b) 3/1 blended, (c) 2/2 blended, (d) 1/3 blended and (e) starch microparticles for incubation times of 3 hrs (top) and 6 hrs (bottom). Error bars are standard deviations ($n = 3$).

microparticle matrices. The results suggest that the % dissolution can be controlled by adjusting the

blended ratio. This is an important advantage for drug delivery application with controllable drug release rate.

4. Conclusions

The SF/starch blended microparticles with different blended ratios were successfully prepared by the W/O emulsion solvent diffusion method. The SF and starch solutions were firstly blended before preparing the blended microparticles. Using FTIR and TG analyses, the intermolecular interactions which exist between SF and starch molecules were determined. The SF conformation changes from random coil to β -sheet form and increasing SF thermal stability can be induced by blending with starch. The increase of starch ratios in the blended microparticles can increase interactions between SF and starch. Blended microparticles with a spherical shape can produce with appropriate blended ratio. The % dissolution in water of

blended microparticles strongly depended upon the blended ratio.

These SF/starch blended microparticles with different random coil/ β -sheet form contents, thermal stabilities and % dissolutions might be of interested for use as controlled-release drug delivery systems.

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