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Cellulose-Silk Fibroin Composite Particles for Hydrophilic Drug-Controlled Release System: Preparation and Characterization

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The objective of this study was to investigate the dissolution of cellulose (C) powder as a potential material for particle formation when combined with a silk fibroin (SF) solution. Each polymer solution as well as a mixture of cellulose and silk fibroin in different ratios were prepared into particles using the solvent emulsion-diffusion method. The particle morphology was characterized by scanning electron microscopy (SEM) and by attenuated reflection Fourier-transformed infrared spectroscopy (ATR-FTIR) and thermogravimetric analyzer (TGA) for the C-SF molecular interaction and thermal properties, respectively. The results showed that the cellulose particles appeared as separate fibers and loosely bound together, while silk fibroin particles were spherical in shape with dense texture and smooth surface. The C/SF mixed particles with different ratios could be formed into spherical particles and a ratio of 1:3 has the most spherical and solid. The ATR-FTIR results indicated the absorption spectra of the main functional groups both cellulose and silk fibroin. Blue dextran helped to enhance the temperature of the maximum decomposition rate ($T_{d,max}$) of the particles *via* hydrogen bond interaction between the blue dextran and polymers. A burst release of blue dextran was observed in the first 12 h due to the swelling of particle surfaces and the slowest release found in the silk fibroin particles. The C/SF composite showed different blue dextran-released profiles and the particle with a high content of silk fibroin-released blue dextran in slowly rate. The results of this work can be used as a basis for applying C/SF composite particles in a hydrophilic drug release-controlled system.

Keywords: Cellulose, Silk fibroin, Property, Blue dextran, Drug-controlled release system.

INTRODUCTION

Solid particles have widespread interest in delivery systems to achieve controlled release [1,2] and applied both in academia and industry including food, pharmaceutical and cosmetic applications [3-5]. In recent, organic materials from biological origins have been increasingly focused, due to the sustainable materials and clean-label products demand as well as high biocompatibility and low toxicity [6]. Natural biopolymers such as proteins, polysaccharides and fat crystals have been explored and produced in particles [5,7-9]. The particles with nano- or micro-sized have been extremely explored. They can be received either from sole material sources, or by combining multiple biopolymers through physical [8], chemical [10] and/or enzyme-catalyzed [11]. Therefore, choosing suitable building blocks is a crucial step to construct particles with desirable properties.

Cellulose (C) is the most abundant natural polymer found in approximately 45% of plants [12]. It shows unique structures by inter- and intra-hydrogen bonds from hydroxyl groups [13], in glucose units linked together *via* β-1,4-glycosidic bonds [14]. Previous reports indicated that cellulose has desirable properties [15,16]. Nowadays, it has been widely exploited in traditional fields and advanced applications [17-21]. Different forms derived from cellulose such as film [22,23], particle [6, 24,25], microfibrillated particle [26], sheet [27], modified fiber [28] have been performed. Nevertheless, the hydrophilic part of cellulose greatly limits its use in stabilizing texture which requires other hydrophobic molecules for balancing structure.

Silk is a natural protein fiber produced by silkworms, composed of two components *viz*. silk sericin (SS) and silk fibroin (SF) [29-31]. The two silk fibroin strands were coated with the silk sericin glue-like protein [32]. In the material field, silk fibroin has been extremely explored and applied in various

applications according to its excellent properties [33-35]. In addition, silk fibroin has been performed in different forms including particles [36,37]. However, cellulose-silk fibroin composite particles found rarely been reported until now.

Therefore, this work aimed to prepare cellulose-silk fibroin (C/SF) composite particles for containing hydrophilic drugs. Each sole polymer was also prepared as a comparison. The morphological shape, chemical interaction and thermal properties of the particles were characterized and discussed. Finally, releasing the blue dextran profile was also investigated.

EXPERIMENTAL

Preparation of cellulose solution: The cellulose solution was prepared from cellulose powder. The cellulose powder (10 g) was weighed and then boiled in 100 mL of 10% (w/v) NaOH at 80 ºC with stirring for 3 h. The slurry was then washed with distilled water until the pH was neutral. The obtained product was then immersed in 5% (w/v) $KMnO_4$ for 1 h at room temperature. Finally, the oxidized sample was hydrolyzed by 5% H_2SO_4 at 50 ºC for 1 h. The cellulose solution was then washed with distilled water until it reached neutral pH, filtered and kept in the refrigerator at 4 ºC for further use.

Preparation of silk fibroin (SF) solution: The silk fibroin (SF) solution was derived from a Thai silk variety known as Nang Lai cocoons. The cocoons were firstly degummed twice in 0.5% (w/v) Na_2CO_3 solution each for 30 min. After washing with distilled water, SF was then dissolved into a solution by boiling in a tertiary mixture of $CaCl_2$:ethanol:H_2O (1:2:8 by mol) at 80 ºC for 1 h. The SF hydrolyzate was then dialysis against distilled water using a dialysis bag with microwave having frequency of 3.5 kDa for 36 h. Finally, the SF solution was adjusted to 2% for use.

Preparation of native cellulose (C) and silk fibroin (SF) particles: The preparation of cellulose and silk fibroin particles was carried out in accordance with a previously documented procedure [37]. This method is called water-in-oil emulsion solvent diffusion. In brief, 0.5 mL of 1% cellulose and 2% SF solution were used as water (W) phase were slowly drop-wised into a beaker containing 2% Span80 (w/v) of 100 mL ethyl acetate (an oil (O) phase) under a magnetic stirrer at 700 rpm. Alumina foil was covered beaker to avoid the evaporation of ethyl acetate. The particle preparation process was performed for 30 min. Finally the obtained particles were gathered and rinsed with new ethyl acetate before drying in a vacuum oven at room temperature for 24 h.

Preparation of C-SF composite loaded drug particles: The drug loaded in the composite particles of different C/SF ratios of 3/1, 1/1 and 1/3 (v/v) were also prepared by the same method. Firstly, the appropriate amount of 1% cellulose and 2% SF were mixed under a magnetic stirrer for 10 min. After that, 1.0 g of blue dextran (BD), a model hydrophilic drug, was directly added to the composite solution. The further step to prepare C/SF composite particles was followed by the process as described for the native particle preparation.

Morphological analysis: All the prepared particles were observed in their morphology under a scanning electron microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). Before observing, the particles were fixed on the stub and then sputtered coated with gold to enhance conductivity before scanning.

Chemical structure analysis: All the particle types were analyzed for their chemical structure by attenuated reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Perkin Elmer-Spectrum Gx, USA) in the spectral region of 4000-400 cm^{-1}.

Thermal analysis: The thermal stability of the all-prepared particles was analyzed by a thermogravimetric analyzer (TGA) (SDTQ600, TA-Instrument Co. Ltd., USA). The suitable weight of particles was loaded on the aluminum pan and then heated from 50-800 ºC having 20 ºC/min of heating rate under a nitrogen atmosphere.

Releasing profile of blue dextran (BD): Blue dextran (BD) loaded particles were immersed in 3 mL distilled water with constant shaking at room temperature. After the interval times of 1, 2, 3, 4, 8, 16, 24, 48 and 72 h, 2.5 mL of water was collected. The same new volume of distilled water was added instead of the collected volume. The blue dextran mixed water's absorbance was measured by UV-Vis-spectrophotometer at 620 nm. By comparing the measured concentration to a reference curve, the amount of blue dextran released by the particles into the water was determined.

RESULTS AND DISCUSSION

Fig. 1 shows the morphology of the native cellulose (Fig. 1a) and silk fibroin (SF) (Fig. 1b). Both cellulose and SF could be performed in the particles even if some differences have appeared. The native cellulose particle is formed from short fibers which are coiled by the stirring force and stable texture by hydrogen bonds *via* hydroxyl groups. At high magnification (Fig. 1aII), the texture of the cellulose particle was non-woven with random orientation, resulting to loosen spaces in its texture. On the other hand, the native SF particle (Fig. 1b) has a more spherical shape and smoother surface and is smaller in size than the native cellulose particle. Moreover, the SF particle has homogeneous in texture without a gap at high magnification (Fig. 1bII). This suggested that the SF particle formed from the SF solution while the cellulose particle was prepared from short fiber (oligosaccharide). This means that the preparation process of the cellulose solution must be improved. The cellulose chain was packed through various hydrogen bonds which makes it very stiff. Therefore, cellulose dissolution is a difficult process [38]. The different solvents used for breaking the intermolecular hydrogen bonds and hydrophobic chain interactions of cellulose have been reported such as LiOH-urea-water [39], cold NaOH-urea [38], thiourea [40]. In addition, strong acidic media including phosphoric acid-water or sulfuric acid glycerol mixtures [41] as well as the ionic liquids and deep eutectic solvents [24,42].

Morphological studies: Fig. 2 shows the SEM images of C/SF composite particles loading blue dextran (BD), a model hydrophilic drug. The results indicated that all the C/SF ratios could be prepared particles in a spherical shape. At higher cellulose content (Fig. 2a), the composite particle has the biggest in size and rougher surface than other ratios. Increasing SF

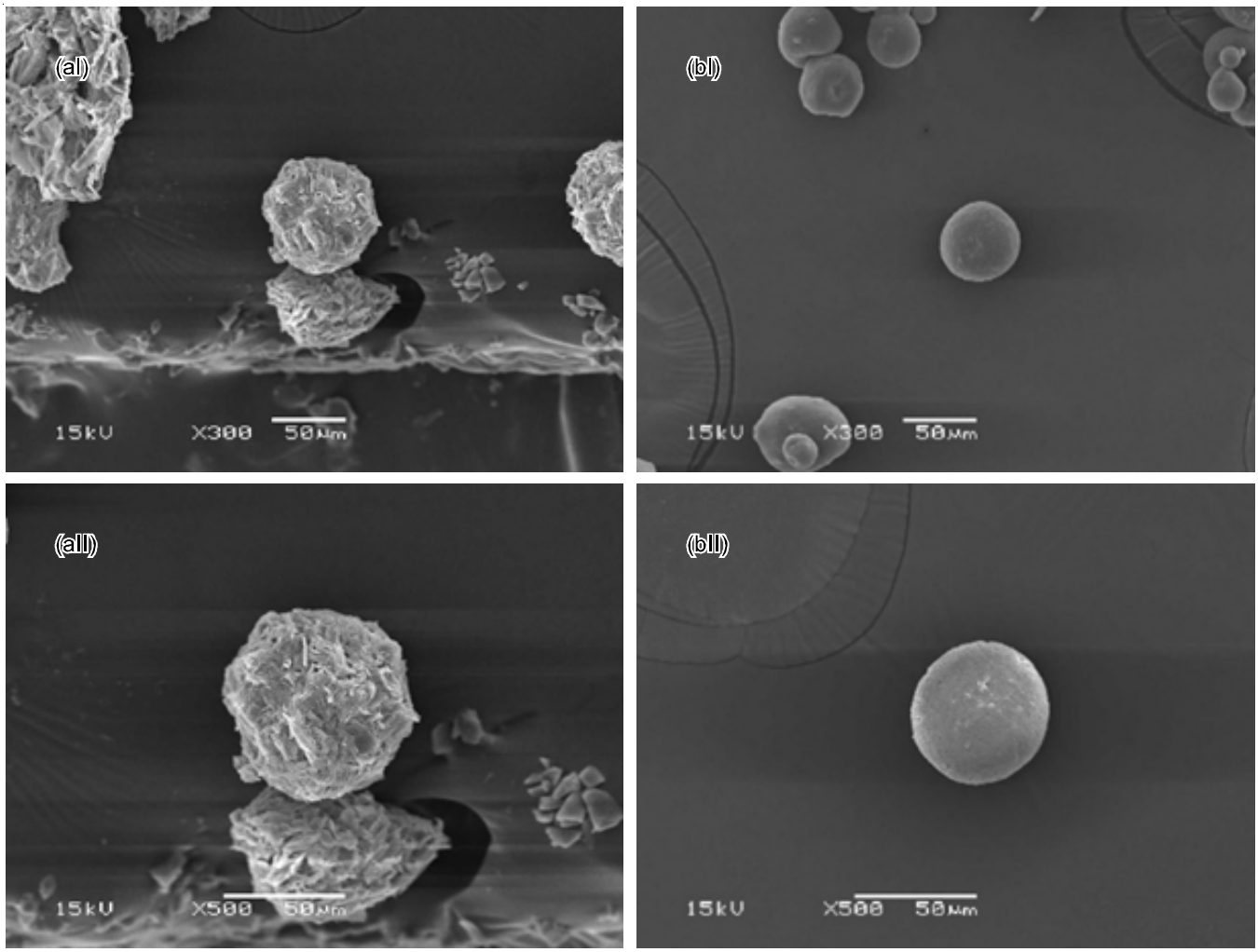

Fig. 1. SEM images of native cellulose (a) and silk fibroin (SF) (b) particles at 300X (I) and 500 (II) magnifications

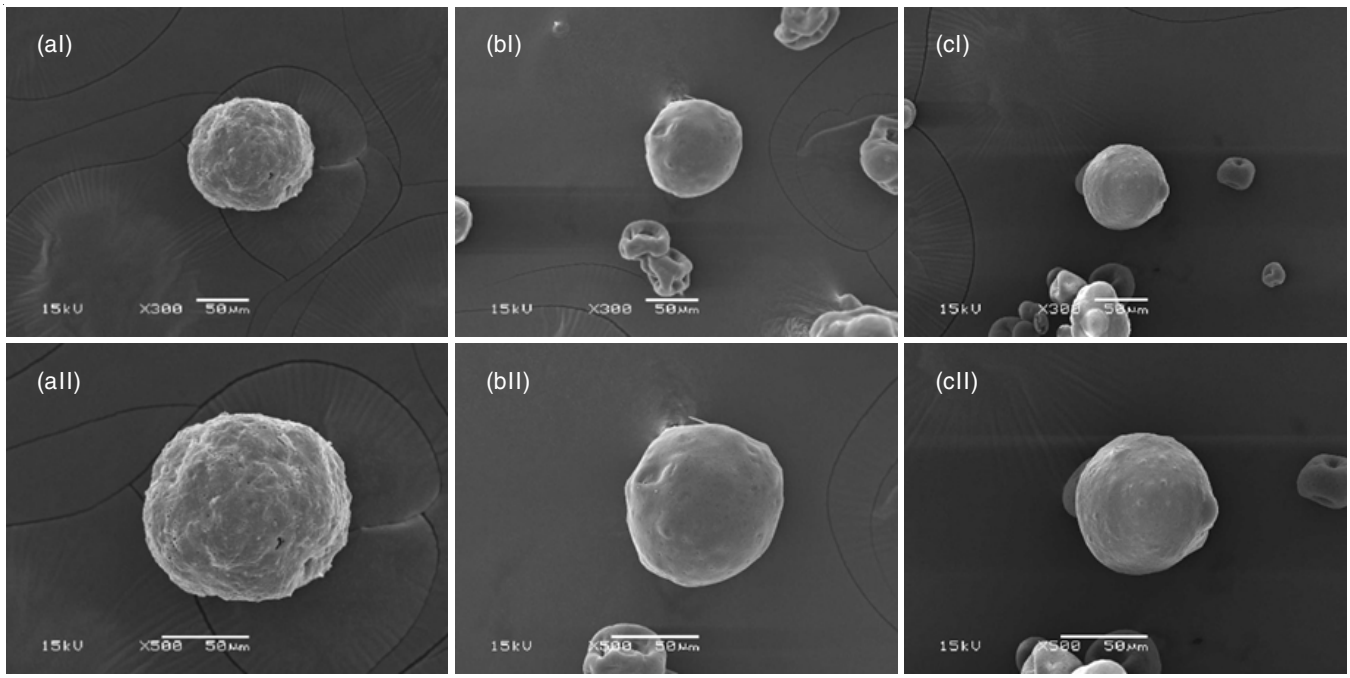

Fig. 2. SEM images of composite particles prepared from different C/SF ratios of 3:1 (a), 1:1 (b) and 1:3 (c) loaded blue dextran at 300X (I) and 500 (II) magnifications

content, the composite particles gradually decreased in size (Fig. 2b,c) as well as smoother surfaces. In comparison to the native SF particle (Fig. 1b), the C/SF composite particles have rough surfaces in all ratios. The rough surface should be caused by the cellulose part. However, the results suggested that both cellulose and SF could be interacted and does not hinder the particle formation. The results exhibited that the particle size of the C/SF composite was arranged from the biggest to smallest as follows; at 3:1 (≈ 100 µm) > 1:1 (≈ 70 µm) > 1:3 (≈ 60 µm), respectively. Silk fibroin (SF) represents a hydrophobic polymer from the main non-polar amino acid components; glycine and alanine which helped to stabilize the hydrophilic cellulose to form solid particles and high structural complexity [6,43].

Infrared studies: Fig. 3 shows ATR-FTIR spectra of all prepared particles loaded blue dextran (BD). The typical bands at 3340-3350 (O-H *str.*), 2890-2900 (C-H *str.*), 1425-1435 (C-H *vib.*), 1020-1015 (C-O-C pyranose ring skeleton) [27] and 870-860 cm^{-1} (β-(1-4)-glycosidic bond of cellulose) were detected [44-47] for the native cellulose particle (Fig. 3a). The ATR-FTIR spectrum of the native SF particle (Fig. 2e) showed absorption peaks of amide I (1629 cm^{-1}) accounted for the carbonyl group (-CO-), amide II (1529 cm^{-1}) accounted for amine group (-NH-) and methyl group (-CH-) and amide III (1230 cm^{-1}) accounted for the -CN- *str.*, plane -NH-, -C-C- and -CO- *str.*, respectively [45,48]. In addition, a small peak at 3348 cm^{-1} is assigned to the CH_2 vibration and H-C=O amide A. This peak was gradually increased in the C/SF composite particles and shifted to a higher wavenumber in the native cellulose. The ATR-FTIR spectra of the C/SF composite particles (Fig. 3b-d) showed that of mixture absorption bands between cellulose and SF depending on the ratio used. This suggests that cellulose and SF could interact together *via* hydroxyl groups in the cellulose structure and polar amino acids in the SF. Moreover, blue dextran (BD) did not affect the chemical structure of both cellulose and SF.

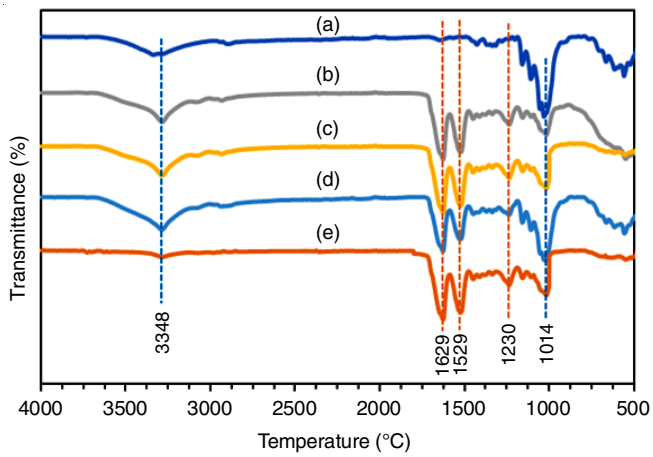

Fig. 3. ATR-FTIR spectra of different particles loaded blue dextran (BD); native cellulose (a), C/SF composite with ratio of 3:1 (b), 1:1 (c), 1:3 (d) and native SF (e)

Thermal studies: The native cellulose (Fig. 4a) and C/SF composite (Fig. 4b-d) particles show decomposition at least 3 points. The first is the temperature less than 100 ºC responds to

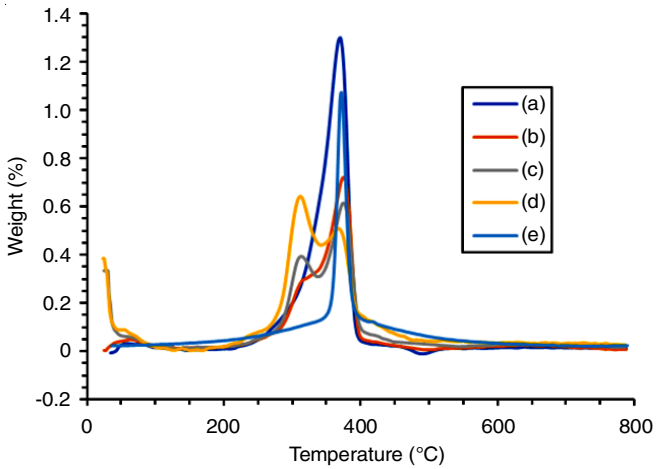

Fig. 4. DTG thermograms of the different particles mixed blue dextran (BD); native silk fibroin (SF) (a), C/SF composite with ratio of 3:1 (b), 1:1 (c), 1:3 (d) and native cellulose (e)

water evaporation [49]. The second point was in the range of 310-325 ºC, which was the decomposition peak of cellulose [50]. The third point was in the range of 365-375 ºC (decomposition of H-bonds between polymer interacted BD). The $T_{d,max}$ for the native SF loaded BD was 370 ºC (Fig. 4e). This was agreed with the previous report for SF decomposition temperature [51]. The results noted that loading BD in the particles could be formed hydrogen bonds between the polar groups in the cellulose or SF structure, resulting in enhanced thermal stability of the particles. The $T_{d,max}$ values of each particle from DTG thermograms are illustrated in Table-1.

TABLE-1
$T_{d,max}$ VALUES OF EACH PREPARED PARTICLES FROM DTG THERMOGRAMS

Types	$T_{d,max}$ values (°C)
Native cellulose	366
C/SF at 3:1	316, 372
C/SF at 1:1	322, 373
C/SF at 1:3	308, 370
Native SF	370

The *in vitro* release profiles of BD from the prepared particles are shown in Fig. 5. Blue dextran (BD), a water-soluble drug was chosen. All prepared particles clearly showed controlled release patterns in the first 12 h of releasing time followed by further slow release until the end of testing. The highest burst release of BD was found in the native cellulose particle (Fig. 5a), while the native SF was the lowest (Fig. 5e). The BD content and released rate from the C/SF composite particles depended on the polymer ratio used. The faster BD released was observed in the C/SF ratio of 3:1, followed by 1:1 and 1:3, respectively. The drug release consisted of two main processes, which are outwards drug diffusion by swelling and degradation or erosion of polymer matrix. The drug release in the first 12 h was due to the drug concentration gradient by matrix swelling, depending on the characteristics of the polymer. The swelling of the cellulose matrix is directly related to the C/SF ratio since a high amount of SF could affect the diffusion barrier. This indicates that the drug release behaviours of the initial burst

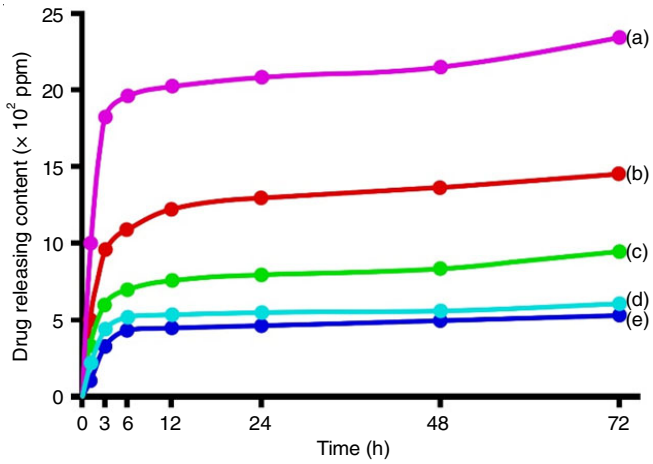

Fig. 5. Releasing profile of blue dextran (BD) from the prepared particles; native cellulose (a), C/SF composite particles with ratios of 3:1 (b), 1:1 (c), 1:3 (d), native silk fibroin (SF) (e)

release were controlled by adjusting the SF ratio. In addition, the slow release of BD may occur by surface erosion of the SF matrix, which might concern the hydrophobic or hydrophilic parts of each matrix [52].

Conclusion

A spherical shape of cellulose (C), silk fibroin (SF) and its C/SF composite particles could be prepared by solvent emulsion diffusion technique. The native SF particle has a more spherical shape and smoother surfaces than others. Moreover, the C/SF composite at high SF content results to obtain small-size particles. The chemical structure of both cellulose and SF did not change by loading blue dextran (BD), but the H-bond formation between the functional groups of the polymer matrix has been found. The formation of H-bonds resulted to increase the thermal stability of the particles. The burst release of BD from the particles was found at the initial time due to the swelling of particle surfaces as well as the degradation of the polymer matrix by hydrophilic parts and water interaction. The controlled release varied by the SF ratio, which suggested that the released BD could be controlled by adjusting the content of the polymer matrix. The obtained results have an advantage for using the C/SF composite particles in a hydrophilic drug-controlled release.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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