Facile Fabrication of Chitosan/Starch Composite Films with Fumed Silica as an Additive

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Abstract. Chitosan/cassava starch (CS/ST) composite films with different CS:ST (w/w) ratios were prepared by solution casting and investigated as a support for fumed silica (fSiO\textsubscript{2}) dispersion. The CS, as a 1\% (w/v) solution in 1\% (w/v) acetic acid, and gelatinized ST, as a 3\% (w/v) solution in 1\% (w/v) acetic acid, were mixed at the appropriate volume ratios with a homogenizer, and then fSiO\textsubscript{2} at 1, 3, or 5\% (w/w of CS) was added and cast as films. The morphology, tensile strength, contact angle, X-ray diffraction (XRD) pattern, and surface functional groups of the films were investigated. The external morphology of the CS/ST composite film revealed that the fSiO\textsubscript{2} particles were homogeneously dispersed into the polymer matrix. The water absorption and surface hydrophobicity of the composite film increased with increasing ST or CS contents. The hydrophobic acetyl groups of CS caused a notable reduction in the wettability as well as the water adsorption ability, which are preferable for a packaging film application. The CS/ST film with a high fSiO\textsubscript{2} content had a higher tensile strength due to the reinforcement effect of the fSiO\textsubscript{2} nanoparticles. The XRD patterns of the CS/ST films revealed that the 2\theta\textdegree\ of the crystalline region of CS were slightly changed when including the fSiO\textsubscript{2}. The shift in the ST diffraction peak was probably due to the change in its chain orientation caused by hydrogen-bonding interactions between CS and ST molecules, resulting in their good adhesion.

Keywords: Cassava starch, chitosan, composite, films, fumed silica.
1. Introduction

Chitosan (CS), 2-acetamido-2-deoxy-β-D-glucose, is a cationic semi-crystalline polysaccharide largely obtained from crustaceans, such as shrimp and crab shells, and produced by the N-deacetylation of chitin. The utility of marine by products is becoming a potential alternative for bio-based materials, due to their abundance, low cost, non-toxic, and biodegradable [1,2]. The structure of chitosan is comprised of glucosamine units linked with β-(1,4) bonds [3]. In addition, chitosan contains a relatively large number of primary and secondary hydroxyl (-OH) and primary amine (-NH2) groups, onto which various ligands can be easily attached [4]. Chitosan is soluble in dilute aqueous organic acids and insoluble in alkaline solutions and so can easily be formed into films and membranes by casting. The reactive functional groups of chitosan have been provided potential for covalent and ionic modifications allowing the improvement of its mechanical properties [5]. In view of the hydrophilicity, excellent film-forming ability, good mechanical properties, and high chemical reactivity of chitosan [1, 5], it is of interest as an alternative bio-based packaging material, and environmentally friendly packaging option for products. The biodegradable properties of chitosan make these films ideal candidates for various applications, such as affinity membranes, protective food coating, and food packaging purposes [6]. However, chitosan films have some disadvantages, including that they can be fragile when neutralized [7] and their unpleasant color affects the general appearance and consumer acceptance of their use in packaging.

Cassava starch (ST) is one of the most abundant agricultural products in Thailand. Starches are considered as one of the most promising biological polymers due to their abundant availability, renewability, biodegradability, and low cost [8-10]. Chemically, starch is a polysaccharide or a polymer of glucose. Structurally, starch has two forms, a straight chain of amylose and a branched chain of amylopectin. The α-1,4 linkages form the chains of glucose molecules and α-1,6 linkages occur at the branch points. However, pure starch is not suitable as a polymer for packaging materials due to its limited ability to form a thin film [11]. Starch films can be brittle and fragile, especially after the drying process. The addition of a plasticizer was found to enhance the brittleness and flexibility of starch films [12-14]. The presence of plasticizer is necessary in order to obtain a more rubbery-like material. The plasticizer can act as an internal lubricant to reduce the friction of the polymer chains. The free volume of the polymer matrix is increased when the plasticizer penetrates between the polymer chains. In general, plasticizers are compatible and miscible with the polymer matrix. Plasticizers, such as glycerol, ethylene glycol, and sorbitol, have been used for improving the physical and mechanical properties of CS films [15-17]. However, the water barrier and mechanical properties of plasticized-CS films with glycerol can change during storage [18]. The development of starch composite polymers by combining macromolecules with inorganic particles for improving some of their mechanical properties and increasing their applications has been reported [19, 20]. Fumed silica (fSiO2), also known as pyrogenic silica or flame-hydrolyzed SiO2, consists of microscopic amorphous SiO2 fused into branched, chainlike, 3-dimensional (3D) secondary particles, which then agglomerate into tertiary particles [21]. Its properties of multihydroxy and high surface area. In literature is possible to find some studies dealing with chitosan/starch blends, the influence of the physico-chemical properties. Many studies indicated that fSiO2 can improve and enhance the performance of polymeric materials for strength and thermal stability [22-24]. However, a relevant study on the starch modified by fSiO2 has rarely been mentioned.

In the present study, chitosan/starch (CS/ST) composite films were prepared via solution casting. The CS/ST film with an optimum CS: ST ratio was selected as a support for fSiO2 NPs. The correlation of the various fSiO2 and CS contents to the physical, mechanical, and chemical properties of the CS/ST-fSiO2 composite films was evaluated.

2. Materials and Methods

2.1. Materials

The CS derived from shrimp shells (commercial grade) with a degree of deacetylation of 81% and an average molecular weight (MW) of 200,000 kDa was obtained from Biolife Co. Ltd., Bangkok, Thailand. The ST (commercial grade, Plamangkorn, Bangkok, Thailand), had an ash content of 0.2%, starch content of 85%, and was used as received. The standardized, well-characterized fSiO2 (CAS no. 112945-52-5, Cab-O-Sil™, Sigma, USA), used as an additive, had a particle diameter of about 0.014 nm, surface area of 200 ± 25 m²/g, and density of 0.0368 g/cm³. The glycerol (analytical grade) with a MW of 92.0938 g/mol and density of 1.261 g/cm³ was from Sukasaph (Thailand) and used as a plasticizer.

2.2. Preparation of the CS/ST Films

First, the incorporation of glycerol in the CS/ST film was predetermined, where the addition of 20% (w/w) glycerol into the CS/ST films was chosen, because it supplied films with a better appearance and was easier to carry out (data not shown). For CS/ST film preparation, 1% (w/v) CS and 3% (w/v) ST solutions in 1% (w/v) acetic acid were prepared, the latter being gelatinized at 70 ºC until a viscous solution was obtained. Then various (w/w) ratios of CS: ST (from 0:100 to 100:0) were mixed with stirring for 24 h to give a visually homogeneous solution. The solution was then filtered collecting the
filtrate. The CS/ST films were prepared by solution casting onto an acrylic mold (12.5 mm width × 16.5 mm length), dried at ambient temperature, neutralized with 1% (w/v) NaOH solution, and then rinsed and soaked with distilled water to remove the residual NaOH. For the CS/ST-fSiO₂ composites, fSiO₂ was added at 1, 3, or 5% (w/w of polymer) solution to the CS/ST mixture and then the films were cast and subsequently treated as per the CS/ST films above.

2.3. Characterization

2.3.1. Measurement of the physical properties

The thickness of the CS/ST film was measured using a flat parallel surface micrometer with a Vernier caliper (Mitutoya, Japan) and the average thickness calculated from five randomly selected points per specimen.

2.3.2. Measurement of the water contact angle (WCA)

The WCA measurements were performed using a contact angle meter. The films formed by the solution casting method had two surfaces with a different morphology. The surface contacting the acrylic mold was obviously smoother than the surface contacting with the air. Herein, all the values of the WCA for the CS/ST films were measured at the acrylic mold contact surface. A droplet of distilled water was dropped on the film surface. The WCA was measured on the side of water droplets and averaged from five droplets taken at different positions on the film surface. Calculation of the WCA was performed using a screen protractor software (http://www.iconico.com).

2.3.3. Water absorption

The dry weight (w₁) of each film (25.4 mm width × 76.2 mm length) was measured with an analytical balance. The film was dried in an oven at 50 °C for 24 h and then kept in a desiccator until use. The sample was immersed in distilled water for 24 h controlling the pH at 7. The film sample was then removed, excess water on its surface blotted off with filter paper, and the wet weight (w₂) of the sample was measured. The swelling property was calculated using Equation (1):

\[ \text{Swelling} = \left( \frac{w_2 - w_1}{w_1} \right) \times 100 \]  

(1)

2.3.4. Tensile strength

The film was cut into slices of 150 mm (length) × 15 mm (width) and the tensile strength was determined using a universal testing machine (model Lloyd LR 100K, UK) on test specimens of 10 mm width and 20 mm gauge length. The tensile test specimens had a width of 10 mm and the tests were performed using a span length of 50 mm. The tensile tests were performed at a crosshead speed of 25 mm/min and a maximum load of 90 Newton, respectively. The average tensile stress at maximum load from five specimens was recorded.

2.3.5. External morphology

The external morphology of each respective CS/ST film was observed with scanning electron microscopy (SEM) using a model XL 30 CP (Phillips, The Netherlands) microscope. The films were cut, placed onto a brass stub and 50 Å gold vapor was sputtered onto the sample.

2.3.6. X-ray diffraction (XRD) analysis

The crystallinity of the polymer (cut into a small piece) was evaluated using XRD (Model PW3710 BASED, Philips Analytical, The Netherlands) operated with Cu Kα radiation (λ = 1.542 Å) at 40 kV and 30 mA. The XRD patterns were recorded in the reflection mode over an angular range of a 2θ of 10–30° at ambient temperature, scan time of 25 min, and scan speed of 0.040 (2θ s⁻¹).

2.3.7. Functional groups of CS/ST Film

The functional groups on the surface of the CS/ST films were analyzed using attenuated total reflectance - Fourier-transform infrared spectrometer (ATR-FTIR) using a model 1760 × (Perkins Elmer, USA) instrument. The plasticized ST, CS, CS/ST films, and CS/ST-fSiO₂ composite films were cut into small pieces and the films scanned over a wavenumber range of 4000–650 cm⁻¹.

3. Results and Discussion

3.1. Physical and Surface Characterization

The transparent, homogeneous, thin, and flexible CS/ST films with 20% (w/w) glycerol plasticizer were obtained by solution casting. Visually, the polymeric films had a slightly yellow appearance increasing in intensity as the CS ratio increased, whereas increasing amounts of ST gave a paler yellow film. The average thickness of the plasticized-CS/ST films ranged from 0.02–0.04 mm (± 0.01 mm). The effect of the fSiO₂ content on the surface hydrophilicity of the films’ surface and degree of swelling was revealed.

The surface wettability of the prepared films was determined by measurement of the WCA, where a WCA of more than 90° was designated as a hydrophobic film [25]. Increasing the amount of CS in the CS/ST film increased the WCA, reaching a WCA of nearly 80° at CS contents higher than 90% (w/w), as seen in Fig. 1. The amount of wetting was found to depend on the surface tension at the interface between the water droplet and the solid surface. The less hydrophilic solid surface of the CS/ST films with a high CS content gave a WCA close to 90°, which may be attributed to the hydrophobic
backbone of the CS chains that contain acetyl groups. Increasing the amount of fSiO\textsubscript{2} in the CS/ST films increased the WCA. These results indicate that the wettability of the CS/ST film decreased with increasing fSiO\textsubscript{2} contents. Silica has a tunable surface comprised of hydrophilic silanol groups and moderately hydrophobic siloxane groups, which can be interchanged through thermal and chemical treatments [26]. In general, fSiO\textsubscript{2} is hydrophilic. Increasing, the fSiO\textsubscript{2} content from 1% to 3% and 5% (w/w) increased the WCA of the CS/ST-fSiO\textsubscript{2} composite films, indicating that the interaction of the CS molecules on the surface of SiO\textsubscript{2} became more hydrophobic and might have restricted the interaction between the silanol groups and water.

In the present work, it seemed to decrease the degree of swelling of the CS/ST films was decreased. It is possibly due to the H-bonds between the silanol groups on the fSiO\textsubscript{2} surface and the amino groups (-NH\textsubscript{2}) of CS and/or hydroxyl groups (-OH) of ST seemed to affect the swelling property. This results in bonding formation, which acts as a barrier to restrict water penetration of CS/ST matrix. When water solvated the polymer chains it would limit their movement.

![Fig. 1. Effect of the CS and fSiO\textsubscript{2} contents on the WCA of the CS/ST-fSiO\textsubscript{2} composite films. Data are shown as the mean ± SD derived from five measurements.](image)

### 3.2. Absorption Properties

Figure 2 shows the percent swelling degree of the CS/ST-fSiO\textsubscript{2} films with increasing levels of CS and fSiO\textsubscript{2} contents. The degree of swelling decreased with increasing CS contents, which reflects the molecular structure of both polymers: CS contains hydrophobic acetyl groups while ST has hydrophilic hydroxyl groups. On the other hand, increasing the ST contents, and so increasing the density of hydroxyl groups to interact with water by hydrogen bonds (H-bonds), increased the swelling degree. Moreover, the inclusion of 20% (v/v) glycerol would have provided more hydroxyl groups to adsorb water molecules. Thus, the moisture affinity of the films was assisted by the glycerol molecules, as seen by the high compatibility with CS and enhanced mobility of the CS chains.

In general, polymer swelling degree depends upon its functional groups of polymer and osmotic pressure [27]. In the present work, increasing the amount of fSiO\textsubscript{2} NPs decreased the degree of swelling of the CS/ST films was decreased. It is possibly due to the H-bonds between the silanol groups on the fSiO\textsubscript{2} surface and the amino groups (-NH\textsubscript{2}) of CS and/or hydroxyl groups (-OH) of ST seemed to affect the swelling property. This results in bonding formation, which acts as a barrier to restrict water penetration of CS/ST matrix. When water solvated the polymer chains it would limit their movement.

![Fig. 2. Effect of the CS and fSiO\textsubscript{2} content on the swelling degree of CS/ST-fSiO\textsubscript{2} composite films. Data are shown as the mean ± SD derived from five measurements.](image)

### 3.3. Mechanical Properties of the CS/ST Films

The tensile strength is a mechanical characteristic value for the evaluation of strength behavior and represents the maximum mechanical tensile strength that a film can be loaded with. Figure 3 shows the tensile strength of the CS/ST-fSiO\textsubscript{2} composite films with various CS: ST ratios and amounts of fSiO\textsubscript{2}. High tensile strength values indicate the films are stronger with a high stress by bonding. The higher the ST amount the more brittle the films were. The tensile strength sharply decreased with increasing CS contents. A high CS content resulted in a tougher film, resulting from the crystalline structure of CS, as seen in the XRD analysis (section 3.4).

Increasing the content of fSiO\textsubscript{2} NPs gave higher tensile strength values supporting that the fSiO\textsubscript{2} NPs strongly influenced the polymer molecules. The CS chains were absorbed on fSiO\textsubscript{2} particles by (1) electrostatic attraction between the negative charges of SiO\textsubscript{2} on the fSiO\textsubscript{2} NPs and NH\textsubscript{3}\textsuperscript{+} of CS molecules, and (2) H-bonds between the hydroxyl and/or amino groups of CS (or ST) and silanol group of fSiO\textsubscript{2} [28]. These functional groups favor intramolecular H-bonding and restrict the CS chain movement. The addition of fSiO\textsubscript{2} NPs in the CS/ST polymer prevents intramolecular H-bonding and leads to the intermolecular bonds instead. The intermolecular interfacial adhesion is improved by the presence of intermolecular interactions between fSiO\textsubscript{2}-ST or fSiO\textsubscript{2}-CS in the CS/ST films [11]. When the fSiO\textsubscript{2} amount increased, the CS/ST film was reinforced by the fSiO\textsubscript{2} NPs and enhanced the film’s mechanical properties. In addition, the tensile strength values were reduced with decreasing fSiO\textsubscript{2} contents.
3.4. External Morphology of the CS/ST Films

Figure 4 shows SEM micrographs of the ST, CS, and CS/ST composite films. The apparent smooth surface of CS and ST films contrasts with the rough-grained appearance of the CS/ST composite films at 50:50 (w/w) ratio of CS: ST with 1% (w/w) fSiO2. Increasing the fSiO2 concentration led to a rougher surface of the CS/ST composite film, where the agglomeration of fSiO2 particles became increasingly dense compared to that at a lower fSiO2 content.

Fig. 4. Representative SEM micrographs (2,000 x magnification) of the (a) ST and (b) CS films, and the (c-e) CS/ST films with a fSiO2 content of (c) 1, (d) 3, and (e) 5% (w/w).

3.5. Physical Structure of the CS/ST Films

Figure 5 shows the XRD patterns of the CS and CS/ST composite films. The native CS had three distinct crystallization peaks at a 2θ of 9.39°, 15.43°, and 20.63°. The high average MW CS revealed a high degree of crystallinity and intensity of diffraction peaks at a 2θ of 20.25°, and higher than at 9.39° (hydrated crystal peaks). In the ST film, the crystallinity could have originated from a clustered amylopectin structure, where the morphology of the crystalline part depends on the plant source [29]. The amylose structure was gelatinized which destroyed the A-style crystallinity. Therefore, the ST exhibited the B-style crystalline form at a 2θ of 5.6°, 15°, 17°, 22°, and 24° [30].

Fig. 5. Representative XRD traces of (a) pristine CS film; and (b, c) the CS/ST-fSiO2 composite films with a fSiO2 content of (b) 1% (w/w), (c) 5% (w/w), and (d) starch.

Figure 5(b) shows the CS/ST films with 1% (w/w) fSiO2, revealing the diffraction peaks of CS and amylose at a 2θ of 20.25° and 24.57°, respectively. Increasing the fSiO2 content to 5% (w/w) of CS shifted the diffraction peaks of CS to a 2θ of 9.38° and 20.25°. This shift in the diffraction peaks for CS and ST probably depends on the breaking of CS H-bonds with a minor microphase separation of the polymer. It has been reported that the X-ray patterns of a fresh potato starch film showed peaks for amylose at a 2θ of 5.5° and 17° [31], and the addition of glycerol at 20% (v/v) gave a sharper peak at 17°. The crystallization of starch could also have occurred due to amylose.

The plasticized-ST film with 20% (w/w) glycerol showed diffraction peaks at a 2θ of 5.76° and 14.96°, which was related to diffraction peaks of the free ST, as shown in Fig. 6(a). The shift in the diffraction patterns was due to the transportation of the plasticizer between the polymer molecules. In addition, the plasticized ST film with 20% (w/w) glycerol showed diffraction peaks at a 2θ of 9.6° and 19.53°, Fig. 6(b). The CS diffraction pattern appeared as a smaller peak than that of the plasticized-ST film, which was probably related to the chain orientation of the CS molecules being disturbed. CS is not completely...
crystallized, as the crystalline domains by H-bonds and electrostatic interactions between the N-acetyl groups in which, some amorphous regions are also present [32].

3.6. Characterization of the Surface Functional Groups of the CS/ST Films

Table 1 and Fig. 7 show the ATR-FTIR spectra of the ST, CS, and CS/ST composite films. For the ST film, the broad absorption bands at 3550–3200 cm⁻¹ were ascribed to O-H stretching, while the strong absorption peaks at 2930 cm⁻¹, 1158 cm⁻¹, 1081 cm⁻¹, and 1015 cm⁻¹ were from C-H (stretching) and C-O (stretching) [33]. The ATR-FTIR spectrum of CS showed strong absorption bands at 1158–1153 cm⁻¹, ascribed to the primary or secondary alcohol on the polysaccharide skeleton. The strong peaks at 1650–1580 cm⁻¹ corresponded to NH₂ deformation vibration and the broad peak at 3450 cm⁻¹ to -NH₂ symmetric stretching vibrations [34]. The strong peaks at 3600–3200 cm⁻¹ were ascribed to C-H stretch vibration, and weak peaks at 2900–2800 cm⁻¹ to the aliphatic carboxylic dimmer. The peaks at 1725–1700 cm⁻¹ (strong) to -OH and CH deformation (ring), at 1420 cm⁻¹ (medium) to C-H₂ stretching bending [35], at 1409 cm⁻¹ (strong) to -OH stretch vibration, at 1383–1377 cm⁻¹ (strong) to CH₃ deformation (bend), at 1267–1263 cm⁻¹ (weak) to CH wag (ring) vibration, and at 1083 cm⁻¹ (strong) to C-O stretching vibration. The other peaks were ascribed as the weak absorption peak at 1625–1500 cm⁻¹ of COOH, C=O stretch and O-H deformation, and at 1560–1548 cm⁻¹ for the NH₃⁺ symmetric and asymmetric deformation vibration. The ATR-FTIR spectrum of the bulk fSiO₂ exhibited the broadband of ≡Si-OH at 3434 cm⁻¹, the strong vibration of Si-O at 1106 cm⁻¹, and Si-O asymmetric (medium) at 809 cm⁻¹. The crystallization sensitive zone was at 1106 cm⁻¹.

Figure 8 shows the ATR-FTIR spectra of the CS/ST-fSiO₂ composite films with CS: ST (w/w) ratios of 80:20, 60:40, 40:60, and 20:80 in the presence of 1% (w/w of CS) fSiO₂ NPs. The transmission peaks of -OH became wider and stronger with higher ST contents and were comprised of 3550–3200 cm⁻¹ for O-H stretching and at 2930 cm⁻¹ for CH stretching. The CS/ST composite films showed a combination of both CS’s and ST’s characteristics, but with shifts in the absorption peaks at wavenumber 1190–950 cm⁻¹ (C-O stretching vibration).

3.7. Water Contact Angle (WCA) Measurements

Glycerol-plasticized CS/ST-fSiO₂ composite films were successfully prepared. The CS/ST films exhibited a deeper thickness with increasing ST amounts. Increasing the proportion of CS increased the WCA of the film, Increasing the CS content of the CS/ST film increased the WCA due to the molecular orientation of CS that contains a hydrophobic acetyl group. Moreover, increasing the CS content decreased the degree of swelling and reduced the tensile stress at maximum load values of the CS/ST film. The mechanical properties of the CS/ST film could be improved by the addition of both CS and fSiO₂, giving a more flexible film due to the interaction of H-bonds of CS molecules (amino-to-amino groups or amino-to-hydroxyl group) being dominant. Increasing the fSiO₂ content
increased the WCA of the composite film due to the Si-OH group on the surface of SiO₂.

The ATR-FTIR spectra of CS/ST-fSiO₂ films revealed characteristic peaks of CS (–NH₂ at 1650–1580 cm⁻¹), ST (C-H stretching at 2930 cm⁻¹), and fSiO₂ (silanol group at 1106 cm⁻¹). The XRD diffraction pattern of the CS film revealed peaks at a 2θ of 9.39°, 15.43°, and 20.63°, while those for the CS/ST film were found at a 2θ of 20.25°, 9.38°, and 20.56° for 1, 3, and 5% (w/w) fSiO₂, respectively. The diffraction patterns of the amyle structure of ST were seen a 2θ of 24.57°. The crystallinity of ST and CS was shifted to a higher θ angle, which was possibly due to the orientation of polymer molecules where the interaction of CS and ST molecules tended to exhibit a microphase separation.

Table 1. Summary of the ATR-FTIR characteristics of the CS/ST composite films

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<th>Sample</th>
<th>Wavenumber (cm⁻¹)</th>
<th>IR band assignment</th>
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<tr>
<td>Chitosan film</td>
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<tr>
<td>Saccharide</td>
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<td></td>
<td>1153–158</td>
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<td></td>
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<td>amide II–N-H deformation and C-N</td>
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<td>1649–1655</td>
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<tr>
<td>Chitin (1st amide)</td>
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<tr>
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<td>1083</td>
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<td>1700–1725</td>
<td>Aliphatic carboxylic dimer</td>
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Acknowledgement

The authors acknowledge the Department of Materials Science, Faculty of Science, Chulalongkorn University for tensile strength measurement.

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