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ABSTRACT
Chitosan film reinforced with nano-sized chitin whiskers and crosslinked using tannic acid was synthesized by the casting-vaporation method. The mechanical and physicochemical properties of several film samples (consisting of different ratio of chitin and tannic acid) were compared with neat chitosan. Tensile tests show that the addition of chitin improves the nanocomposite films mechanical properties up to 137% compared to neat chitosan, but this is slightly degraded when tannic acid is introduced. However, tannic acid and chitin whisker content greatly reduced moisture content by 294% and water solubility by 13%. Transmission electron microscopy (TEM) and Fourier-transform-infrared spectroscopy (FTIR) were used to investigate the morphology and molecular interaction of film. X-ray diffraction results indicated that the samples with chitin whiskers had a more rigid structure. The addition of tannic acid changed the structure into an anhydrous crystalline conformation when compared to neat chitosan film.

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1. Introduction

The production of synthetic polymers based on petroleum has dominated global commercial industries for decades, however their non-biodegradability has caused environmental concerns. The need for biocompatible material has inspired the development of “green” synthetic polymers like polyactic acid (PLA) or polyhydroxybutyrate (PHB), but they are expensive and still have problems with ecological compatibility (Simkovic, 2013). Bio-nanocomposite materials are replacing petroleum based materials for many packaging and structural applications. Polysaccharide composites are abundant, biodegradable, and non-toxic. Cellulose and chitosan are types of polysaccharides used for the structural material in food packaging and other bio-based applications. Although they are biodegradable, their mechanical and physicochemical properties are inferior to petroleum based polymers (Tuhin et al., 2012). The structures inherent in many biological organisms consist of a biocomposite matrix, reinforced with fibrous biopolymers (Neville, 1993). This has inspired much research focused on modifying biopolymer matrix materials with nano-sized fillers (1–1000 nm) as a reinforcement.

Chitosan is a biodegradable and biocompatible natural polysaccharide. Its excellent film forming capability gives it great potential for use in many applications. The inspiration for the research in this paper is its potential application as a wing membrane in a biomimetic micro air vehicle (BMAV). This is a type of miniaturized aircraft that generates lift by mimicking the flapping wing motion of flying organisms (e.g., insects or small birds). It is envisioned that future BMAV will be mass produced and used in one-way (disposable) missions. This requires that the wings be composed of biodegradable and biocompatible materials so not to pose risk to the biosphere. However, the poor mechanical properties of neat chitosan make it unsuitable for this and other applications (e.g., packaging). Therefore it is important to study methods of improving its mechanical and physicochemical properties. Past research has indicated that the addition of reinforcement fillers or crosslinking agents may improve these properties (Liu, Su, Lee, & Lai, 2005). The addition of non-biodegradable fillers, such as carbon nanotubes, parallel-aligned graphene oxide and unzipped multiwalled carbon nanotube oxides into a chitosan membrane, will yield a higher mechanical strength that is unique to the filler material selected (Fan et al., 2012; Pan, Wu, Bao, & Li, 2011). But since these fillers are not biodegradable, their applications are limited.

Polysaccharides, such as cellulose and chitin, are one of the most abundant biomaterials on earth with added advantage of low cost, environmental friendly, renewable and biodegradable properties (Muzzarelli, 2012). Chitin is usually found in stiff extracellular coatings typified by the arthropod exoskeleton and developed as...
robust structures for insect exoskeletons and wings (Muzzarelli, 2011a). Research has been done examining the many potential biomedical applications of chitin (Muzzarelli, 2011b). The use of chitin is also of interest in BAVM materials research and development. The polymers form a highly-structured crystalline network, consisting of three types of polymorphs: α, β, and γ. The α-polymorph is the most abundant and it is also more stable than the other two types (Mathew, Laborie, & Oksman, 2009). Despite its wide availability, the utilization of chitin has been restricted by its intractability and insolubility. Chitin whiskers have been successfully prepared from crab shells (Marchessault, Morehead, & Walter, 1959). Nano-sized whiskers exhibit a high crystallinity and rigidity that can elevate a composite film’s mechanical property if it is used as a filler material. Both chitin and chitosan are composed of glucosamine and N-acetylated glucosamine (2-acetylamino-2-deoxy-ß-glucopyranose) units linked by (1→4) glycosidic bonds (Möller, Greller, Pardon, & Costa, 2004).

A polymer matrix can also be modified by introducing a crosslinking agent (Chambi & Grosso, 2006; Sung, Huang, Huang, & Tsai, 1999). This chemical process alters the stability and the mechanical properties of the polymer. Two different reactions are associated with crosslinking: Schiff base or Michael-type adducts. (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Glutaraldehyde is a highly effective crosslinking agent for biopolymers, yielding superior mechanical properties and water resistivity than most other agents (Bigi, Coazeli, Panzarovela, Rubini, & Roveri, 2001). However, its cytotoxicity hinders its biocompatibility. Several chemicals have been studied as alternative crosslinking agents. These include ferulic, tannic acid, genipin and citric (Cao, Fu, & He, 2007; Muzzarelli, 2005; Reddy & Yang, 2010). Tannic acid is extracted from plants and microorganisms. It is fully biodegradable and less expensive to produce compared to other chemical derivatives. It is a high molecular weight, polyphenolic compound containing a central carbohydrate core that is esterified by phenol acids (Božič, Grgieva, & Kokol, 2012). Tannic acid has a high antioxidant capacity and can interact with other biological macromolecules (Shutava, Prouty, Kommireddy, & Livo, 2005).

Despite its high potential, little has been published on the use of tannic acid as a crosslinking agent for chitosan nanocomposites. Therefore, the objective of this research is to synthesize a chitosan based biopolymer and study the effects of tannic acid and chitin whiskers on its mechanical and physicochemical properties.

2. Materials and methods

2.1. Materials

High molecular weight chitosan powder (with minimum deacetylation degree of 75%) and chitin flakes (from crab shell) were purchased from Sigma-Aldrich (Malaysia). Glacial acetic acid grade AR and tannic acid were purchased from Friedemann Schmidt. Hydrochloric acid (HCl 36–38wt%) and other agents were provided by Fisher Scientific (and used as received).

2.2. Preparation of chitin whiskers

Chitin whiskers were prepared by the hydrolysis method to remove the amorphous region of the chitin flakes, using a modification of the method reported in Gopalan Nair and Dufrasne (2003a). The preparation begins by dispersing 2g of chitin flakes into 150ml of a 3N HCl solution (weight ratio of 1:30) in a flat bottom flask fitted with a magnetic stirrer under reflux for 3h at 90°C. After acid hydrolysis, the suspension was centrifuged at 10,000 rpm for 10min. This process was repeated three times followed by removal of the HCl solution from the container. The samples were diluted with distilled water for each run. Next, the chitin whiskers slurry was collected and transferred into a dialysis bag. The slurry was dialyzed in running water for 2h and then soaked in distilled water for 24h. Once neutralized, the pH of the chitin whiskers suspension was corrected to 2.5 by adding diluted HCl. The dispersion was completed by 10min of ultrasonic treatment for every 30cm³ aliquot. Finally, the chitin whiskers suspension was refrigerated in an air tight container at 6°C with a final concentration of 2.5 weight% of chitin whiskers in the suspension.

2.3. Preparation of chitin whiskers/chitosan composite film

The chitosan solution was prepared by adding 2wt% of chitosan powder into a 2% (v/v) acetic acid solution under magnetic stirring for 3h at 35°C. Then, the chitin whiskers were slowly added into the chitosan solution for 1h, until a mixed suspension was obtained. The desired mass ratio of chitin whiskers to chitosan was controlled at 0:100, 10:90, 20:80, and 30:70. The mixed suspension was cast onto a plastic Petri dish. The casted suspensions were left overnight in a dry cabinet to remove bubbles and later evaporated in a drying oven for 48h to obtain dry composite films. Theoretical dry weight of each sample was 0.4607g.

2.4. Preparation of crosslinked chitin whiskers/chitosan film

Chemical crosslinking was achieved by incorporating different amounts of tannic acid (20 and 40mg of tannic acid per 1g of chitosan) into the suspensions under vigorous magnetic stirring for another 1h at 35°C until it was homogenized. The mixed suspensions were cast with the same procedure described in Section 2.3. Table 1 shows twelve different types of composite films (Set I: neat chitosan film, serving as the control sample; Set II: crosslinked chitosan film; Set III: chitosan film embedded with chitin whiskers; Set IV: crosslinked chitosan film embedded with chitin whiskers). All samples have an average thickness of 0.1mm.

2.5. Microstructural studies by transmission electron microscope (TEM)

The size and morphology of chitin whiskers were examined using a Zeiss ETEM Libra 120 transmission electron microscope (TEM). The chitin whiskers were dispersed in distilled water after ultrasonic treatment and dropped on a carbon coated copper grid, as reported in Ma, Qin, Li, Zhao, and He (2014). The copper grid was stored in dry cabinet for 48h before viewing.

<table>
<thead>
<tr>
<th>Sample set</th>
<th>Sample code</th>
<th>Chitosan (%)</th>
<th>Chitin (%)</th>
<th>Tannic acid (mg/g)</th>
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2.6. Fourier-transform-infrared spectroscopy (FTIR)

The Fourier-transform-infrared (FTIR) spectra of the films were recorded on a Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer. A total of 32 scans were performed at 4 cm⁻¹ resolution. Measurements were recorded between 4000 cm⁻¹ and 400 cm⁻¹.

2.7. X-ray diffraction (XRD)

Composite films were analyzed by an X-ray diffractometer (Siemens D5000) equipped with Cu Kα radiation source (λ = 1.540600 Å) operating at 40 kV and 40 mA (at room temperature). The relative intensity was recorded in the scattering range of 2θ = 5–80° with a step size of 0.1°.

2.8. Mechanical properties

Tensile properties were measured at room temperature using a Shimadzu AGS-X series with 100 N load cell and a crosshead speed of 1 mm/min. Composite films were cut in a rectangular shape (80 mm by 8.5 mm). Tensile strength, Young’s modulus, and elongation at the break were calculated according to the ASTM D882-02 method. At least five samples were tested for each film.

2.9. Water uptake test

The film was cut into 15 mm × 12 mm pieces. Samples were weighed to the nearest 0.0001 g in a dry state after heating in oven at 105°C for 1.5 h. Water uptake was measured by immersing dried film pieces in 75 ml of distilled water (under constant agitation) for 1 h at 25°C. Then samples were recovered and dried with filter paper to remove excess surface water and weighed again. The average value of water uptake was calculated using Eq. (1):

\[ \% \text{ water uptake} = \left( \frac{W_f - W_i}{W_i} \right) \times 100 \]  

(1)

where \( W_i \) is the weight of the swollen sample and \( W_f \) is the weight of the sample after heating it in a convection oven to make sure the film is in a completely dry state.

2.10. Solubility test

Immediately after recording the swollen weight (Section 2.9), the samples were dried in a convection oven at 105°C for at least 1 h 30 min until it reaches a constant dry weight. The samples were weighted to the nearest 0.0001 g. This dry weight was then used in Eq. (2) to find the solubility:

\[ \% \text{ solubility} = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \]  

(2)

3. Results and discussion

3.1. Microstructural studies by transmission electron microscope (TEM)

The morphology of the chitin whiskers was studied using a transmission electron microscope (TEM). Fig. 1 shows TEM images of nano-scale chitin whiskers in dilute HCl. It was found that chitin suspension is composed of individual and aggregated nanocrystals in a lump state. The individual nanocrystals are shaped like long, slender rods with an average aspect ratio of 8.8. The TEM image in Fig. 1a shows that the chitin whiskers have a length of 200–300 nm (with an average value being ~246 nm). Fig. 1b shows that the diameters of the individual chitin whiskers range from 20 to 40 nm (with an average value being ~28 nm). These measurements accord with the dimensions of crab shell chitin whiskers reported in past studies (Zeng, He, Li, & Wang, 2011). The chitin whisker suspension exhibits colloidal behavior, due to the protonation of the amino groups (–NH₂)⁺ which induces positive charges on the surface of the crystallites and promotes the stability of the suspension as described by Watthanaphanit, Supaphol, Tamura, Tokura, and Rujiravanit (2008).

3.2. Fourier-transform-infrared spectroscopy (FTIR)

A Fourier-transform-infrared (FTIR) spectroscopy identifies unknown materials and the chemical components in a mixture by emitting infrared radiation through a sample. The resulting spectrum creates a unique representation of the molecular absorption and transmission. This was used to characterize the
Fig. 2. FTIR spectra of all sample sets, (a) chitosan film without crosslinker, (b) 40 mg tannic acid crosslinked chitosan film, and (c) 20 mg tannic acid crosslinked chitosan film.

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