Tensile Properties and Crystallinity of Crosslinked Nanocrystalline Cellulose/Chitosan Composite

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Abstract. This study evaluated the properties of the polymeric composites obtained from nanocrystalline cellulose and chitosan using citric acid as the crosslinking agent prepared via solvent casting method. The tensile strength and Young’s modulus of composites increased, but the elongation at break decreased with the nanocrystalline cellulose (NCC) loading. The addition of citric acid has further enhanced the tensile strength and Young’s modulus. The morphology of the non-crosslinked and the citric acid crosslinked NCC/chitosan composites was investigated by the scanning electron microscopy (SEM) analysis. The X-ray diffraction (XRD) revealed that the relative crystallinity of composites was enhanced by the incorporation of NCC but the value declined by the addition of citric acid.

1. Introduction
Chitosan, a natural polymer consisting of 1,4-linked 2-amino-deoxy-β-D-glucan, can be obtained from the N-deacetylation of chitin. It is a promising and an attractive material for future applications due to its unique properties such as non-toxic, biodegradable, biocompatible and anti-microbial [1]. Chitosan can be applied in many areas such as packaging [2], biomedical purpose [3] and wastewater treatment [4], however, the chitosan film has poor barrier and mechanical properties which make it unsuitable for the aforementioned application [5]. Therefore, chitosan is often blended with reinforcing agent to improve its properties.

Nanocrystalline cellulose (NCC) is a highly crystalline nanometre rod-like cellulose which produced by acid hydrolysis with sulphuric acid being the most common-used acid. Acid hydrolysis involves the extraction of crystal from cellulose by hydrolysing the amorphous domains, resulting in highly crystalline nanoparticles [6]. The NCC synthesised (from plant cellulose) usually has a diameter of 5-20 nm and length of 100 -500 nm [7]. NCC reinforces the polymer matrix by forming the percolation network which interconnecting the NCC with hydrogen bonding. The presence of well-dispersed NCC as reinforcement in polymer matrices offers remarkable performances such as superior mechanical properties, biodegradable and highly crystalline leading to a new generation of biomaterial [10].

Besides adding reinforcing filler, polymers can also be chemically modified to improve its properties by using crosslinkers to alter the chemical structure of the matrix [10]. The crosslinking reaction occurs between the amino groups of chitosan and active groups of crosslinkers forms a three-dimensional network which limits the mobility of chitosan [11]. In this study, citric acid was used as the crosslinking agent for NCC/chitosan composites. Citric acid is a low cost and non-toxic poly(carboxylic) acid that widely used as a crosslinker for food and drug industry. Citric acid is
categorised as safe for food usage due to its renewable and biodegradable properties [12]. Chitosan composites crosslinked with citric acid can undergo the amidation reaction at an elevated temperatures [13]. It was reported that addition of citric acid enhanced the mechanical properties of poly(vinyl alcohol)/starch significantly [14]. In the present study, the incorporation effect of NCC from empty fruit bunch (EFB) used as reinforcement and citric acid as crosslinking agent on mechanical properties of NCC/chitosan film was investigated. The morphology of the fracture surfaces of nanocomposites was also studied using field emission electron microscopy (FESEM).

2. Materials and Methods

2.1. Materials
EFB fibres was kindly provided by Taclico Company Sdn. Bhd. Chitosan with a degree of deacetylation 90.5% was provided by Cleo Shanghai Pharmaceutical. Sodium chlorite, acetic acid and sodium hydroxide were purchased from Sigma-Aldrich while citric acid and sulphuric acid were purchased from Merck. Potassium carbonate was provided by HmbG.

2.2. Preparation of Nanocrystalline Cellulose
NCC was isolated from EFB cellulose by acid hydrolysis. EFB fibres was grounded and sieved into a diameter of less than 125 µm. The fibres were pre-treated with deep eutectic solvent (DES) and bleaching treatments. DES was prepared by mixing and heating potassium carbonate and glycerol in a molar ratio of 1:7. The bleaching solution consisted of equal parts of acetate buffer, aqueous chlorite, and distilled water. Both DES and bleaching were performed at 80°C for 2 hours and repeated for three times. The treated cellulose was washed with distilled water and dried in oven at 60 °C for 2 hours.

Acid hydrolysis was carried out for 45 minutes at 45°C with a 64 wt% of sulphuric acid. After acid hydrolysis, the suspension was washed with distilled water and centrifuged at 9000 rpm for 10 minutes repeatedly until the suspension become turbid. 3 wt% of sodium hydroxide was added to neutralized the suspension. Ultrasonic treatment was applied for 10 minutes. The resulting suspension was kept in the refrigerator at 4°C for further usage.

2.3. Preparation of Chitosan Composites Reinforced with Nanocrystalline Cellulose
The chitosan solution was prepared by dissolving 1.5 wt% of chitosan powder into 2 v/v% acetic acid solutions at 60 °C for 4 hours. 10 wt% of glycerol and NCC were added into the chitosan solution and stirred for another hour. After removal of air bubbles, the NCC/chitosan solution was casted onto a 12 cm x 10 cm petri dish and dried in an oven at 50 °C for 48 hours. The dried film has an average thickness of 0.09 mm.

For crosslinking process, 5wt% of citric acid was added into the NCC/chitosan solution. The solution was stirred vigorously in order to obtain homogeneous solution. The dried NCC/chitosan composite was cured in the oven at 105 °C for 10 minutes for the curing process.

2.4. Characterisation

2.4.1. Tensile Properties
Tensile properties of non-crosslinked and citric acid crosslinked NCC/chitosan composites were obtained using Instron universal tensile testing machine (UTM) according to ASTM standard method D882-A. The samples were prepared by cutting into rectangular specimens of 10 cm x 1 cm (length x width). A total of 10 measurements of were performed for each formulation and the average values were determined.

2.4.2. Scanning Electron Microscopy (SEM)
SEM was used to study the morphological fractured surface of non-crosslinked and citric acid crosslinked NCC/chitosan composites. To avert electrostatic charging, the external surface of samples
was coated with platinum using auto fine coater JFC 16000 before scanning. The samples were observed under the magnification of 5000 and the accelerating voltage of 10kV.

2.4.3. X-Ray Diffraction (XRD) Analysis
Crystallinity of non-crosslinked and citric acid crosslinked NCC/chitosan composites was determined using a Bruker D2 Phaser X-ray diffractometer at the operating voltage and current at 40 kV and 30 mA. Data were collected in a 2θ angle between 5 and 40°.

3. Results and Discussion

3.1. Tensile Properties

Figure 1: (a) Tensile strength, (b) elongation at break and (c) modulus of elasticity of non-crosslinked and citric acid crosslinked NCC/chitosan composites

Figure 1 shows the tensile properties of non-crosslinked and citric acid crosslinked NCC reinforced chitosan composites. The addition of NCC increased the tensile strength and modulus of elasticity values with respect to the neat chitosan film, proving the reinforcing effect induced by the NCC. Chitosan composite with the incorporation of 4 wt% NCC records the highest tensile strength of 45.7 MPa for non-crosslinked set. The enhancement could be attributed to the homogeneous dispersion between the NCC and chitosan matrix [15]. The good dispersion between filler and matrix also led to a better interfacial adhesion and enhanced the stress transfer to the filler which in turn to improve the tensile strength [16]. The crosslinking process has further increased the tensile strength by forming a more stable network between crosslinker and matrix. Similar result was also reported by Rivero et al.
[17] in which the addition of crosslinking agent has led to a formation of more rigid structure and hence, improved the tensile strength significantly.

However, the elongation at break of composites declined significantly with the incorporation of NCC in chitosan matrix as shown in Figure 1 (b). This observation may be attributed to the stiffening effect of the highly crystalline NCC which increased the rigidity of the composite. Furthermore, the addition of crosslinkers has limited the polymer chain mobility and flexibility of composites [1]. Besides, the structural reorganisation and moisture loss during curing process also led to the reduction of elongation at break [17].

The elasticity of modulus of NCC/chitosan composites increased linearly with the addition of NCC. This could be due to the formation of strong intramolecular or intermolecular hydrogen bonding of NCC with chitosan, as well as the stiffening effect, which resulted in the increment of elasticity of modulus [18]. Introduction of citric acid as crosslinker also interfered the NCC network formation in the chitosan matrix and restricted the mobility of chitosan segmental chain, consequently increased the modulus of elasticity dramatically [13]. The high performance of citric acid crosslinked NCC/chitosan composites proposed the favourable interactions between citric acid, NCC and chitosan matrix.

![Figure 2: Fracture surface of the non-crosslinked chitosan composites with (a) 0% NCC, (b) 1% NCC & (c) 4% NCC and the citric acid crosslinked chitosan composites with (d) 0% NCC, (e) 1% NCC & (f) 4% NCC at 5000X magnification](image-url)
The fracture surfaces of neat chitosan and chitosan composites were determined by SEM (Figure 2), in order to investigate the effect of NCC addition and the influence of crosslinker on the chitosan microstructure. The tensile fracture surface of non-crosslinked and citric acid crosslinked neat chitosan composites in Figure 2 (a) and (d) displayed a homogeneous and smooth surface with matrix tearing. The micrograph of non-crosslinked NCC/chitosan composites showed a rough surface. Furthermore, the fracture surface of 4 wt% NCC/chitosan composite showed a higher roughness due to the higher concentration of NCC with respect of 1 wt% NCC/chitosan composite. After the chemical modification using citric acid crosslinking agent, the film became more porous [5]. SEM analysis further verified the improved mechanical properties of chitosan composites due to the incorporation of NCC and citric acid.

3.2. X-Ray Diffraction (XRD) Analysis

![X-ray diffractograms of non-crosslinked and citric acid crosslinked NCC/chitosan composites](image)

Table 1: Crystallinity index of non-crosslinked and citric acid crosslinked NCC/chitosan composites

| Samples                           | Crystallinity index (%) |
|-----------------------------------|-------------------------|
| Neat chitosan                     | 42.1                    |
| 5 wt% NCC/chitosan                | 54.8                    |
| Citric acid crosslinked chitosan  | 31.4                    |
| Citric acid crosslinked 5 wt% NCC/chitosan | 39.5            |

The X-ray diffraction patterns of non-crosslinked and citric acid crosslinked NCC/chitosan composites were exhibited in Figure 3. All composites showed major crystalline peaks at $2\theta = 12.5^\circ$, $22.6^\circ$ and $34.7^\circ$, which corresponded to the hydrated “tendon” conformation of chitosan [19]. The chitosan composite with the addition of NCC has significantly elevated the peak at $2\theta = 22.6^\circ$. This indicated that NCC has a higher degree of crystallinity [20]. The introduction of NCC did not cause any significant shift in peaks in the NCC/chitosan composites. The addition of 5 wt% of NCC has increased the crystallinity index of composites from 42.1% to 54.8% as shown in Table 1.

In comparison with the non-crosslinked chitosan composites, the crosslinked chitosan composites have a significantly weakened peak at $2\theta = 22.6^\circ$ which indicated the effective crosslinking between chitosan and citric acid [21]. The average crystallinity index of crosslinked neat chitosan and
NCC/chitosan composites were reduced to 31.4% and 39.5% respectively. The crosslinking process hindered the close packing of the polymer chains by limiting the degree of freedom in the 3-D conformation, restricting or even inhibiting the formation of crystalline region. The introduction of crosslinking agent disturbed the intra and inters molecular hydrogen bonds of chitosan network which in turn lower the crystallinity index of chitosan composites [22].

4. Conclusion

NCC/chitosan composites were successfully produced and chemically crosslinked with citric acid. The tensile strength was improved notably by the addition of citric acid as crosslinking agent. The incorporation of NCC has increased the crystallinity of the chitosan composites, however, the crosslinking process has reduced the crystallinity index due to the breaking of crystalline domains of chitosan polymer.

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