Thermoplastic sago starch nanocomposites wound dressing fortified with antibiotic-modified HNT

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Abstract. Starches were reported to promote wound healing. However, the hydrophilicity of starch help absorbs the exudates from the wounds during the healing process, but it also enables a bacterial infection that slows the healing process. Halloysite nanotubes (HNT) are attracting many biological technologies because of their high loading capacity and biocompatibility. This paper investigates the modified HNT as a carrier for antimicrobials agent in wound healing materials. Halloysite was modified by dispersing it with chloramphenicol solution using a magnetic stirring method. Thermoplastic sago Starch (TPSS)/modified HNT (MHNTs) biocomposite films of different compositions (0.25, 0.5, 0.75 and 1 wt. % HNT) were then developed using the solution casting method. SEM revealed that modified HNT shows good dispersion on the TPSS matrix. With the introduction of modified HNT, the FTIR peaks of TPSS have altered at the peak of 3693.21 cm⁻¹ and 1040.05 cm⁻¹. In addition, modified HNT reduced the water absorption rate of the TPSS films. Furthermore, modified HNT showed good resistance to bacterial culture and significantly reduced the biodegradability rate of TPSS compared to pristine HNT. From the findings, HNT can be a potential carrier for antibacterial agents to withstand bacterial attacks.

1. Introduction
Nowadays, there seems to be an increasing interest in biodegradable polymers as wound healing materials to reduce environmental pollution caused by synthetic dressing materials. In addition, the need to minimize the carbon footprint of synthetic polymers is driving new biomaterials developed from these renewable sources. Environmental issues and a lack of fossil resources have indeed prompted the growth of new biomaterials. Starch is a suitable choice for replacing toxic and non-biodegradable polymeric materials commonly used in the industry. In addition, the use of starch in non-food products is considered to be of innovative interest.

Starch has several benefits, including low cost, highly available, and absolute compostability with no harmful substances. This renewable resource is the primary ingredient for most biopolymers on the market. starch-based biopolymers are accounting for 85 % to 90 % of all biopolymers available.[1] Native starch, on the other hand, is brittle and has weak mechanical properties. [2, 3] Starch-based materials have several drawbacks, including low processability, long-term stability, and high water sensitivity. [1] As a result, sago starch must be thermoplasticized by adding plasticizers, including
glycerol, to improve plasticity and minimize brittleness. Several studies have used biopolymers in medical applications because of their biocompatibility and environmental sustainability [4]. Furthermore, researchers have proven polysaccharides starches to be effective wound dressing materials [5][6]. Wound dressing compounds polysaccharides, such as sago starch, help to facilitate and promote healing. However, starch hydrophilicity absorbs the exudates from the wounds during the healing process, which most likely enables a bacterial infection that delays the healing process. As a result, an antimicrobial agent must be used to address this problem [5].

Halloysite nanotubes (HNT) were commonly used in drug delivery and tissue engineering scaffolds in the medical industry. However, halloysite has no antibacterial effect, and several studies have shown that HNT must be modified to provide an antibacterial effect [7][8]. In addition, the hollow structure of the halloysite makes it an excellent carrier for an antimicrobial agent. The need for HNT to be modified is to prevent bacterial infection that thermoplastic sago starch (TPSS) promotes during the wound healing process. Therefore, HNT is proposed to be modified with an antibacterial agent for wound dressing films. Henceforth this paper investigates the effect of modified HNT on thermoplastic sago starch nanocomposites.

2. Materials and Methods

2.1. Materials

Four main materials were used in this experiment: sago starch, glycerol, distilled water, and halloysite nanotubes (HNTs). In this study, the Malaysian sago starch powder (25% amylose) with an average diameter of 53.66µm, length of 388.76µm, and density of 0.571g/cm³ was used. Glycerol (95% purity) with a density of 1.232g/cm³ was purchased from Merck Sdn. Bhd. Ultrafine grade HNTs were obtained from Imerys Tableware Asia Limited, New Zealand.

2.2. HNT modification

HNT was modified by dispersing 5 grams of HNT in 20 ml of distilled water and magnetically stirred in a hotplate stirrer for about 30 minutes at room temperature. Afterward, 2.0 ml of liquid antibiotics (chloramphenicol) were added to the HNT solution and mixed for 15 minutes. Finally, the solution of HNT was placed in an oven for 24 hours at 30 °C. After 24 hours of drying the HNT solution. Shear stress ball mill homogenization was used to reduce the HNT conglomerate to achieve good homogeneous dispersion of HNTs [9].

2.3. Film Preparation

The sago starch used in this experiment was previously oven-dried at 80°C for 8 hours and sieved using a mechanical shaker with a sieve size of 50µm. Based on the previous study, the optimum composition used for the preparation of the composite films was 6.5% sago starch, 3.5% glycerol, and 90% distilled water. Stock solution with the desired weight percentage of modified HNT according to Table 1. The solution contains modified HNT, sago starch, glycerol and distilled water while magnetically stirred at a gradually elevated temperature. The mixture was continuously stirred for 20min under the controlled temperature until it reached 75°C, where the starch mixture started to gelatinize around 70°C – 75°C. The mixture was then solvent casted on a smooth and flat surface and oven-dried for 20 hours at 40°C. The resultant composite film was peeled off the surface and stored in a sealed plastic bag for at least 40 hours at room temperature for further testing.
Table 1. Formulations for modified HNT/TPSS nanocomposites films

| HNT (wt%) | Sago Starch (g) | Glycerol (g) | Distilled Water (ml) | MHNT (g) |
|-----------|-----------------|--------------|----------------------|----------|
| TPSS      | 6.500           | 3.500        | 90.000               | 0        |
| TPSS/MHNT 0.25 | 6.484       | 3.491        | 89.775               | 0.25     |
| TPSS/MHNT 0.50 | 6.468       | 3.483        | 89.550               | 0.50     |
| TPSS/MHNT 0.75 | 6.452       | 3.471        | 89.326               | 0.75     |
| TPSS/MHNT 1.00 | 6.435       | 3.465        | 89.100               | 1.00     |

3. Materials Characterization and Evaluation

Morphological properties of wound dressing films were investigated using a JEOL JSM-IT100 scanning electron microscope (SEM) with a 10.0kV operating voltage. Thin-film specimens were sputter-coated with gold-palladium to avoid sample charging throughout imaging. Meanwhile, the Nicolet iS50 FTIR spectrometer was used to determine the chemical group functionalities of the thin films. The films were tested using a 20-scan mode with a resolution of 4 cm\(^{-1}\) and a wavelength range of 650-4000 cm\(^{-1}\).

Moreover, the water absorption of wound healing films was determined using the ASTM D570 method. Specimens were cut into 20 mm x 20 mm squares, dried for 24 hours at 50 °C, and afterward weighted. The specimens were then submerged in distilled water for 24 hours at a temperature of 23±1 °C. After 24 hours of immersion, specimens were removed and gently wiped clean of any water collected on the specimen surface, followed by a weight measurement to the nearest 0.001g. The percentage of water absorption, \(W_a\), was determined using equation (1). Where \(m_a\) represents the sample's weight after immersion and \(m_b\) represents the sample's weight prior to immersion.

\[
W_a = \frac{m_a - m_b}{m_b} \times 100
\]  

Furthermore, the soil burial method was conducted to check the biodegradability of the materials [10]. In this method planting plastic pots filled with black soil that have an approximately 18% moisture content. Test samples were cut into 20 mm x 20 mm squares, dried for 24 hours at 50 °C, and afterward weighted. Specimens were gently and cautiously brushed to clear the sticking soil after the burial period of 10 days. After that, the samples were dried in a 40°C oven for 30 minutes. The antimicrobial susceptibility of the prepared films was measured using the Kirby-Bauer method in an agar diffusion disk. Filter papers with a diameter of 1cm were dipped in different HNT/TPSS composite gel and then put on top of the agar disk surface, inoculated with E. coli bacteria. The disks were placed in an incubator at 37°C for 24 hours, after which the effects were observed. The region of the inhibition zone can be calculated using equation (2).

\[
Area \ of \ inhibition \ zone \ (mm^2) = Area \ of \ clear \ zone \ (mm^2) - Area \ of \ well \ (mm^2)
\]  

4. Results and discussion

4.1. Surface morphology

Figure 1 (a) shows the SEM morphology of TPSS, which reveals a smooth and clear surface resulting from the effective integration of amorphous sago starch granules with glycerol and water due to starch plasticization. The surface of the TPSS is smoothed with the addition of glycerol. On the other hand, the surface morphology of TPSS with the addition of 0.25, 0.5, 0.75, and 1 wt.% of MHNT is showed in Figure 1 (b)-(e). The introduction of small amounts of MHNT to the matrix results in proper dispersion. However, at higher concentrations, MHNT causes certain agglomeration in
the TPSS matrix, and only a few sago granules were often visible on the matrix surface. Higher HNT loading results in more aggregates in the matrix. This may be attributed to the modification of halloysite, which resulted in a weaker interfacial interaction with the polymer matrix. In addition, the modification of HNT with a strongly hydrophobic material (chloramphenicol) may explain the weaker interfacial interaction, which leads to weaker interfacial interaction between TPSS (hydrophilic) and modified HNT with hydrophobic substance [12].

![Figure 1. SEM micrograph of TPSS nanocomposite films at (a) 0 wt.% MHNT (b) 0.25 wt.% MHNT, (c) 0.5 wt.% MHNT, (d) 0.75 wt.% MHNT and (e) 1.0 wt.% MHNT](image)

4.2. Chemical characterization

The OH stretching vibration and deformation and Si-O groups and stretchings can be seen in the FTIR spectra of MHNT in Figure 2. The antibiotics chloramphenicol did not show any alteration on the TPSS/MHNT peaks. According to early studies, the absorption peaks of substances encapsulated within the HNTare normally not revealed [13]. Chloramphenicol molecules are encapsulated within the tubes of MHNT, according to the current findings. These results are also backed up by antimicrobial and biodegradability studies. The inner-surface hydroxyl group's O-H stretching, which mildly changed from 3693.86 cm\(^{-1}\) for MHNT to 3691.88, 3692.45, 3692.97, and 3693.12 MHNT/TPSS at 0.25, 0.5, 0.75, and 1 wt. %, respectively. This is attributed to reactions between the inner surface hydroxyl groups of MHNT and the C-O-C group of starch or glycerol. With the introduction of more MHNT, the Si-O bonding increases. The notable peak of the TPSS at 1042.50 cm\(^{-1}\) had moved towards the peak of MHNT at 1041.17 cm\(^{-1}\), as shown. For MHNT/TPSS at 0.25, 0.5, 0.75, and 1 wt. %, MHNT peaks generated at 1041.75 cm\(^{-1}\), 1041.72 cm\(^{-1}\), 1041.69 cm\(^{-1}\), and 1041.57 cm\(^{-1}\), respectively. The Si-O bond represents the typical MHNT-matrix interconnection through bond water and CH\(^2\) stretching, which decreases the biocomposite's water absorption rate [14]. Previous studies have shown that modified antimicrobial HNT has a similar impact on TPSS FTIR spectra [14][15].
4.3. Water absorption

The addition of MHNT reduces the water absorption rate from 72.96% to 60.00%, 54.17%, 54.55%, and 53.13% when adding 0.25, 0.5, 0.75, and 1 wt.% of HNT, respectively, as illustrated in Figure 3. When MHNT is mixed into the TPSS matrix, it reduces water absorption. The increase in crystallinity of the composites is responsible for this. HNT also creates a tortuous path that makes it difficult for water molecules to move through. Also, modified halloysite lumen was loaded with chloramphenicol, a highly hydrophobic antibiotic that makes the films containing MHNT have less capacity for water absorption [16].

The interaction between the nanocomposite films and the water molecules would be reduced as a result. The reduction is also due to the chloramphenicol antibiotics, a highly hydrophobic material being encapsulated in MHNT. Water absorption is essential for wound healing since it increases the films' ability to absorb wound exudates, which speeds up the healing process [15][16].
4.4. Biodegradation
Weight loss and crack formation were reduced with the addition of MHNT compared to composite films without MHNT. Films containing 0.25 and 0.5 wt. % MHNT developed cracks after 40 days, but they were less severe than the composites with only TPSS, as seen in Figure 4. The composites display no cracks or damage when the MHNT content is increased to 0.75 and 1 wt. %. The high amount of MHNT in the composite resulted in better antimicrobial properties and a lower degradability rate. The cracks will impact the weight loss and degradation because the surfaces would be exposed to the activity of microbes as a result of the cracks [17]. Higher levels of MHNT help slow down the rate of weight loss and degradation due to increased structural rigidity and the antibacterial property promoted by chloramphenicol.

![Figure 4. The degradation condition of the nanocomposites after certain burial days](image)

4.5. Antimicrobial inhibition test
The disc diffusion method in Figure 5 revealed that halloysite is not an antimicrobial agent in nature. However, it showed strong resistance to bacterial culture after being modified with an antimicrobial material. Halloysite was modified in this study by solution mixing, and when combined with TPSS, a successful inhibition area was observed, as seen in Figure 5. With further increasing the MHNT in TPSS, a wider inhibition area is obtained. This demonstrates modified halloysite's ability to serve as an antibacterial container for wound dressings.

![Figure 5. Anti-microbial disk diffusion test for TPSS/HNT & TPSS/MHNT composites.](image)
5. Conclusion
The MHNT/TPSS nanocomposite films were successfully produced via the solvent casting method, revealing that HNT was well dispersed in the TPSS matrix at lower loading. Furthermore, based on the FTIR spectra obtained, it can be confirmed that the addition of HNT into TPSS films led to the formation of hydrogen bonding and Si-O, Al-OH, and Al-Si-OH bonding in the film matrix. TPSS with 0.25 wt. % MHNT was found to be the best biocomposite in this study to be used as a wound-healing material. This candidate had a higher water absorption rate compared to the other biocomposites with higher MHNT loading. Water absorption is crucial in wound healing because it helps the exudates from the wounds during the healing process. In addition, the biocomposite's biodegradability was high at 0.25 wt. % MHNT, compared to other higher loadings; as a result, this candidate is less damaging to the environment. Finally, the candidate demonstrated adequate antimicrobial capacity, suggesting that it could be used for wound healing applications.

6. References
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