SYNTHESIS AND CHARACTERIZATION OF STARCH SILVER
NANOCOMPOSITE FILMS

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ABSTRACT
Starch has been widely used as a raw material in films production due to its low cost and biodegradable properties. In this project, silver nanoparticles will be added to starch solution to form Nano composites films with anti-microbial properties. Parameters such ratio of starch and silver nanoparticles used together with amount of cross-linkers were investigated and optimized to produce starch-silver Nanocomposite films with desirable physical and anti-microbial properties. FTIR, SEM, FEM and UV-Visible spectrophotometer were used to characterized morphology, particle size and changes in chemical composition of starch-silver nanocomposite films.
Keywords : Nanoparticles, anti-microbial, nano-composite films

ABSTRAK
Keywords: nanopartikel, anti-mikrob, filem nano-komposit
1.0 Introduction

Due to the increase number of waste generated from plastic in the world, researchers started to find alternative ways of replacing plastics. In recent years, degradable films have attracted widespread interest due to increasing environmental pollutants. Degradation of conventional plastic requires years to fully biodegrade, which leads to secondary pollutants and costs. Thus, an environmentally friendly biodegradable film has been developed.

Among many natural biopolymers to prepare degradable films, starch is one of the most commonly used as raw materials due to its abundance, low cost, renewability, and biodegradability (Avenous et al., 2001). However, the application of starch films was limited because of its own weakness such as water solubility and brittleness (Mathew et al., 2006). In order to overcome the shortcomings and get functional properties, starch is often combined with other natural biopolymer to form composite films.

Starch is a polymer which occurs widely in plants. Starch consists of replicating units of glucose. Starch contains 30% of amylase and 70% amylopectin and less than 1% lipids and proteins from plants is a fine additive for forming membrane owing to its insoluble properties (Fujun et al., 2009). In its application in biodegradable plastic, starch is physically mixed in with its active granules, kept intact, or melted and blended on a molecular level with the appropriate polymer.

The fraction of starch in mixture which is accessible to enzymes can be degraded by either, or both amylases and glycosidase. Term sago is used to describe starch from the stem of palms, cycads and from maniac tubers. Sago palm contains a large amount of starch in its trunk and
its productivity was calculated to be four times that of paddy rice (Ishizuka et al., 1995). Sago starch produced from pith of sago palm, is a useful resource for commercial raw materials and food stuff also important product in South Asia (Yatsugi et al., 1986).

According to Takashi (1998), the properties of sago starch can be easily gelatinized, as it gelatinization occurs at low temperature, it easily to mould and gel syneresis is also low and highly viscous. Previously, due to the good film-forming property, excellent biocompatibility and certain antimicrobial activity (Park et al., 2004; Xiao et al., 2010) chitosan has often been chosen as raw material to prepare the composite films.

Recently, silver nanoparticles exhibiting antimicrobial activity have been synthesised (Zhu et al., 2006). Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization on dental materials, stainless steel materials, and human skins (Maillard et al., 2002; Mock et al., 2002). Due to its high reactivity as large surface to volume ratio, nanoparticles play crucial role inhibiting bacterial growth in aqueous and solid media. Silver-nanoparticles containing materials can be employed to eliminate microorganisms on textile (Zhu J.J et al., 2001) or use for water treatment (Mock J.J et al., 2002).

In this study, starch-silver nanocomposite films were prepared by incorporation of silver nanoparticles into starch films. The physical and antimicrobial properties of the starch-silver nanocomposite were tailored by varying the composition of the films.
1.1 Objectives

The objectives of this research project are:

1. To synthesize starch-silver nanocomposite films

2. To characterize the physical and antimicrobial properties of the starch-silver nanocomposite films.
2.0 Literature Review

2.1 Starch as raw material

In its application in biodegradable plastics, starch is physically mixed in with native granules, kept intact, or melted and blended on a molecular level with the appropriate polymer. Starch molecules have two important functional groups, the -OH groups that susceptible to substitution reactions and C-O-C bond that is susceptible to chain breakage. The hydroxyl group of glucose has a nucleophile character. By reaction of its -OH group, modification of various properties can be obtained. Cross-linking or bridging of the -OH groups changes the structure into a network while increasing the viscosity, reducing water retention and increasing its resistance to thermo mechanical shear. Starch films possess low permeability and thus attractive materials for food packaging.

However, due to it’s the strong intermolecular and intramolecular hydrogen bond in starch, native starch is not a true thermoplastics. In presence of plasticizer, for example, water and glycerol, at high temperature. Among the plasticizer, water is most commonly used in thermal processing of starch based polymer. Bio-plastic starch containing only water alone, possess poor mechanical properties such as brittleness due to the fast retro degradation (recrystallization). Thus, other non-volatile plasticizer is investigated to improve the processing ability and product properties of bio-plastic starch such as glycerol, glycol, sugar, urea and citric acid.

Besides, the chemical used for cross-linking starch a relatively toxic, expensive or do not provide the desired improvement in properties. Blending synthetic polymers with starch at high temperature could damage starch and lead to poor properties of the material developed.
2.2 Citric acid cross-linking starch films

Poly(carboxylic acid), such as citric acid, is inexpensive and non-toxic chemicals that had been used to improve the performance properties of cellulose and proteins in textile applications (Yang C. et al., 1991; Yang C. et al., 1997; Yang Y. et al., 1996). Cotton and silk fabrics have been cross-linked with carboxylic acid, mainly improve their appearance and resistance to wrinkling, and also shown that the mechanical properties and water stability of regenerated protein fibers can be improved by cross-linking with poly(carboxylic acid) (Yang Y. et al., 1996). Carboxylic acid cross-link the hydroxyl group of cellulose and the cross-linking reaction is reported occurring at temperature between 165°C to 175°C (Yang Y. et al., 1996; Yang C. et al., 1997).

Scheme 1: Mechanism of conventional dry cross-linking of cellulose using carboxylic acid with presence of catalyst, R represents cellulose (Yang and Wang 1996 et al., 1997)

Cross-linking poly(carboxylic acid) of cellulose occurs mainly through the hydroxyl group. Since starch also contains considerable amount of hydroxyl group and starch is more easy accessible to chemical than cellulose (refer Sheme1), poly(carboxylic acid) can be expected to cross-linking starch and improve its properties (Narendra et al., 2010). Citric acid has not been
used to cross-link starch widely, it has been reported that citric acid can form strong hydrogen bond interaction with starch improves its thermal and water stability and inhibit retrodegradation (Yu J. et al., 2005).

Incorporating citric acid substantially reduce the tensile stress of the products developed under the condition (Yu J. et al., 2005). Citric acid was also used as additive to starch-PVA films, due to the improvement in tensile strength and concentration above 5% reduce the tensile strength of films. At high concentrations, there is excess cross-linking that limits the mobility of starch molecules, leading to lower tensile strength (Yang Y. et al., 1996; Yang C. et al., 1997).

2.3 Silver nanoparticles

Silver nanoparticles are of interest due to its unique properties, such its size and shape which can be incorporated into antimicrobial applications. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles (Senapati et al., 2005; Klaus-Joerger et al., 2001). The most popular chemical approaches, including chemical reduction using variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of nanoparticles.

2.3.1 Synthesis of silver nanoparticles

The most common approach for synthesis of silver nanoparticles is by chemical reduction using organic or inorganic reducing agents. Generally, different reducing agents such, sodium citrate, sodium borohydride NaBH₄, polyol process and Tollen reagent are used for reduction of silver ions (Ag⁺) and lead to formation of metallic silver (Ag⁰) which followed by agglomeration into oligomeric cluster. These cluster can eventually formed a metallic colloidal
silver particles (Wiley et al., 2005; Evanoff and Chumanov 2004; Merga et al., 2007). Thus, it is important to use protective agents to stabilize dispersive nanoparticles during the course of metal nanoparticles preparation, and protect the nanoparticles that can be absorbed on or bind onto nanoparticles surfaces, avoiding their agglomeration (Oliveira et al., 2005).

Silver nanoparticles were prepared using water as an environmentally-friendly solvent and polysaccharides as capping/reducing agents. The synthesis of satrch-silver nanoparticles was carried out with starch (as a capping agent) and ß-D-glucose (as reducing agent) in a gently heated system (Raveendran et al., 2003). The binding interactions between starch and produced silver nanoparticles were weak and could be reversible at higher temperatures, allowing for the separations of the synthesized nanoparticles. Besides, silver nanoparticles were synthesized by the reduction of silver ions inside nanoscopic starch templates (Raveendran et al., 2003, 2005). Extensive network of hydrogen bands in templates provided surface passivation or protection against nanoparticles aggregation.

Smaller silver nanoparticles were synthesized by mixing two solutions of silver nitrate containing starch (as a capping agent), and NaOH solutions containing glucose (as reducing agent) in spinning disk reactor with a reaction less than 10 mins (Tai et al. 2008). Silver nanoparticles with controllable size were synthesized by reduction of [Ag(NH$_3$)$_2$]$^+$ with glucose, galactose, maltose and lactose (Panacek et al., 2006). In addition, conventional heating approach (simultaneously polymerization-reduction method) to polymerize acrylonitriles reduces silver ions resulting in homogeneous dispersal and narrow size distribution of the silver nanoparticles in silver-polyacrylonitrile composite powders (Zhang et al., 2003).
2.3.1.2 Applications of silver nanoparticles

Nanoparticles exhibit size and shape-dependent properties which are of interest for applications ranging from bio-sensing and catalyst to optics, antimicrobial activity and wireless electronic logic. These particles also have many applications in different fields such as medical imaging, nano-composite, filters, drug delivery and hyperthermia of tumors (Tan et al., 2006; Lee et al., 2008; Pissuwan et al., 2006).

Silver nanoparticles have been used extensively as antimicrobial agents in health industry, food storage, textile coatings and a number of environmental applications. It is shown that silver nanoparticles mainly in range of 1-10nm attached to the surface of *Escherichia coli* (*E. coli*) cell membrane, and disturbed its proper function such as respiration and permeability (Morones et al., 2005). Introduction of nano-sized silver into poly(vinyl alcohol) provides antibacterial activity, which highly desired in textiles used in medicine, wound dressing applications, clothing and household products (Hong et al., 2006).

These silver/TiO$_2$ composite films were prepared by incorporating silver in the pores of titanium dioxide films with an impregnation method via photoreduction (a versatile and convenient process with advantage of space-selective fabrication). It was reported that these composite films are promising in application of antimicrobial and self-clean technologies (Liu et al., 2008).
3.0 Materials and Methods

3.1 Materials

Sago starch (OPAC brand) was used in this study. Citric acid monohydrate (CA), sodium hydroxide (NaOH), silver nitrate (AgNO₃), β-D-glucose. Ultra-pure water was used throughout the experiment (Water Purifying System, Ultra Genetic, and Model : ELGA,UK ).

3.2 Methods

3.2.1 Preparation of Citric Acid Cross-linked Starch

The reactions were carried out between the esterification reaction of sago starch and citric acid monohydrate. Sago starch was added into the water to form a gelatinized starch solution, and stirred at temperature of 75°C, NaOH solution was added as a catalyst. The mixture was heated for 15 minutes followed with addition of citric acid. The heating was continued for 40 minutes.
3.2.2 Synthesis of Starch citrate with Silver Nanoparticles.

Starch citrate with silver nanoparticles can be obtained by addition of AgNO₃ and glucose to starch citrate solution and stirred for 15 minutes at temperature of 55-75°C. Then addition of NaOH drop by drop with vigorous stirring till colorless solution turns to reddish brown.
3.2.3 Samples Characterizations

Fourier Transform Infrared Spectroscopy (FT-IR) was used to examine functional group of modified starch. Starch-silver nanocomposite films were sent for Scanning Electron Microscope (SEM) to observe its surface morphology and morphology of nanoparticles. UV-visible Spectrophotometer was used to confirm the formation of silver nanoparticles.

3.2.3.1 FT-IR

Prior to the characterization, the modified starch film was washed with ultra-pure water to remove unbound citric acid monohydrate and a film was dried. The thin films was cut into smaller pieces and grind with dried KBr pellet by ratio 1:100. Then, the pellet was scanned.
through FT-IR at the wave number in the range between 400-4000 cm$^{-1}$.

3.2.3.2 SEM

A starch-silver sample solution was dropped on stainless steel plate and dried in oven. The samples were coated with plantinum to avoid charges during characterization. To observe the size distribution of nanoparticles surface morphology were characterized with SEM.

3.2.3.3 UV-Visible Spectrophotometer

The starch-silver solution was isolated and placed in a small glass bottle and wrapped with aluminum foiled. Ultra pure water in a blank cuvette was run as a baseline, and followed with ultra pure water with a drop of starch-silver and run as sample. Samples were scanned at range of 200-700 nm.

3.2.3.4 Swelling degree

The swelling degree of the samples was characterized at 37$^\circ$C, for which samples with 1 cm diameter were placed in deionized water. The experiments were carried out by measuring the weight gain as a function of immersion time in 20 ml water. The swelling degree was calculated by using the following equation,

\[
\text{Swelling degree} = \frac{W_t - W_o}{W_o} \times 100
\]

Where, $W_t$ is the wet weight of the samples, $W_o$ is the original dry weight of the sample.
3.2.3.5 Zone of inhibition

Zone inhibition test was performed in Lysogeny broth (LB) agar plates with starch citrate AgNPs films. 25ml of LB agar was poured in autoclaved petri plates, and 1.0ml of active bacterial culture was homogeneously spread in agar plates and starch films were placed in the agar. Method adapted from standard protocol based on National Committee for Clinical Laboratory Standards (NCCLS) recommendation. The plates were incubated at 37°C for 24 hours. The zone size was observed.
4.0 RESULTS AND DISCUSSION

4.1.0 Preparation of Starch Citrate

There is an optimum amount citric acid that is necessary to obtain good increase in the tensile strength of the films. Cross-linking interconnects the starch molecules in the film, which could increase the molecular weight of the starch and also provides better inter-molecular interaction between molecules, leading to better tensile strength, compared to the non-cross-linked starch films.

<table>
<thead>
<tr>
<th>Curing time (mins)</th>
<th>Amount of citric acid (wt./v)%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>20</td>
<td>Transparent</td>
</tr>
<tr>
<td></td>
<td>Quite Brittle</td>
</tr>
<tr>
<td></td>
<td>Weak Strength</td>
</tr>
<tr>
<td>30</td>
<td>Transparent</td>
</tr>
<tr>
<td></td>
<td>Flexible</td>
</tr>
<tr>
<td>Sample (starch to citric acid)</td>
<td>Synthesis Parameters</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>Native sago Starch (g)</td>
</tr>
<tr>
<td>SCA 1 (4:3)</td>
<td>2.0236</td>
</tr>
<tr>
<td>SCA 2 (1:1)</td>
<td>2.0062</td>
</tr>
<tr>
<td>SCA 3 (4:1)</td>
<td>2.0066</td>
</tr>
<tr>
<td>SCA 4 (2:1)</td>
<td>2.0210</td>
</tr>
</tbody>
</table>

Table 1: Effects of curing time and amount of citric acid to properties of modified films.

Table 2: Synthesis parameters used in preparation and citric acid modified starch.

At low amount of citric acid, there is not enough cross-linking between the starch molecules to
improve the tensile strength of the films. At high amount of citric acid, there is excess cross-linking that limits the mobility of starch molecules, leading to lower tensile strength (Yang et al., 1996).

Further increase in reaction time substantially decreased the strength of the films. Reaction time is one of the most important factor determining the efficiency of the carboxylic acid cross-linking and hence the properties of the cross-linked materials. Suitable reaction time is necessary for the cross-linking reaction to occur but excess reaction time will damage the starch molecules, leading to decrease in tensile strength and unwanted changes in the color of the films.

An optimum reaction time of about 5 minutes at about 167-170°C was reported for citric acid cross-linking of cotton fabrics. In this research, temperature was adjusted to 55°C and thus, the reaction time was increased to 40 minutes. Longer reaction times were maybe suitable, if lower temperatures were used, but shorter reaction time for large-scale processing of films due to economic and technical reasons.

Figure 3: FTIR result for native sago starch
The FTIR spectrum of native sago starch (Figure 4) showed broad absorption peak at 3448 cm\(^{-1}\) indicating presence of intermolecular hydrogen bonding hydroxyl group having polymeric association. Peak expressed on 2929 cm\(^{-1}\) refers to C-H stretching and 1423 cm\(^{-1}\) indicates C-H bending as presence of hydrocarbon chromospheres in native sago starch. Comparing with cross-linked sago starch with native sago starch, both films have similar peaks, except additional peak in the cross-linked films at 1736 - 1741 cm\(^{-1}\). The band is ascribed to carboxyl and ester carbonyl bands (Yang et al., 1991).

The presence of carbonyl group shows the reaction condition applied is feasible for esterification of citric acid with sago starch. Since the films were thoroughly washed to remove unreacted citric acid, the presence of carbonyl peak confirmed the chemical linkages
between citric acid monohydrate with starch. Based on the result obtained, ratio of 4:3 provides good properties of starch modified films and maintained in the next proceedings.

4.1.1 Effect on morphology of starch citrate films by varying amount of citric acid used

Figure 5: SEM images on surface of (a) Native starch films, (b) SCA4, (c) SCA3, (d) SCA2, (e) SCA1
Scanning Electron microscope images of the control and cross-linked films does not show any appreciable change in surface morphology due to the cross-linking as seen Figure 5. These films are homogenous and have no cracks, it shows well dispersed starch molecules (Garg & Jana et al., 2007) but the surface are less smoother than native starch films.

### 4.2.0 Preparation Starch citrate with Silver nanoparticles

The formation of silver nanoparticles in this experiment is where silver nanoparticles were prepared using water as an environmentally-friendly solvent and starch as capping/reducing agents. In this experiment starch acts as capping agent and β-D-glucose acts as the reducing agents. This method were reported by (Raveendran et al., 2003) which it said that the synthesis of starch-silver nanoparticles was carried out with starch (as a capping agent) and β-D-glucose (as reducing agent) in a gently heated system.

As the silver nanoparticles possess antimicrobial properties, thus, the effectiveness of the antibacterial activities in different types of starch films was tested. Different types of starch films was prepared which are, native starch with silver nanoparticles (SS), starch citrate doped with silver nanoparticles (SCS) and starch citrate with AgNO₃ (SCA). In order to ensure the formation of silver nanoparticles, the starch solution with nanoparticles were tested using UV-visible spectrophotometer.
4.2.1 Different Preparation Method Of Ag-NPs in starch citrate film

Figure 6: Different method on preparation of Ag-NPs in sago starch solution.

The different between two method above are, in method A, the sago starch was being modified before synthesize of Ag-NPs take place, compared with method B the pure sago starch solution proceed with synthesize of Ag-NPs then only cross-linked process take placed. Method A, the sago starch was cross-link with citric acid before AgNPs was synthesis. The hydroxyl group in the starch helps in reduction of silver ions. Due to the modification, the hydroxyl group is being converted to ester carbonyl. Thus, there is less availability of free hydroxyl group to reduce the Ag$^+$ ions, addition of glucose aids the process of reduction.
Figure 7: FT-IR result on (a) SS film (b) SSC film (c) SCS film

Based on the result in Figure 7, method B shows that, the crosslinking with citric acid are not preferable after addition of Ag-NPs, as peak on 1736 - 1741 cm$^{-1}$ were not observed, this is more probably because the hydroxyl group presence in sago starch reduce the AgNO$_3$ and thus the availability of free hydroxyl group to be cross link is minimized. From this research, it shows that, synthesis of Ag-NPs starch citrate solution is more preferable.
Figure 8: Comparison on native starch films and SCS films.