Development of Biopolymer film with different ratios of Gelatine to Chitosan reinforced with Zinc Oxide Nanoparticles for food covering/preservation

DY Yi, BM Siddique*, JC Lai

School of Chemical Engineering and Science, Faculty of Engineering, Computing and Science, Swinburne University of Technology Sarawak Campus, Kuching, Malaysia.

E-mail: msiddique@swinburne.edu.my

Abstract

In this research project, gelatine and chitosan have been chosen to form a composite film and the zinc oxide nanoparticles have been used to increase the properties of the composite film. In the experiment, the ratio of gelatine to chitosan as well as the amount of zinc oxide nanoparticles have been manipulated. These composite film with the gelatine (G) to chitosan (Ch) ratio of 40G:60Ch, 50G:50Ch, 60G:40Ch have incorporated with 0 mg, 1 mg and 10 mg of ZnO NP in each film. A layer of smooth film with 30 μm thickness were produced. From the experiment, the higher the ratio of chitosan in the composite have resulted a stronger adhesive properties of the film. Due to the high adhesive properties, ratio of 50G:50Ch and 60G:40Ch composite films were not able to be peeled off from the dish. Hence, only ratio of 40G:60Ch composite film with different amount of zinc oxide nanoparticles were evaluated. The transparency of the film decreased while increasing the amount of zinc oxide nanoparticles. Despite that, 10 mg zinc oxide loaded film has 10 % decrease in its water solubility as compared to the film that without loaded with nanoparticles. Moreover, the film loaded with 10 mg zinc oxide have a better ability to withstand in an acidic condition. When contact with 1 % acetic acid solution, the film could maintain its original shape but for the film loaded with 0 mg and 1 mg nanoparticles have rolled up when contacted with the acid solution. All the test samples were dissolved completely in acid solution after 24 hours.

1. Introduction

The properties of the films are a key requirement for an organic product to extend their shelf life after harvested. In contrast with petrochemical-based film, a biopolymer-based film has provided advantages not only for the environment but also to consumers. Biopolymer film possesses the biodegradable properties thus self-decompose after certain period of time. The biodegrading properties of the film have greatly reduced the environmental waste. Hence, the organic that wrapped with biopolymer film has contributed to a higher quality of the product.

In the past decades, edible films and coating such as wax were introduced and used for various fruits to prevent loss of moisture while create a glossy surface for decorative purposes. These practices are still carried out in the present day even though their researchers and associated chemistries were understood and accepted long before. According to Pavlath & Orts [1 p.1], the edible film had been only related to food applications in the past 50 years. The terms, film and coating had been used interchangeably to show the surface of a food is covered by a thin layer of material of certain
composition. The only difference between film and coating are the notion that film is a stand-alone wrapping material, whereas a coating is applied and form directly contact on the surface of the food. In the early years on 1967, the idea of edible films are not popular and have been limited mostly to the wax layer that used on the fruits [1 p.1].

In the years of 1986, the products that wrapped or coated with edible films have offered only by nearly 10 companies. During intervening years, it shows significant changes in business who applied the concept of edible films, whereas it resulted in more than 600 companies offering such products in 1996 [1 p.1]. Nowadays, edible film uses have expanded widely in order to retain the quality of various horticultural product. Edible film wrapping technique has been developed rapidly because it extended the period of fresh horticultural products after harvest and also extend the useful marketing distances. Edible films have greatly reduced the economic waste in advanced and developed countries.

Nanotechnology is a popular research and has gained momentum in enhances 21st century. Due to their distinctive properties, nanoparticles have been widely used in various field. Among these metal nanoparticles, zinc oxide nanoparticles are most important as they have been used as biomedical, cosmetic, agriculture, and etc. The unique properties of zinc oxide nanoparticles against bacteria have attracted the attention of researchers toward food preservation [2 p.4-5]. The relatively low cost of zinc oxide as compared to silver or gold nanoparticles also have promoted the usage of zinc oxide nanoparticles. Besides that, zinc oxide nanoparticles have less harmful to human health as they have been used as an additive in food products such as breakfast cereals.

The constraints of natural polymers food packages can be recovered by applying composite polymer, which combined two or more biopolymers to form a single food package or implanting nanoparticles that used to strengthen the packages without causing any harm toward humans. The main aim of this research project was to study the properties and the effectiveness of the films which was loaded with zinc oxide nanoparticles and with various ratios of gelatine to chitosan content.

2. Methodology
2.1 Materials
A medium molecular weight of Chitosan with 75-85 % deacetylated, 30 g of gelatine powder (Type-A) from cold water fish, 20 mg of zinc oxide nanoparticles with average particle diameter of 30 nm, 15 g of glycerol and 1 % acetic acid were purchased.

2.2 Films Preparation
2.2.1 Preparation of Gelatine Solution
The gelatine solution was prepared by dissolving 0.5 g, 0.75 g and 1 g of gelatine powder in different beaker. Each of the powder in different beaker was dissolved by 50 ml of distilled water with a percent concentration of 1 % (w/v), 1.5 % (w/v) and 2 % (w/v) respectively. The mixture was stirred continuously in room temperature for 30 min.

2.2.2 Preparation of Chitosan Solution
The chitosan solution was prepared by dissolving 0.75 g of chitosan powder with 5 ml of glacial acetic acid. The solution filled up with distilled water to obtain a total volume of 50 ml mixture. The mixture was stirred overnight at room temperature and with a percent concentration of 1.5 % (w/v). The impurities will be filtered by using gravity filtration method.

2.2.3 Preparation of Gelatine-Chitosan Film Loaded with Zinc Oxide Nanoparticles
The film formation are based on Hosseini et al. [3 p.1491] but with slightly modification. The gelatine and chitosan film forming solution was prepared by adding 1 % (w/v), 1.5 % (w/v) and 2 % (w/v) gelatine solution with 1.5 % (w/v) chitosan solution in a proportions of 1:1 (v/v) to obtain different
proportions respectively (solution 40G:60Ch, 50G:50Ch, 60G:40Ch). All the mixture was stirred for 15 minutes to obtain a good blend. Then, glycerol (0.3 g/g gelation plus chitosan) was added to each mixture as a plasticiser and the solution was again stirred for 15 min. After that, 10 ml of each mixture was distributed into three beakers for the addition of 0 mg, 1 mg, 10 mg zinc oxide nanoparticles. All the solution were stirred for 5 min and homogenised for 10 min with 500 rpm.

A 3ml of each aliquots film forming solution was pipetted and pour onto the petri dishes. The solution was separated evenly until the solution has covered the surface of the dish. The dishes that filled with the solution were dried in an incubator at 35 °C for 72 hr. The dried films were manually peeled off and stored in the incubator at 35 °C for further analysis.

2.3 Film Morphology
The optical microscope (Nikon Eclipse 1500) equipped with capture system was used to observe the surface of the film. A magnification of 20x, 50x and 100x were used on microscope. The surface of the film has been captured by the installed image capture system.

2.3.1 Thickness
The optical microscope (Nikon Eclipse 1500) equipped with capture system was used to measure the thickness. A magnification of 5x will be used on microscope and the image will be capture by using the capture system.

2.3.2 Light Transmission and Transparency
The light transmission and transparency was determined according to the method reported by Hosseinib et al. [3 p.1491-1492]. By using UV-Vis Spectrometer (Lambda-35), the wavelength between 200 nm and 800 nm (200, 300, 400, 500, 600, 700, 800) have been selected to measure the barrier properties of films against ultraviolet (UV) and visible light. Each sample was cut into a portion of 7 mm × 40 mm and placed into the cuvette. An empty cuvette has been placed into the spectrophotometer which used as the reference. The transparency of the film can be calculated by using the following equation. According to the equation, high transparency indicates opaque.

\[
\text{Transparency} = \frac{A_{600}}{mm} = - \frac{\log T}{x}
\]

\[T = \text{Fractional Transmittance} ; x = \text{Thickness of the film (mm)}\]

2.3.3 Moisture Absorption
The sample films with 20 × 20 mm² were first stored in 0 % relative humidity (RH) (calcium sulphate) for 24 hr. The sample films were conditioned in a desiccator containing calcium nitrate saturated solution at 20-25 °C to ensure a relative humidity of 55 % after weighed the films. At desired intervals of time, the sample films were weighed until the equilibrium state was reached. The moisture absorption of the films was calculated as follows:

\[\text{Moisture Absorption (\%)} = \left[ \frac{W_t - W_0}{W_0} \right] \times 100\]

\[W_t = \text{Weights of the sample after } t \text{ time at 55\% RH} ; W_0 = \text{Initial weight of the sample}\]

2.4 Water Solubility
The solubility of the film in distilled water was determined according to the method reported by Benettaïeb et al. [4 p.2411] with slightly modification. Each of the film was dried in the incubator at 60 °C for 2 hr. After drying, each of the film has been cut into 3 mg in pieces. The dried film was immersed in 5 ml of distilled water under room temperature. After 24 hr immersion, film pieces were taken out from the distilled water and re-dried the film in the incubator at 60 °C for 2 hr to measure the
weight of dry matter, which was not solubilized in water. The final dried weight of the film was weighed and recorded. The water solubility of the film (% \( FS_w \)) was calculated using the following equation:

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

\( FS_w = \) Film solubility in water (%)
\( W_i = \) Initial dry weight (mg); \( W_f = \) Final dry weight (mg)

2.5 Acid Solubility
The solubility of the film in acetic acid was determined according to the method reported by Cardoso et al. [5 p.88] with modification. Each of the film was dried in the incubator at 60 °C for 2 hr. After drying, each of the film has been cut into 3 mg in piece. The dried film was immersed in 5 ml of 1 % of acetic acid under room temperature. After 24 hr immersion, film pieces were taken out and re-dried the film in the incubator at 60 °C for 2 hr. The final dried weight of the film was weighed and recorded. The acid solubility of the film (% \( FS_A \)) was calculated using the following equation:

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

\( FS_A = \) Film solubility in acid (%)
\( W_i = \) Initial dry weight (mg); \( W_f = \) Final dry weight (mg)

3. Results & Discussion
From the experiment, 40G:60Ch, 50G:500Ch and 60G:40Ch composite films with each addition of 0 mg, 1 mg and 10 mg of zinc oxide nanoparticles have been produced. But only 40G:60Ch composite film loaded with 0 mg, 1 mg and 10 mg have been characterized. The 50G:50Ch and 60G:40Ch composite films unable to be peeled off from the petri dish due to the high adhesive properties which laminated on the petri dish. In can be observed that, increases the ratio of gelatine in the composite film have increase the adhesive properties of the film.

3.1 Physical Appearance & Thickness
The texture of the film is an important requirement for them to be used in product packages. From the experiment, the samples film which prepared was transparent and flexible. Figure 1 shows the appearance of the film and its clear from the image that it was transparent. The surface of the films was smooth especially the side that exposed to the air while drying on the petri dish. The other side of the sample contacted with the glass petri dish remained shining but it possesses an adhesive property which allow the films to stick on the surface of the food product. Apart from that, the acidic smell of the film was very slight or non-discernible. The appearance and texture of the films were in accordance with the description reported by Sukkunta [6 p.43] for the film which made by gelatine and chitosan. The levels of ZnO NPs added has no significantly effect on the appearances of the film.

Figure. 1 Physical appearance of the film
Figure. 2 Thickness of 40G:60Ch film reinforced with 10 mg
Despite that, the 40G:60Ch composite film with addition of different amount of zinc oxide nanoparticles had been determined to have an average thickness of 30 μm which measured by an optical microscope. The image for the thickness of the films have been captured from the microscope and illustrated in Figure 2. The rest of the films thickness was determined through same method. However, the addition of zinc oxide nanoparticles was found to have insignificant effect on the thickness of the film.

3.2 Film Morphology
The morphology of the film was observed under an optical microscope with magnification of 100×. The captured images for each of the film have been illustrated in Figure 3 and 4. There was no white spot being observed from the microscope. For the film with loaded with 1 mg and 10 mg of zinc oxide nanoparticles, white spots being observed and a relatively higher amount for the film loaded with 10 mg of nanoparticles. The zinc oxide nanoparticles which observed from the microscope was, most probably, caused by an unevenly distribution of the nanoparticles on the film and part of the nanoparticle have clumped together during the mixing of film forming solution with zinc oxide nanoparticles.

3.2.1 Light Transmission and Transparency
In this study, the light transmission and transparency of each film incorporated with ZnO NPs at different levels were conducted by using range of 200-800 nm wavelength. The result of light transmission with different wavelength were shown in Table 1. From the result obtained, the control film (without ZnO NPs) has a good barrier property in the UV-range of 200-300 nm. Regardless of zinc oxide nanoparticles, gelatine as protein based are generally reported to have an excellent barrier properties which contributed by the presence of high content of aromatic amino acids that absorb the UV light [3 p.1493]. When the visible wavelength ranges at 400-700 nm, the transmission for all the films were greater than 80 % and only control film (without ZnO NPs) have greater than 90 % of transmission in 800 nm wavelength.
Table 1: Light transmission and transparency of films obtained with different amount of ZnO NPs

| Film                     | Light transmittance (%) at different wavelength (nm) | Transparency (A_600/mm) |
|--------------------------|------------------------------------------------------|-------------------------|
| 40G:60Ch with 0mg ZnO NPs | 0.10 76.17 87.53 89.69 89.25 89.94 90.09             | 1.65                    |
| 40G:60Ch with 1mg ZnO NPs | 0.09 70.17 84.45 86.97 87.27 87.89 88.49             | 1.97                    |
| 40G:60Ch with 10mg ZnO NPs | 0.10 68.04 82.55 84.79 85.82 86.63 87.45             | 2.21                    |

From the graph that depicted in Figure 5 shows that the higher the levels of zinc oxide nanoparticles added into the 40G:60Ch composite film has lower the percent of light transmission through the film. The results indicated that zinc oxide nanoparticles have the ability to impede the light transmission through the film. According to Arfat et al. [7 p.106-107], the phenomena was mainly due to the obstacle of the light passage by the presence of the nanoparticles dispersed in the film matrix.

Despite that, the transparency of the film at wavelength of 600 nm were computed. The results show that the control film has higher transparency values than the film loaded with 10 mg zinc oxide nanoparticles indicating the control film are more transparent. The transparency values increase as the level of zinc oxide nanoparticles content increased indicating the decrease in the transparency of the resulting films. These results were in agreement with Arfat et al. [7 p.107] who reported that films based on fish skin gelatine added with zinc oxide nanoparticles was less transparent.

![% Transmission vs Wavelength](image)

Figure 5: Percentage transmission against different wavelength

3.3 Solubility in Acid
The acid solubility is essential for the film because it allowed the film to withstand in an acidic medium while environment pH decreases. From the experiment, the acid solubility was found to be 100 %, all the dissolved completely after 24 hr immersion in 1 % acetic acid solution. Since the composite film was majorly made up by chitosan, the amine group of chitosan are protonated to form NH₃⁺ when chitosan contact with the acid solution, thus chelating and polyelectrolyte properties of chitosan were mainly governed by the acidity of NH₃⁺ [6 p.27].
Besides that, it was observed that the composite film loaded with 0 mg and 1 mg of zinc oxide nanoparticles have rolled up and became curled instantly when contact with 1% acetic acid solution. The composite film loaded with 1 mg of ZnO NPs was inadequate to hold and maintain the film in shape in an acidic medium. Only the composite film loaded with 10 mg of ZnO NPs have maintain in its original shape when contacted with the acidic solution. According to Baeza et al. [8], the nanoparticles ‘entangled’ in the film matrix acts as a reinforcement to the film that allow the film to reduce its segmental mobility and able to maintain its shape in an acidic medium. Hence, the addition of ZnO NPs has provided an advantage for the film to sustain in an acidic environment.

4. Conclusion
The properties of composite biopolymer (40G:60Ch) film can be improved by addition of zinc oxide nanoparticles. The film had increased the water resistance after incorporated with ZnO NPs. From the results, the higher the levels of ZnO NPs present in the film had significantly decreased their water solubility. Furthermore, the presence of nanoparticles played an important role when contact with acidic solution. It allowed the film to maintain in their original shape.

All the films were observed to have a smooth, flexible and shining surface. The transparency of the film which determined on wavelength 600 nm have showed that the film without zinc oxide nanoparticles have the most transparent property as compared to 1 mg and 10 mg nanoparticles incorporated film. Besides that, each of the film possesses a high UV absorption regardless of the presence of ZnO NPs.

Acknowledgement
This research was conducted as per the final year research project for Chemical Engineering discipline at the Swinburne University of Technology Sarawak Campus, with the help of the FYRP 1, 2016 research grant from the Swinburne University.

References
[1] Pavlath, AE & Orts, W 2009, 'Edible Films and Coatings: Why, What, and How?', in KC Huber and ME Embuscado (eds), Edible Films and Coatings for Food Applications, Springer New York, New York, NY, pp. 1-23.
[2] Sabir, S, Arshad, M & Chaudhari, SK 2014, 'Zinc Oxide Nanoparticles for Revolutionizing Agriculture: Synthesis and Applications', The Scientific World Journal, vol. 2014, p. 1-8.
[3] Hosseini, SF, Rezaei, M, Zandi, M & Ghavi, FF 2013, 'Preparation and functional properties of fish gelatin–chitosan blend edible films', Food Chemistry, vol. 136, no. 3-4, pp. 1490-1495.
[4] Benbettaïeb, N, Kurek, M, Bornaz, S & Debeaufort, F 2014, 'Barrier, structural and mechanical properties of bovine gelatin–chitosan blend films related to biopolymer interactions', Journal of the Science of Food and Agriculture, vol. 94, no. 12, pp. 2409-2419.
[5] Cardoso, GP, Dutra, MP, Fontes, PR, Ramos, AdLS, Gomide, LAdM & Ramos, EM 2016, 'Selection of a chitosan gelatin-based edible coating for color preservation of beef in retail display', Meat Science, vol. 114, 4, pp. 85-94.
[6] Sukkunta, S 2005, Physical and mechanical properties of chitosan-gelatin based film, thesis, Faculty of Graduate Studies, Mahidol University.
[7] Arfat, YA, Benjakul, S, Prodpran, T, Sumpavapol, P & Songtipya, P 2016, 'Physico-Mechanical Characterization and Antimicrobial Properties of Fish Protein Isolate/Fish Skin Gelatin-Zinc Oxide (ZnO) Nanocomposite Films', Food and Bioprocess Technology, vol. 9, no. 1, pp. 101-112.
[8] Baeza, GP, Dessi, C, Costanzo, S, Zhao, D, Gong, S, Alegria, A, Colby, RH, Rubinstein, M, Vlassopoulos, D & Kumar, SK 2016, 'Network dynamics in nanofilled polymers', Nature Communications, vol. 7, 04/25/online, p. 11368.