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Analysis of Biofilms from Biosynthesized Zinc Oxide Nanoparticles

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Authors' contributions

This work was carried out in collaboration between both authors. Authors FMD and KMCL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author FMD managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Abstract: Kappa-carrageenan, a natural polymer that is a product derived from seaweeds has been gaining vast attention on researches because of its biodegradability and low cost production. On the side, Nanotechnology, the emerging Science of nanoparticles has been indulging the world with its new approaches on improving the characteristics of the medium used. This study differentiated the physicochemical properties of the films without and with the incorporation of zinc oxide nanoparticles (ZnOnps) to kappa-carrageenan (kc).
Materials and Methods: Incorporation of ZnOnps to kc was prepared, the physicochemical properties: moisture content, degradation time, structural composition, and surface morphology were differentiated between bio composite films with and without the ZnO nps.
Results and Conclusion: After several tests, zinc oxide nano particles incorporated to kappa-carrageenan exhibited some changes to its physicochemical properties, that is the lowering of moisture content and longer degradation time was observed; using SEM magnifications ZnOnps was seen in the surface of the biocomposite film. However, structural composition analysis showed

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that no new formation of the functional group was seen. The biocomposite films could be a substitute with the existing drug capsule because of its longer decomposition, low water content and secured safety of the drug's quality.

Keywords: Zinc oxide nanoparticles, biofilms, kappa-Carrageenan, physicochemical properties.

1. INTRODUCTION

Seaweeds produce a carbohydrate form known as carrageenan. A natural polymer like polysaccharides, as such, it has been receiving a great deal of attention by many scientists, because of its biodegradability and availability at low cost. The innate properties and structure of carrageenan may be used for non-food applications.

Kappa-carrageenan (kc) is a natural polymer product from the extract of seaweeds. Nanotechnology, as an emerging field of Science, catered researches with modifications of the characteristics of a substance. This research targeted the changes on the physicochemical property of the kc biocomposite films filled with ZnOnps. The incorporation of metal oxide in polymer-based substances modified some of the characteristics based on the research conducted. The biofilm with ZnOnps created the new products aligned to the pharmaceutical and packaging industries. Products that can be developed like capsules and packaging plastics with higher degradation time for preserving the medicine and foods. Pharmaceutically produced capsules and industry-produced packaging film could be replaced with these developed biocomposite films. With the alteration on the properties, kappa-Carrageenan with zinc oxide nanoparticles will make a debut in the market and could replace the existing products.

The wide range of applications is possible as ZnO has key advantages. It is bio- safe, biocompatible and can be used for biomedical applications without coating. With these unique characteristics, ZnO could be one of the most important nano materials in future research and applications (Kathirvelu et. al, 2008). The nano-sized ZnO suspension clearly has much higher activity than the micron-sized ZnO [1].

The usage of acidic electrolyzed water in the production of carrageenan and gelatin hydrosols and hydrogels has not caused undesirable changes in their chemical and texture properties [2].

In other researches, when zinc oxide nanoparticles were used as an antimicrobial agent it exhibited decrease in microbial population. The decrease depended on the concentration of ZnOnps [3]. Zinc oxide nanoparticles and zinc nanoparticles coated with soluble polymeric material may be used for treating wounds, ulcers, and microbial infections besides being used as a drug carrier in cancer therapy [4]. With this evidences, the development of medicinal capsules from zinc oxide nanoparticles is deemed because of its unique clinical property.

This study focused on evaluating the physicochemical properties of biofilm from kappa-carrageenan filled with zinc oxide nanoparticles.

2. METHODOLOGY

2.1 Preparation of Zinc Oxide Nanoparticles from Zinc Nitrate

The preparation was done with the procedure of Rao [5] with some modifications made. Zinc oxide nanoparticles was prepared by using Zinc nitrate and sodium hydroxides precursors and kappa-carrageenan as a stabilizing agent. Kappa-carrageenan about 0.1 g was dissolved in 500 mL of lukewarm distilled water. Zinc nitrate, 14.874 grams (0.1 mol), was added in the above solution, and then followed by constant stirring for an hour to completely dissolve the zinc nitrate. After complete dissolution of zinc nitrate, 0.2 M of NaOH solution was added drop by drop under constant stirring. The reaction was allowed to proceed for 2 hours. After the completion of reaction, the solution was kept overnight and the supernatant solution was kept overnight and the supernatant solution was discarded carefully. Rest of the solution was centrifuged at 10,000 g for 10 min and the supernatant was discarded. Thus, the nanoparticles were obtained and washed thrice using distilled water. Washing was carried out to remove the by-products and the excessive starch bound with the nanoparticles. After washing, the nanoparticles were dried at 80°C overnight.

2.2 Preparation of Biofilms from Biosynthesized Zinc Oxide Nanoparticles

The preparation was done using Nafchi et al. [6] method, with some modifications made. Grams of ZnOnps were dispersed in a volume of water, stir for 1 hour, and then sonicated in an ultrasonic bath for 30 minutes. The solution will be used to prepare the aqueous dispersion with grams' addition of kappa-carrageenan. A mixture of sorbitol and glycerol (3:2) was added as plasticizer. The biocomposite solution was heated to and held for 45 min to allow gelatinization. Upon completion of starch gelatinization, the solution was cooled to room temperature. A portion of the solution was dispersed to a petri dish. Films were dried under controlled conditions in a humidity chamber. Control films were prepared similarly and stored at and relative humidity (RH) until experimentation. A concentration 0.06 mol·L^{-1} of zinc oxide nanoparticles was used in this study, this followed the research conducted of Brayner et. al (2010) for zinc oxide nanoparticles.

2.3 Determination of the Physicochemical Properties of Biofilms from Biosynthesized Zinc Oxide Nanoparticles

2.3.1 Test for moisture content of the biofilm

Films were conditioned at 58% RH and for 7 days. The weight difference was determined after drying of the equilibrated films in an oven at for 24h [6]. The samples were measured in three replicates and the percentage of total moisture content of the biofilm was calculated as follows:

$$Moisture\ Content\ (\%) = \frac{initial\ weight\ of\ film - final\ dried\ weight\ of\ film}{initial\ weight\ of\ film}$$

2.3.2 Test for degradation time of the biofilm

In this analysis, thermogravimetric analysis was used. Temperature range from 95- 600°C was used. TGA determined the degradation time of the sample. The temperature range was based on the degradation of kappa-carrageenan and zinc oxide nanoparticles.

2.3.3 Test for structural formation of the biofilm

FTIR spectra was recorded using an attenuated total reflection (ATR) method in Smart Itr. The thin films were applied directly onto the ZnSe ATR cell. For each spectrum, 64 consecutive scans at 400 to 4000 cm^{-1} resolutions. T – test was done to compare the results from the biofilms with and without ZnOnps.

2.3.4 Test for surface morphology of the biofilm

The conditioned bio-nanocomposite samples were placed in a Scanning Electron Microscope (SEM); the surface microstructure of films was investigated with this machine. Magnifications from 500 to 4000 μm was used.

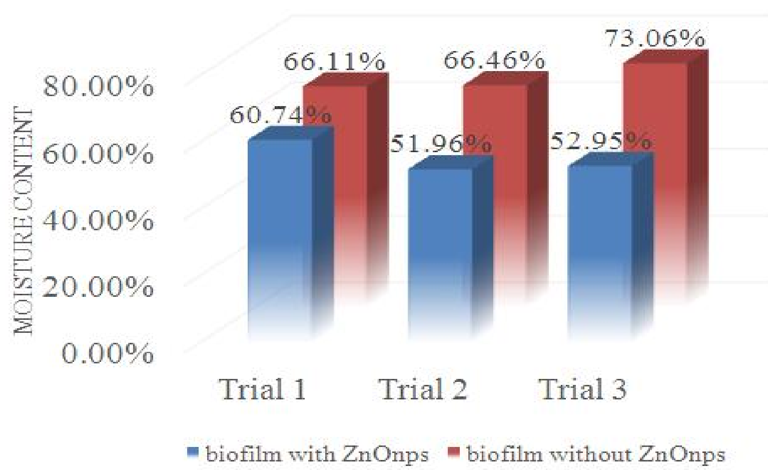

Fig. 1. Moisture content of the biofilms

3. RESULTS AND DISCUSSION

Zinc Oxide was successfully synthesized from Zinc Nitrate, from white chunky bulks to powdery white substance. The biocomposite films exhibited changes in its wavelength spectra, moisture content, degradation time, surface morphology. No new formation of the functional group was seen in the structural formation analysis.

Moisture content place an important role in this film, greater moisture content indicates that the degradation is rapid because of the high water content. Nafchi [6] cited in his work that increasing the nanoparticle (ZnO) content of films results in the formation of more hydrogen bonds the ZnO and the matrix components, thus, free water molecules do not interact as strongly with nanocomposite films compared with composite alone. Hence, an increase in ZnOnps levels leads to decreased moisture content and with high degradation time, products will be preserved in a longer period of time.

The result of the t-test showed that there was a significant difference between the biofilms with and without the ZnOnps. Thus, the kc with ZnOnps films can replace the commercially available drug capsules or the plastic films for the packaging industry.

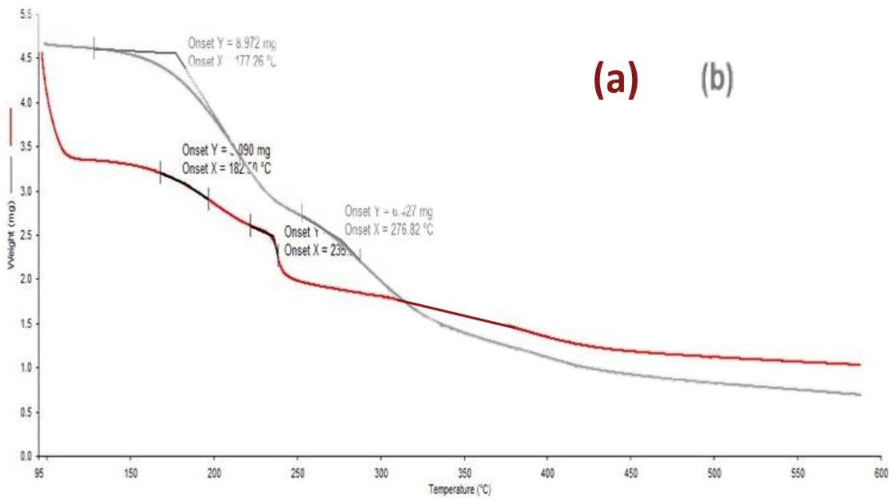

Fig. 2. Degradation time of the biofilms without ZnOnps (a) and with ZnOnps (b)

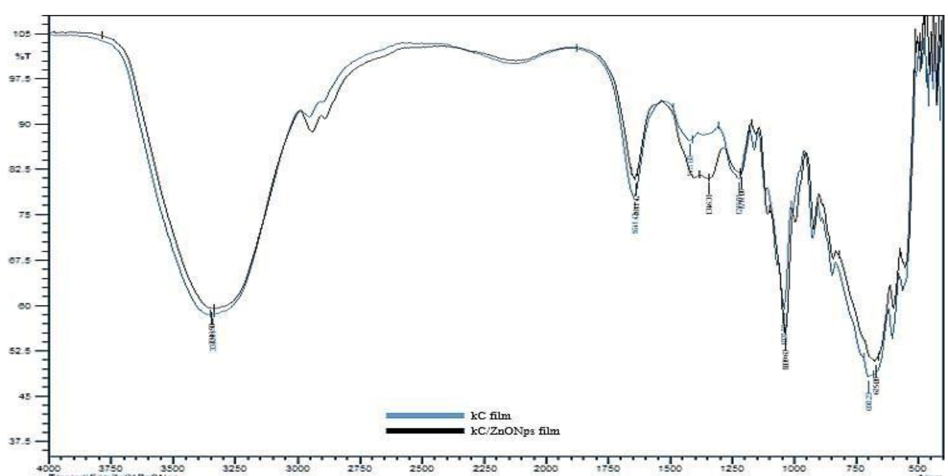

Fig. 3. FTIR result of the biofilms

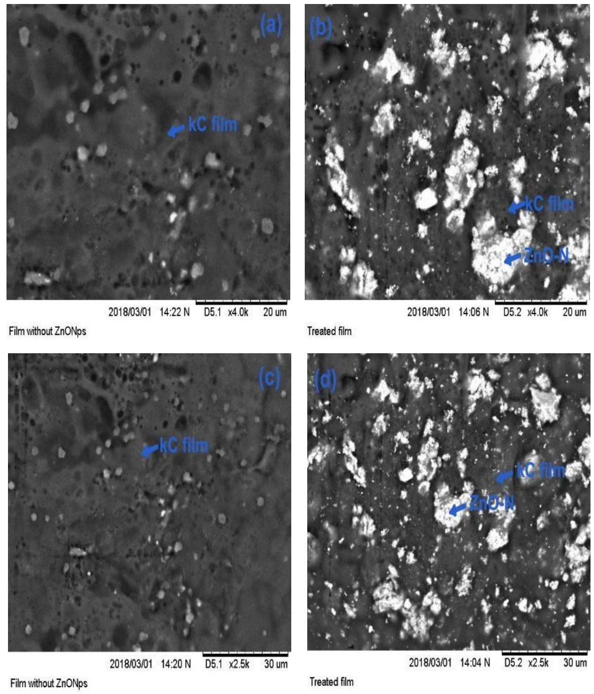

Fig. 4. SEM micrograph of the kc film without ZnOnps (a) magnification = 4,000, (c) magnification = 2,500. The films with ZnOnps (b) magnification = 4,000, and (d) Magnification = 2,500

Between 95 – 150°C the biofilms from kc started to degrade rapidly and lost approximately 1.5mg of mass and continue as the temperature increases (a). The kc with ZnOnps started to degrade at 150-200°C. This only implies that the incorporated ZnOnps to the kc manages to extend the degradation time of the biofilm (b). Thus, biocomposite material incorporated with metal oxide gives longer time to degrade. In layman's term, kc with ZnOnps have been upgraded to have a longer shelf life. Products in the market may decompose as it is not consumed, but products that use biocomposite films with zinc oxide nanoparticles will have longer time to decompose.

In order to confirm the presence of zinc oxide nanoparticles, an IR spectra examination was done. A broad peak was seen at ~600 cm^{-1}. The appearance of broad peak around 600cm^{-1} explains the distinct stretching mode of zinc oxide (Wang et al. 2003). A broad peak also was seen at ~3250 cm^{-1}, this peak corresponds to an alcohol (-OH) functional group.

Using a scanning electron microscope (SEM), the result revealed that films without ZnOnps have dumps at magnifications 4000 with 20 µm (a) and 2500 with 30 µm (c). Furthermore, results for the film with ZnOnps revealed that the metal oxide was attached to the surface of the biofilms. Also, it is seen that zinc oxide nanoparticles covered the dumps present in the untreated film at magnifications 4000 with 20 µm (b). This implies that the metal oxide incorporated is in the surface of the biocomposite film. This implies that the biofilm remains the same but with the presence of a metal oxide that enhanced its physicochemical property. This result is in congruent with the findings of Pulit-Prociak et al. [7] that zinc oxide nanoparticle does not penetrate through dermal membrane which is the desired effect concerning their toxicity.

The physicochemical properties of the treated film as seen in the previous presentations were much better compared to the biofilm without ZnOnps. Moisture content was higher in biofilm without ZnOnps than the biofilm with ZnOnps which indicates that the untreated biofilm has more water content than the treated biofilm. The degradation time proves the claim of the moisture content analysis, biofilm without

ZnOnps degraded and loss weight faster than treated biofilm. Structural composition revealed that the film developed no new functional group and around 600 cm^{-1} a broad peak appeared as to the presence of zinc oxide. Surface morphology tells us the presence of ZnOnps in the biofilm.

4. CONCLUSION

With this, the biofilm with ZnOnps exhibited unique properties that can be used in the pharmaceutical and packaging industries. In this starting point, this biofilm could replace the existing medicine capsules for better drug protection and resistance to bacterial contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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