Research Article

Antimicrobial properties of biocomposite films from kappa-Carrageenan (kC) filled with Nanorod-rich Zinc Oxide (ZnO-N)

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Abstract

Objective: A natural polymer, carrageenan is a product derived from the extract of seaweeds. With its bioavailability at low cost and biodegradability, carrageenan has been gaining vast applications and researches this past year. On the other hand, nanoparticles have indulged the world of science with new techniques and approaches**. Materials and methods:** Nanorod-rich zinc oxide has been used against *E. coli* and *S. aureus* in different researches with results in the decrease of bacterial population. In this approach, kC bionanocomposite films was filled with ZnO-N and used against four different bacteria: *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes,* and *Pseudomonas aeruginosa.* Bacterial effects of the film were determined using the zone of inhibition**. Results and conclusion:** Results showed that, the biocomposite kC/ZnO-N film exhibited a good antimicrobial property to the bacteria sample except *P. aeruginosa*, a multi-drug resistant bacteria.

Keywords: Nanorod-rich Zinc Oxide (ZnO-N), biocomposite films, kappa-carrageenan (kC), antimicrobial property

Introduction

Seaweeds produce a carbohydrate form known as carrageenan. A natural polymer like polysaccharides, as such, it has been receiving 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.

In this time of technology enhanced matrix, nanoparticles have been greatly used in advancement of Science. A breakthrough in its ability to enhance polymer properties. Also, supplies a specifically usable platform and establishing enhanced properties with general wide-ranging applications specifically for pharmaceutical and medicine.

Recent studies have been conducted on starch filled with metal nanoparticles. Results showed that nanoparticles enhanced the starch physicochemical properties and including its antimicrobial properties. In addition, nanoparticles of any kind are used today. Zinc oxide and polyvinyl chloride are some great examples of nanoparticle. With this, nanoparticles have greatly affected the lives of human. From the permeability of the sample to its enhanced and flexible outcome.

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 nanomaterials in future research and applications (Kathirvelu et al., 2008).

The antibacterial activity increase with increase nanoparticle concentration and increases with decreasing particle size. Particle concentration is observed to be more important than particle size. Another cause of bacetria inhibition, which is the electrostatic force interaction between ZnONps and cell surface (Zhang et al., 2008). The interaction will create opposing charges between the

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bacteria and nanoparticles that will generate an electrostatic force. This electrostatic force will strongly bind the bacteria and ZnO nanoparticles together and consequently cause cell membrane damage (Rahman et al., 2013).

The dominant mechanisms of such antibacterial behavior are found to be either or both of chemical interactions between hydrogen peroxide and membrane proteins, and the chemical interactions between other unknown chemical species generated due to the presence of ZnONps with the lipid bilayers (Zhang et al., 2009).

The surface of Zinc oxide nanoparticles reacts with water to produce reactive oxygen species that destroy the bacteria cell membranes. And, if the nanoparticles are made small enough, the bacteria will actually internalize the nanoparticles, resulting in even higher killing efficiency as the cells are attacked from the inside too (Anita and Ramachandran, 2012).

Effects of sago starch film reinforced with ZnO-N on the growth of S. aureus were investigated (Nafchi et al, 2012). To continue, the inhibition zone of nano-incorporated films was significantly increased by increasing the ZnO-N contents, suggesting that sago starch film incorporated with ZnO-N can act as an active film against microorganism. Excellent antimicrobial activity of ZnO nanoparticles against *S. aureus* and *E. coli* and the corresponding mechanism of action have also been demonstrated by other researchers.

Nanorod-rich zinc oxide carries antimicrobial properties. The interaction affects the cell permeability between the ZnO-N and the bacteria, causing the ZnO-N to enter the bacterial cell and inducing the oxidative stress in the cell. Thus, the inhibition zone approved to the ZnO-N and will cause bacterial death.

This study focused on the application of biocomposite film from kappa-Carrageenan filled with nanorod-rich Zinc oxide (ZnO-N) in antimicrobial analysis of *E. coli, S. aureus, Pseudomonas aeruginosa,* and *Enterobacter aerogenes*.

Materials and methods

Preparation of Zinc Oxide from Zinc Nitrate

The preparation was done with the procedure of Rao (2015) with some modification made. Nanorod-rich Zinc oxide was prepared by using Zinc nitrate and sodium hydroxides precursors and starch as a stabilizing agent. Kappa-Carrageenan about 0.1g 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 1 hour using magnetic stirrer 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^{0}C overnight.

Preparation of Biocomposite Films

The preparation was done using A.M. Nafchi et al. (2012), with some modification made. Five grams of (5g) ZnO-N was dispersed in 95mL water (0.92% ZnO-N solution), stir for 1 hour, and then sonicated in an ultrasonic bath (if not available, use the magnetic stirrer as substitute) for 30 minutes. The solution will be used to prepare the aqueous dispersion with 2 g addition of kappa-carrageenan. A mixture of sorbitol and glycerol (3:2) was added as plasticizer. The biocomposite solution was heated to

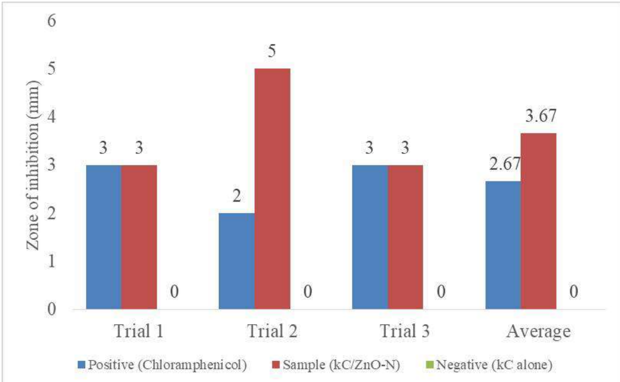

Figure 1. Comparative Chart on the Zone of Inhibition for *Escherichia coli*

In addition to the claim of Nafchi (2012), figure 1 shows that sample film has greater inhibitory effect than the other two. Thus, the incorporation of ZnO-N truly had a great effect in the changes of the properties of the kC. Using the t-test, results showed that there was no significant difference between the two. Thus, whether you use the chloramphenicol or the kC/ZnO-N films, results will be the same. But kC/ZnO-N films as biocomposite material will be a vital use to humans. With less chemicals intact, this film will be safer.

Enterobacter aerogenes

Figure 2 shows that sample film has greater inhibitory effect in *Enterobacter aerogenes* than the other two. Thus, the added nanorod-rich zinc oxide truly affected the properties of the kappa-Carrageenan. Using the t-test, results showed that there was no significant difference between the two.

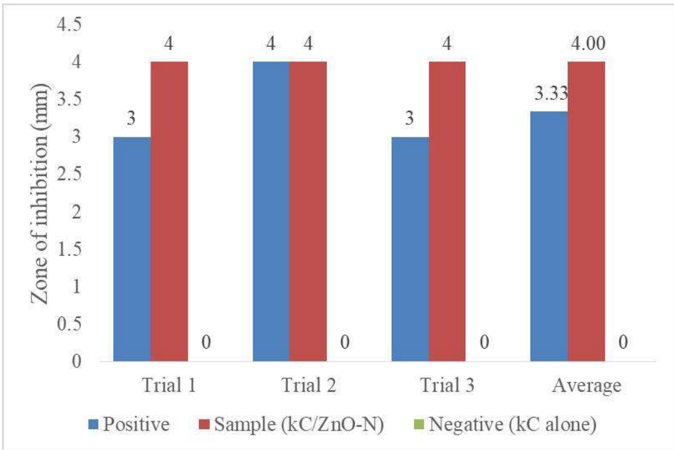

Figure 2. Comparative Chart on the Zone of Inhibition for *Enterobacter aerogenes*

Pseudomonas aeruginosa

Figure 3 shows that sample film has no inhibitory effect in *Pseudomonas aeruginosa*. Thus the results showed that ZnO-N at low concentrations has no inhibitory effect in *P. aeruginosa* also cited by Paul and Bam (2014). The reason was perhaps the multi-drug resistance that was developed by the bacteria. But studies showed that ZnO applied in higher concentration will result to antimicrobial property with different bacteria. ZnO produced during the entrapment between the cell membrane a reactive oxygen species that kills and coat the bacterial cell membrane. Thus, antimicrobial property of ZnO depends on its dosage.

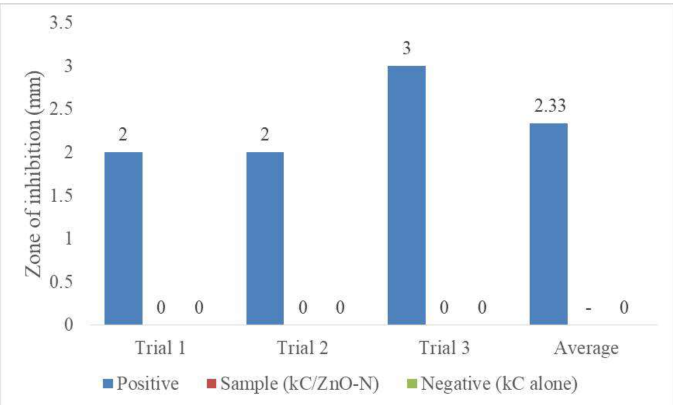

Figure 3. Comparative Chart on the Zone of Inhibition for *Pseudomonas aeruginosa*

Staphylococcus aureus

Cited by Rajendran et al. (2010), nanoparticle and bulk sized ZnO-N has an excellent antimicrobial activity against *S. aureus*. In this figure, the inhibitory effect of the kC/ZnO-N shows great result than the other two. ZnO produced during the entrapment between the cell membrane a reactive oxygen species that kills the bacterial cell membrane and wraps it when the cell dies to prevent the flowing of the liquid inside.

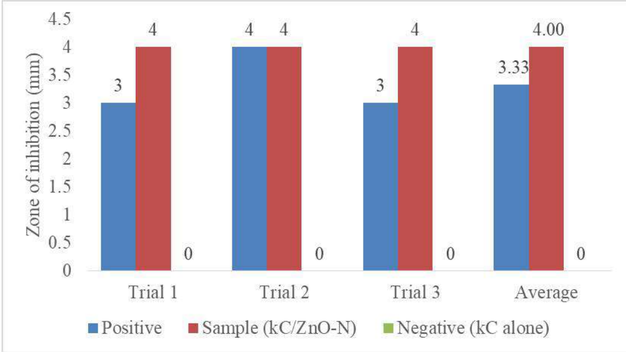

Figure 4. Comparative Chart on the Zone of inhibition for *S. aureus*

Comparative analysis between the kC/ZnO-N films and kC films

In this area, results between the untreated and treated films will be compared.

Table 1. Comparative Data of the Sample films

Parameters	kC films	kC/ZnO-N films
E. coli	No zone of inhibition	3.67 mm, zone of nhibition
Enterobacter aerogenes	No zone of inhibition	4.00 mm, zone of nhibition
Pseudomonas aeruginosa	No zone of inhibition	No zone of inhibition
Staphylococcus aureus	No zone of inhibition	4.00 mm, zone of nhibition

The data revealed that ZnO-N has great effect after its incorporation. The metal oxide enhanced the properties tested in the untreated film. The treated film has greater antimicrobial property than the untreated film except for *P. aeruginos*a. This bacterium is a multi-drug resistant. But, with higher concentration of ZnO, this bacterium could be killed (Paul and Ban, 2014).

With this, the kC/ZnO-N film exhibited unique properties that can be used in the pharmaceutical and packaging industries. In this starting point, this film could replace the existing medicine capsules for better drug protection and resistance to bacterial contamination.

Conclusions

The biocomposite films from kC filled with ZnO-N exhibited good antimicrobial property with the three bacteria except *Pseudomonas aeruginosa*. *P. aeruginosa*, is a multi-drug resistant bacteria and at lower concentration of ZnO is hard to kill. Biocomposite film from kC/ZnO-N has more chances of being substituted in the market as the new drug capsule and as packaging plastic. With its unique and enhanced property kC/ZnO-N developed a good antimicrobial property, efficient than the kC film.

Conflicts of interest: Nil

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