PREPARATION OF SILVER-CHITOSAN NANOCOMPOSITES COLLOIDAL AND FILM AS ANTIBACTERIAL MATERIAL

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ABSTRACT

Colloidal nanocomposites silver-chitosan have been made. Silver nanoparticles were produced by chemical reduction methods assisted microwave irradiation using chitosan from crab shells as a reducing agent and stabilizer, $AgNO_3$ as a precursor and NaOH as an accelerator. This study investigated $AgNO_3$ concentration toward localized surface plasmon resonance (LSPR) phenomenon of nanocomposites colloidal. The size and shape of the silver nanoparticles were confirmed by TEM. Furthermore, the stability of the storage was observed for twelve weeks. Colloidal and film nanocomposites silver- chitosan have been made by casting method by drying at room temperature. After that, the film characterization was carried out, including swelling with gravimetry methods and surface morphology using scanning electron microscopy (SEM). Diffusion methods tested colloid antibacterial activity and silver-chitosan nanocomposite film's against *E. Coli* and *S. Aureus*. The results showed that the formation of silver nanoparticles was identified by the LSPR absorption band's appearance at 413-419 nm. The increasing of $AgNO_3$ concentration increased the intensity of the LSPR absorption band. Silver nanoparticles with sizes of about 3-9 nm are spherical. The silver nanoparticles were stable at 12 weeks of storage. The higher $AgNO_3$ concentration tends to increase the swelling of the film. The surface of the silver-chitosan nanocomposite film's was rougher than that of the chitosan film. The higher the silver nanoparticle concentration, the higher the colloid and film antibacterial activity against *E. Coli* and *S. Aureus.*

Keywords: nanocomposites, silver nanoparticles, chitosan, colloidal, film, antibacterial

INTRODUCTION

The medical world is a health sector that plays an essential role in human life so that everything continues to be developed. One of the things developed in the medical world is a polymer. Currently, synthetic polymers are still widely used, such as polyurethane, polyethylene, polylactides, polyglycolide, and polyacrylonitrile, where there is a weakness, low biocompatibility. Therefore, biopolymers are continuously being developed to replace synthetic polymers. The requirements for biomedical polymers include, among others, that it must be non-toxic, not allergenic, easily sterilized, have adequate mechanical properties, strong, elastic, durability and biocompatibility. Meanwhile, medical textiles' main require-ments include absorption, strength, elasticity, smoothness, and biodegradation [1].

Chitosan is one of the ingredients that is widely used as a basic material for making

biofilms. Chitosan has many useful properties, including non-toxic, antibacterial, biodegradable and biocompatible [2]. Chitosan is a material that is widely used as a base for making biofilms. It has many useful properties, including non-toxic, antibacterial, biodegradable and biocompatible [2]. Chitosan is obtained from the isolation of fishery waste such as shrimp shells, small crab shells, crab shells, clamshells, and other shelled marine animals. Crab shells contain chitin and chitosan, the second-largest biopolymer compounds found in nature after cellulose [3]. Crab contains the highest percentage of chitin (70%) among crustaceans, insects, worms and fungi. This chitin is later deacetylated to become chitosan [4]. Chitosan is appropriate for use as a polymer in the medical field because it has an antibacterial and antifungal activity which can inhibit infection, reduce contractions, accelerate wound closure and healing [5]. Chitosan is a polymer compound in the form of a long chain of glucosamine with a chemical formula (2-amino-2-deoxy-β-D-Glucose). Chitosan is also a multifunctional polymer because it contains an amine group and a hydroxyl group. The existence of this functional group causes chitosan to have high chemical reactivity [6].

Chitosan has antibacterial properties, but its antibacterial properties can still be improved by adding nanoparticles. One of the nanoparticles that are currently being developed is Ag (Silver) nanoparticles. The addition of silver nanoparticles to chitosan will form silver-chitosan nanocomposites biopolymers. Chitosan acts as a matrix or binding component, while silver nanoparticles act as a filler component [7]. Ag nanoparticles' anti-bacterial ability is influenced by nanomaterial physical characteristics, such as size, shape, and surface properties. Nanoparticles have many uses, such as detectors, catalysts, surface coating agents, and antibacterial agents. Among metal nanoparticles, silver nanoparticles have received much attention because of their physical and chemical properties. Silver has been used to treat medical ailments for more than 100 years because of its natural antibacterial and antifungal properties, and because it is non-toxic to humans [8].

Silver-chitosan nanocomposites biopolymers can be prepared by first synthesizing Ag nanoparticles. Chemical reduction methods can carry out the preparation process. Besides easy and quite effective, the costs required are also relatively affordable. There are 2 important components that must be involved in the synthesis process of silver nanoparticles using chemical reduction; 1) a reducing agent that will reduce Ag^+ to Ag^0 and 2) a stabilizer (stabilizing agent) which will provide stability to silver nanoparticles formed from oxidation, agglomeration and deposition processes [9], [10].

Besides acting as a matrix, chitosan can act as a stabilizer and a reducing agent in the synthesis of silver nanoparticles. [11-12] used chitosan as a stabilizer, while [13] used chitosan as a reducing agent-microwave irradiation for 11 minutes with a break of 45 seconds every 1 minute. Thus, microwave irradiation assistance can provide time efficiency in the synthesis process of silver nanoparticles[13].

The synthesis of silver nanoparticles can also be added with an accelerator to increase the effectiveness and efficiency. Based on research conducted by Susilowati [14], the use of NaOH as an accelerator can increase the effectiveness and efficiency of the silver nanoparticle synthesis process with glucose as the reducing agent. The use of NaOH as an accelerator can reduce the size of silver nanoparticles and increase their number.

This research was carried out on the preparation of colloid and film filled with silver nanoparticles (Ag) with a reduction method with chitosan from crabs shell as both reducing agent and stabilizing agent assisted by microwave irradiation became silver-chitosan nanocomposite film's and colloidal to be used as an antibacterial material. NaOH is used as an accelerator to increase the effectiveness of silver nanoparticles producing. Besides, chitosan also acts as a biopolymer matrix in the synthesis of silver-chitosan nanocomposites.

METHODS

The material used in this study was chitosan from crab shells produced by Biotech Surindo Indonesia. Acetic acid (CH_3COOH), silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) was produced by Merck. Aquades is produced by Laboratorium of Chemistry Education Sebelas Maret University, Indonesia.

1. Preparation of silver-chitosan nano-composite colloidal (SCNC)

Initially, 1% chitosan solution was made in 1% (v / v) acetic acid solution. As much as 2.5 grams of chitosan from crab shells dissolved in 250 mL of 1% acetic acid, then stirred until homogeneous for 2 hours. Then the chitosan solution was left to stand for 24 hours so that the chitosan was completely dissolved. Then a solution of $AgNO_3$ 0.12 g / ml and NaOH 2 M was also made.

The preparation of colloidal silver-chitosan nanocomposite was started by taking 12.5 mL of 1% chitosan solution and then adding 0.12 g / ml AgNO3 as much as 0.25 ml stirring for 5 minutes. After that, 1.75 ml of 2 M NaOH was added and stirred vigorously for 5 minutes, and a white gel was formed. This gel was then irradiated with a microwave with a power of 100 watts for 4 minutes, and the gel changed colour to brown. This gel was then mixed with 47.5 chitosan and stirred until all the gel was dissolved and homogeneous to form colloids which in this study were called colloidal silver-chitosan nanocomposites (SCNC1).

The same step was taken to variation the volume of AgNO3 0.12 g / ml, namely 0.50 ml; 0.75 ml; 1.00 ml; 1.25 ml and 1.5 ml with coded samples respectively are SCNC2, SCNC3, SCNC4, SCNC5 and SCNC6.

2. Preparation of silver-chitosan nano-compocite film

Nanocomposites silver-chitosan film was prepared by casting method. A volume of 60 mL of colloidal silver-chitosan nanocomposites (SCNC 1-6) was poured into a mould made of polypropylene with a size of 12 x 18 cm2. Then it is dried at room temperature until a completely dry film is formed. Then the film was removed by adding

1 M NaOH and shaking slowly. The film was then neutralized with distilled water to pH seven and dried again on a glass board. After drying, the film is peeled off and stored in a desiccator. These films are coded SCNF 1-6) and ready to characterization.

3. Characterization

a. Identification of silver nanoparticles production

The silver nanoparticles formed were identified based on the LSPR phenomenon using the UV-Vis spectrophotometer Shimadzu UV3150. Before scanning, the colloidal sample was diluted ten times using distilled water. The sample with the smallest silver nanoparticle concentration was poured into a cuvette at a certain volume and then scanned with a wavelength of 300-600 nm. Furthermore, measurements were taken for samples with higher concentrations of sequentially.

The analysis was carried out using a transmission electron microscope (TEM) JIOL JEM-1400 series to confirm silver nanoparticles' formation. Colloidal samples were dropped on a copper grid and absorbed with a tissue and then observed at a certain magnification.

b. Stability test of colloidal silver-chitosan nanocomposites

The stability of colloidal silver-chitosan nanocomposites is based on the LSPR phenomenon by measuring the absorbance value at the UV-Vis spectra's maximum length. Silver nanocomposites samples (SCNC 1-6) were stored in transparent vials at room temperature. Samples were stored for 0, 2, 4, 6, 8, 10 and 12 weeks. Before taking measurements, the sample was diluted ten times using distilled water. Then scanned at a wavelength of 300-600 nm.

c. Swelling test of the silver-chitosan nanocomposites film

The swelling test procedure weighs the sample's initial weight (SCNF 1-6) to be tested (W_0), then placing it in a container containing phosphate buffer for 30 minutes. Remove the sample from container then dry it or remove the phosphate buffer on the surface of the film with tissue or filter paper, then weighing it so that the wet film wight (W) is obtained. Then the film was dried and repeated the soaking and weighing procedure three times. (triple).

$$Swelling\ (\%) = \frac{W - Wo}{W} x\ 100\% \dots\dots\dots\dots (1)$$

d. Analysis of the morphology of silver-chitosan film

SEM was used to observe the surface morphology of the film. Chitosan and SCNF4 films were observed using a scanning electron microscope (Zeiss DSM 960) with an acceleration voltage of 10 kV. The morphology observed was the surface and the cross-section.

e. Antibacterial activity test of silver-chitosan nanocomposites colloidal and films

The antibacterial activity test was carried out qualitatively and modified by referring to the research Wahyudi [10] and Fatihin [15]. The test was carried out by observing the inhibition zone of the sample on bacterial development. For silver -chitosan

nanocomposite films, the antibacterial test was carried out by disk diffusion method. The step is to cut the resulting film into a circle and then prepare an antibacterial test medium in Nutrient Agar (NA) media. Take one bacterial colony using loop needle and put it in NA media, then leave it in the incubator for 24 hours. Sticking the edible film that has been cut into the media containing the bacteria then leave it in the incubator for 24 hours. Observe the results and see the edible inhibiton zone of the film against bacterial growth. For colloids, the test was carried out using the well diffusion method. The step is to make a well on NA media then take a bacterial colony using loop needle and put it in NA media, the leave it in the incubator for 24 hours. Then drop the colloid on the well on the media that already contains the bacteria then leave it in the incubator for 24 hours. Observe the results and see the colloidal inhibiton zone against bacterial growth

RESULT AND DISCUSSION

The silver-chitosan nanocomposites colloid process's preparation produces homogeneous colloids with darker colour with increasing Ag concentration, as shown in Figure 1. The brown colour that arises is due to the formation of Ag nanoparticles in the colloid. The formation of silver nanoparticles was carried out by chemical reduction methods and the aid of microwave irradiation. Then the silver nanoparticles formed were dispersed on the chitosan polymer to form colloids [16].
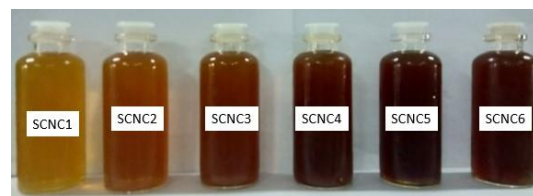

Figure 1. Silver-chitosan nanocomposites colloidal with various Ag concentrations: SCNC1. 0.5%; SCNC2. 1%; SCNC3. 1.5%; SCNC4. 2%; SCNC5. 2.5%; and SCNC6. 3%

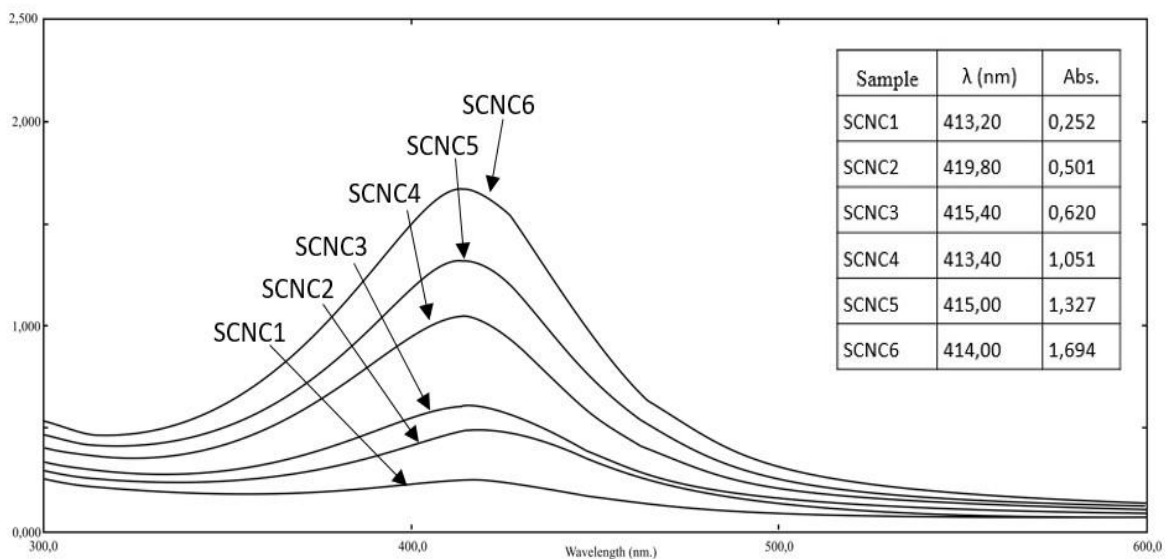| Sample	λ (nm)	Abs.
SCNC1	413,20	0,252
SCNC2	419,80	0,501
SCNC3	415,40	0,620
SCNC4	413,40	1,051
SCNC5	415,00	1,327
SCNC6	414,00	1,694

Figure 2. UV-Vis Spectra of silver-chitosan nanocomposites colloidal with various Ag concentrations, SCNC1. 0.5%; SCNC2. 1%; SCNC3. 1.5%; SCNC4. 2%; SCNC5. 2.5%; and 3%; SCNC6.)

The formation of silver nanoparticles in silver-chitosan nanocomposites colloidal can be identified using UV-Vis spectroscopy. The UV-Vis spectra of each silver-chitosan nanocomposites colloidal sample can be seen in Figure 2.

Figure 2 shows that each sample's maximum absorbance occurs in the range of wavelengths of 413-420 nm. This indicates that silver nanoparticles have been formed. These results follow Raghavendra's research [13] and Shah [12], which state that the SPR band silver nanoparticles on UV-Vis spectra can provide characteristic peak absorbance spectra wavelength range of 400-450 nm. Based on UV-Vis spectra (Figure 2), the increase in the concentration of $AgNO_3$ used is generally directly proportional to the increase in absorbance. Increased absorbance shows that the concentration of silver nanoparticles also formed increases. These results are following the research conducted by Susilowati [14].

Silver-chitosan nanocomposite film's was formed from silver-chitosan nanocomposite colloidal which was moulded and dried at room temperature to be further neutralized.

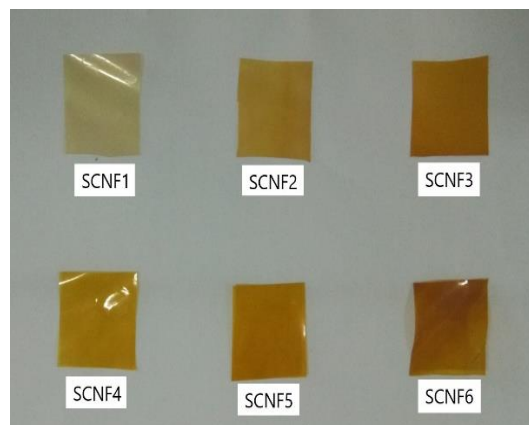The film that is created has a darker colour variation, as shown in Figure 3.

Figure 3. Silver-chitosan nanocomposite film with various Ag concentrations: SCNF1. 0.5%; SCNF2. 1%; SCNF3. 1.5%; SCNF4. 2%; SCNF5. 2.5%; and SCNF6. 3%

1. Stability test of silver-chitosan nanocomposites colloidal

To determine the colloidal stability of silver nanoparticles by measuring the absorbance at various concentrations of $AgNO_3$. Measurements were made every two weeks for 12 weeks in room temperature storage. Figure 4 shows the absorbance chart of silver-chitosan nanoparticles for 12 weeks.
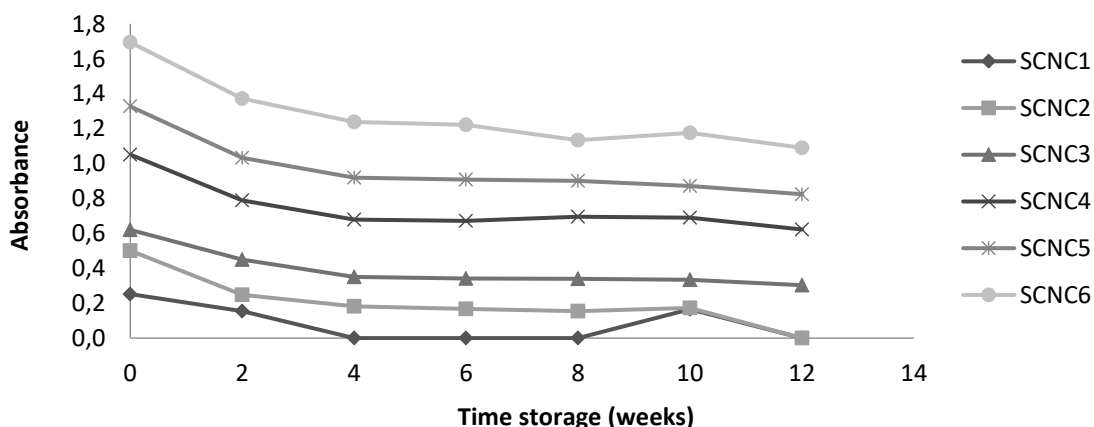

Figure 4. The effect of storage time at room temperature on the absorbance intensity of the UV-VIS spectra

Based on the graph (Figure 4), it can be seen that the longer the storage time, the absorbance value tends to decrease. For SCNC1 samples starting from week four, the absorbance value was 0. For samples SCNC1 and SCNC2 at week 12, it already showed an absorbance 0. This 0 value of absorbance indicates that silver nanoparticle undergoes aggregation. The absorbance peaks that tended to be stable for samples SCNC3 to SCNC6 showed that in storage for 12 weeks at room temperature, nanoparticles silver remains stable and does not undergo significant particle aggregation [14].

2. Swelling test of the silver-chitosan nanocomposite film's

The swelling test shows that the addition of AgNO3 affects the absorption of the film layer into water. The results of the swelling test are shown in Figure 5. The greater the addition of $AgNO_3$, water absorption will increase, and the increase in the concentration of $AgNO_3$ is directly proportional to the increase in water absorption.
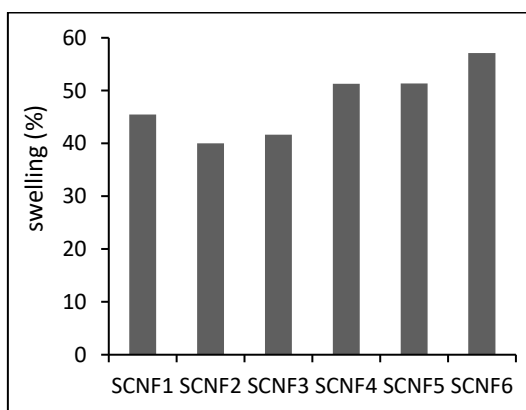

Figure 5. The swelling test results of the silver-chitosan nanocomposite film's

Since the increase in the concentration of $AgNO_3$ is directly proportional to the

increase in the concentration of the Ag nanoparticles and the decrease in size, water absorption also increases with increasing concentration and decreasing the size of the Ag nanoparticles. This is consistent with research conducted by Shah [12] which states that water absorption is influenced by the size of the nanoparticles, surface tension, and surface morphology of the nanocomposite film. The growth of silver nanoparticles can cause the chitosan matrix to expand and encourage the formation of gaps between the chitosan polymer matrices so that the liquid can more easily enter and bond to the silver-chitosan nanocomposite film's. The contribution of amino and hydroxyl groups of chitosan is also known to increase the silver-chitosan nanocomposite film's water absorption capacity [12].

3. TEM Analysis

The shape and size of silver nanoparticles in silver-chitosan nanocomposites colloidal were analyzed using TEM, the results of which can be seen in Figure 6. The samples analyzed were SCNC3 and SCNC6 only to compare the shape and size of colloids with low and high silver nanoparticles concentrations.
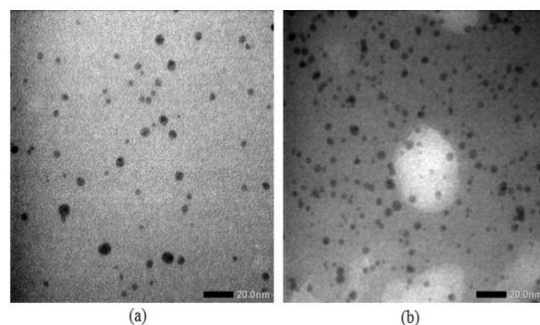

Figure 6. TEM image of (a) sample SCNC3 (b) sample SCNC6

Based on Figure 6, the resulting silver nanoparticles have a spherical shape with the size of about 3-9 nm. When the SCNC3 and SCNC6 samples were compared, it was seen that the SCNC6 samples had more silver nanoparticles than the SCNC3 samples. The spherical shape of silver nanoparticle in line with LSPR band about 400 nm [16]. The increase in the concentration of $AgNO_3$ is directly proportional to the increase in the concentration of silver nanoparticles that formed that have proved from TEM image [16].

4. Morphological analysis of silver-chitosan nanocomposite film's

The morphology of the silver-chitosan nanocomposite film's was analyzed using SEM. The results of SEM analysis in Figure 7. Sample SCNC0 is a pure sample of chitosan without the addition of Ag nanoparticles.

Based on the SEM results in Figure 7, the addition of $AgNO_3$ causes the surface of the film to become uneven. This occurs because Ag nanoparticles' addition causes chitosan polymer chains to develop when interacting or binding with Ag nanoparticles. Besides, there was clumping on the silver-chitosan nanocomposite film's surface, which indicated that the silver nanoparticles were experiencing agglomeration. This is consistent with a study conducted by Susilowati [16] which stated that the higher the concentration of silver nanoparticles on the film, the higher the nanoparticles' tendency to experience agglomeration [16].
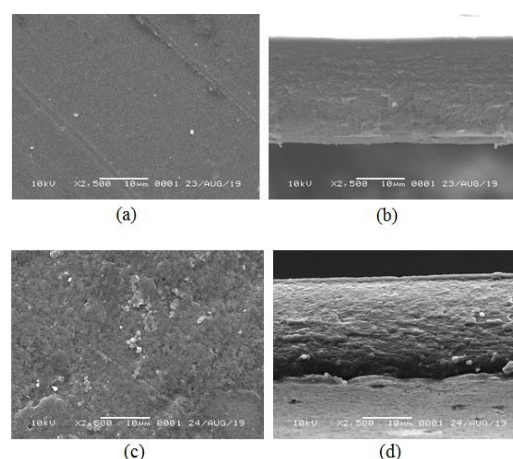

Figure 7. SEM image with magnification 2,500 times of (a) sample SCNF0 surface, (b) sample SCNF0 cross-section, (c) sample SCNF4 surface and (d) sample SCNF4 cross-section film.

5. Antibacterial activity test of silver-chitosan nanocomposite film's and colloidal

Antibacterial activity test of silver-chitosan nanocomposites colloid by well diffusion method, and silver-chitosan nanocomposite film's by disc diffusion method. Antibacterial activity test against Staphylococcus aureus (gram-positive bacteria) and Escherichia coli (gram-negative bacteria).The results of this antibacterial test can be seen in Figures 8 and 9.
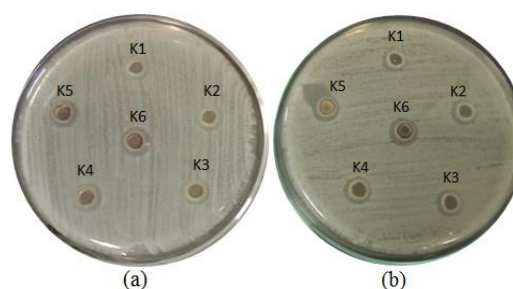

Figure 8. The results of colloidal testing against bacteria (a) *S. Aureus* (b) *E. Coli*
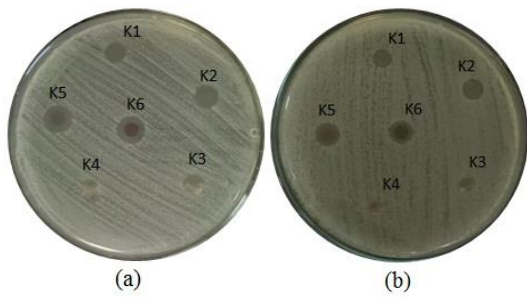
Figure 9. The results of film testing against bacteria (a) *S. Aureus* (b) *E. Coli*

Table 1. Data on the diameter inhibition zone of colloidal against *S.aureus* and *E. coli* bacteria

Sample	Inhibition zone diameter (mm)	
	E. Coli	*S. Aureus*
SCNC0	0	0
SCNC1	8.76	7.54
SCNC2	9.38	8.58
SCNC3	9.27	9.16
SCNC4	9.92	9.96
SCNC5	10.46	9.64
SCNC6	10.52	10.58

The silver-chitosan nanocomposites colloidal showed good antibacterial activity against both bacteria, as evidenced by the appearance of an inhibition zone (clear areas) around the wells on agar media that had been overgrown with bacteria. The larger clear area indicates that the inhibition of the compound against bacterial growth is getting stronger. Based on Table 1, the silver nano-particle dispersion to chitosan biopolymer was proven to affect the colloidal antibacterial activity of silver-chitosan nanocomposites.

The silver-chitosan nanocomposite film's showed good antibacterial activity against both bacteria, as evidenced by the appearance of inhibition zone (clear area) around the film disc on agar media that had been overgrown with bacteria [16]. The larger clear area indicates that the inhibition of the compound against bacterial growth is getting

stronger. Based on Table 2, silver nanoparticle dispersion to chitosan biopolymer was proven to affect silver-chitosan nanocomposite film's antibacterial activity.

Table 2. Data on the diameter inhibition zone of the film against *S. aureus* and *E. coli* bacteria

Sample	Inhibition zone diameter (mm)	
	E. Coli	*S. Aureus*
SCNF0	0	0
SCNF1	6.81	6.87
SCNF2	7.71	7.70
SCNF3	7.19	7.28
SCNF4	7.19	8.01
SCNF5	8.39	9.76
SCNF6	8.60	10.08

When compared, the diameter of the colloidal inhibition zone of silver-chitosan nanocomposites was slightly larger than the silver-chitosan nanocomposite film. According to a study conducted by Haryati [17], the good diffusion method will produce a larger diameter of the inhibition zone than the disk diffusion method [17]. This is because the well method occurs at a higher osmolarity process than the disk method. In the well diffusion method, the osmolarity that occurs is more thorough and more homogeneous, and the concentration is higher so that it is stronger to inhibit bacterial growth [17]. Dissolved chitosan and its derivatives are more effective at inhibiting bacterial growth. Dissolved chitosan has a broad conformation, which allows the reaction to be effectively and efficiently, whereas the solid chitosan only comes into contact through its surface [18].

The trend of increasing the inhibition zone for both bacteria was accompanied by an increase in the concentration of $AgNO_3$,

which means that the increase in the concentration of silver nanoparticles formed was directly proportional to the increase in the area of the colloidal inhibition zone and silver-chitosan nanocomposite film's against both bacteria. Antibacterial activity in colloids and films comes from silver nanoparticles because, without silver nanoparticles (SCNC0 and SCNF0), the inhibition zone is 0 for both bacteria.

CONCLUSION

Silver-chitosan nanocomposite film's and colloidal can be made using the reduction method assisted with microwave irradiation. Chitosan from crab shell acts as a reducing agent and stabilizer (stabilizing agent), and NaOH is used as an accelerator. The formation of silver nanoparticles identified the appearance of the LSPR absorption band at 413-419 nm. Silver nanoparticles are spherical shape with a dominant size of 3-6 nm. Silver nanoparticles were stable in twelve weeks of storage at room temperature. The higher concentration of $AgNO_3$ tends to increase the swelling of the film. The surface of the silver-chitosan nanocomposite film is rougher than that of the chitosan film. The higher the silver nanoparticles' concentration in the film layer, the higher the colloid antibacterial activity and the film layer on *E. Coli* and *S. aureus* bacteria.

ACKNOWLEDGEMENTS

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