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SYNTHESIS AND CHARACTERIZATION CHITOSAN FILM WITH SILVER NANOPARTICLE ADDITION AS A MULTIRESISTANT ANTIBACTERIAL MATERIAL

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ABSTRACT

The synthesis of chitosan films with the addition of silver nanoparticles (AgNps) has been carried out in 2 stages. The first stage is to make colloidal silver nanoparticles with chitosan as a stabilizer and a reducing agent with NaOH as an accelerator and assisted by microwave irradiation. The second stage is to make a film from colloidal silver nanoparticles-chitosan by casting method. The formation of silver nanoparticles was identified using a UV-Vis spectrophotometer and Transmission electron microscopy (TEM). Chitosan films with the addition of AgNps were characterized by chemical, physical and mechanical properties. Furthermore, the antibacterial activity was tested against *E. Coli* ATCC, *S. Aureus* ATCC and multi-resistant bacteria ESBL and MRSA. The results showed that the formation of AgNps was indicated by the appearance of an absorption band at 400-413 nm with a size of less than 6 nm. The FTIR spectra showed that there was a slight shift at the 1604 cm-1 peak which indicated that the AgNps interacted with the NH_2 group on D-glucosamine of chitosan. The films morphology with addition Ag NPs tends to be rough in surface and cross-sectional. The presence of AgNps tends to increase the swelling value, tensile strength and film elasticity. Chitosan film with the addition of silver nanoparticles has antibacterial activity against multi-resistant bacteria ESBL and MRSA. The antibacterial activity of the film was derived solely from the silver nanoparticles.

***Keyword:** Chitosan film, silver Nanoparticles, antibacterial, multiresistant*

INTRODUCTION

The medicine field is very important in human life so that matters related to the medical world continue to be developed. One of the things that are often developed in the medical world is the polymer used for wound dressings. The wound dressing polymers that are widely used today are synthetic polymers such as polyurethane, polyethylene, polylactide, polyglycolide, and polyacrylonitrile. However, these synthetic polymers have deficiencies against living things, namely, they are weak in adapting to the conditions of living things or have weak biocompatibility. So that the development of biopolymers for medical purposes is needed to replace synthetic polimara wound dressings that are incompatible with living things. The biopolymer properties must be present are non-toxic or non-toxic for living things, do not trigger allergies, are easily sterilized, durable, have

good elasticity, are strong and are biocompatible or suitability with high living things [1].

Chitosan is a natural polymer from isolated chitin that can be obtained from fishery waste such as shrimp shells and crab shells with chitin levels about 65-70 percent. Chitin is a natural polymer that has the most abundance after cellulose with the chemical formula poly (1,4)-acetamide-2-deoxy-beta-D-Glucose or poly (beta-1,4-N-Aceilglucosamine). The deacetylation process of chitin will eliminate the acetyl group and leave a positive charge amino group in acid condition (cationic) and determine the functional properties of chitosan [2].

Chitosan is one of the materials that can be used for biofilm production [3]. The properties of chitosan are non-toxic, biocompatible, can be decomposed, mucoadhesive, bioadhesive, easily chemical modified [3]. Biofilm of chitosan can be used as a good wound dressing because it has antibacterial properties and antifungals so it can inhibit infection and recover wounds faster [4]. In addition, chitosan film has good mechanical properties. But antibacterial properties of chitosan biofilm is low that can be seen in an experiment that showed the inhibition zone of chitosan biofilm did not exist [5]. Therefore the research of making chitosan biofilm that can increase antibacteria properties is needed so it can be used as a wound dressing.

Antibiotic is the most important thing that can overcome a disease caused by bacteria or other microorganisms in the latest 60 years. Resistant bacteria against antibiotics extremely increased [6]. Antimicroba chemotherapy is the most cause of the uncontrolled rise in this 20 century. However, the increased resistant microbe against antibiotic cause health problem case in society [7]. For example, in the latest of this year, the resistant of methicillin-resistant *Staphylococcus aureus* against antibiotic fluoroquinolones cause raising nosomical infection of *Klebsiella pneumonia*, *Serratia marcescens* and *Pseudomonas aeruginosa*. Also more important is the resistance of *Escherichia coli*, *Salmonella species*, *Campylobacter species* dan *Neisseria gonorrhoeae* against fluoroquinolones [8].

Skin and Soft Tissue Infection (SSTIs) is an infection caused by bacteria [9]. Annually observation by SSTIs office showed that there is a raising trend from a pathogen that caused SSTIs such as *S. aureus*, *Pseudomonas aeruginosa*, *E. coli* dan *Enterococcus sp* [9]. The infection trend rose cause of resistant bacteria. Resistant bacteria such as Methicillin-resistant *S. aureus* (MRSA) dan Extended-spectrum beta-lactamase (ESBL) [9].

Anti-bacteria properties of chitosan can be increased with silver nanoparticle addition [5]. Silver is anti bactericide metal that is safe and effective because of its non-toxicity against eucaryotic but is very toxic in bacteria cells such as *E. coli* and *S. aureus* [10]. Based on its properties, silver nanoparticle-chitosan can be a good base material as a covering material in biomedical techniques [11].

The silver nanoparticle can be synthesized using the chemical reduction method [12], [13]. This method is very effective and usually used because it is cheap and easy to do on a large scale. This

method used reductor and stabilizer that influence the quality of nanoparticles [14].

Sodium Borohide ($NaBH_4$) and Hydrazine are reduction agents but have a high toxicity. Using a nature reductor is highly recommended because it is cheap and safe for our environment. Some research claimed nature reductors in making silver nanoparticle are gelatine [15] and chitosan [16] that can also be used as nature stabilizers. (Darraoudi, M., et al., 2010) claimed that using an accelerator can influence the amount and size of silver nanoparticle production [13]. NaOH can be used as an accelerator that can decrease the size and increase the amount of silver nanoparticles that can be comparable with the amount of added NaOH [17].

Synthesis of silver nanoparticles using the chemical reduction method can be accelerated with microwave irradiation. For example, research claimed that the synthesis of silver nanoparticles for 3 minutes using microwave irradiation [18] compared with other research that used the same reductor and stabilizer needed 3 hours in making silver nanoparticles at room temperature (25^oC) [15].

In this research, chitosan films were synthesis with silver nanoparticle addition using microwave irradiation and NaOH as an accelerator was needed. This research studied the influence of precursor $AgNO_3$ against the number of silver nanoparticles based on the *Localized Surface Plasmon Resonance* (LSPR) phenomenon that can be identified using a UV-Vis Spectrophotometer. The film was also characterized by chemical, physical and mechanical properties, and examined antibacteria activity against multiresistant bacteria MRSA (Methicillin-resistant *S. aureus*) and Extended-spectrum beta-lactamase (ESBL).

METHOD

1. Chemical

Chitosan from shrimp waste with the high molecular weight used in this study was purchased from Biotech Surindo Cirebon, Indonesia. Glacial acetic acid, Sodium hydroxide and silver nitrate produced by Merck, and aquadest produced by UPT Laboratorium Terpadu Universitas Sebelas Maret Surakarta Indonesia.

2. Preparation of AgNps chitosan colloidal.

Chitosan solution 1% (w/v) in glacial acetic acid 1 % (v/v) was prepared by stirring using a stirrer until homogeneous. Chitosan solution is stored for 24 hours for complete dissolution. Chitosan solution of 12.5 mL was mixed with $AgNO_3$ 1.2% (w/v) in at various of volume (of 0.25; 0.50; 0.75; 1.00; 1.25; and 1.50 ml) and was stirred for 5 minutes. NaOH solution 2M was added and was being mixed by a stirrer for 5 minutes. The mixed solution was entered in the microwave and was irradiated by microwave with 100 watts for 4 minutes. The solution was added with 47.5 ml chitosan solution and was stirred until homogenous. Variations in $AgNO_3$ concentrations represent variations in $AgNO_3$ concentrations, namely 0.5%; 1.0%; 1.5%; 2.0%; 2.5% and 3.0% (w/w; $AgNO_3$/Chitosan) with the codes A_1, A_2, A_3, A_4, A_5, A_6.

3. Preparation of AgNps chitosan film

The film was formed from AgNps chitosan colloidal with codes A_1, A_2, A_3, A_4,

A_5, A_6 through casting into polyethylene molds, then it was left until it was dried. The dried film was neutralized by adding 1ml NaOH Solution and then was peeled off from the molds and it was rinsed using distilled water until neutral of PH. Then the film was put on a glass plate until it was dry. The films are coded FA1, FA2, FA3, FA4, FA5.

4. Film Characterization

Identifiying Ag nanoparticle was carried out at 300-600 nm using Spekrofotometer UV-Vis Shimadzu UV3150. Before analysis, silver nanoparticle-chitosan colloidal was dilution for 5 times. The shape and size of silver nanoparticles were identified by Transmission Electron Microscopy (TEM) JEM-1400 with power 80KV.

Scanning Electron Microscope (SEM) analysis. The morphology of film across the surface and the upper surface can be identified by SEM Zeiss DSM 960 with 2500 times of magnification. Fourier Transform Infra-Red (FTIR) analysis. To identify the interaction between silver nanoparticle and chitosan in the silver nanoparticle-chitosan film the transmitant was measured using FT-IR 8201PC in the wavelength 4000-1000 cm^{-1}

Swelling property measurements. Testing of swelling properties or water absorption capacity on Ag NPs chitosan film was measured using phosphate buffer solution (pH 7.4) at room temperature. The film was cut to 2x1 cm size and then weighed initially (W_0). Then the film was immersed in phosphate buffer solution for 30 minutes, and then the film was dried using filter paper and then weighed back (W_t). Do this step three times with each film. Percent swelling (S) was formulated as:

$$\%S = (W_t - W_o)/W_o$$

Mechanical properties measurement. The mechanical test of chitosan film with the addition of Ag NPs was carried out to determine the tensile strength, elongation and elasticity of the film using tool l. Testometric material testing machines.

Antibacterial Test. Antibacterial activity tests were carried out toward *E. Coli* ATCC, *S. Aureus* ATTC, ESBL, MRSA. Antibacterial activity testing was carried out with the diffusion method [19]. The test was carried out by observing the inhibition zone of the sample on bacterial growth. Sticking the cut film into the media that already contains the bacteria. Observe the results and see the edible inhibition zone of the film against the growth of bacteria.

RESULTS AND DISCUSSION

1. Preparation of Silver Nanoparticle Colloids in Chitosan

The synthesis of chitosan colloid containing silver (Ag) was carried out through a reduction process using microwave radiation. The addition of $AgNO_3$ was done by dropping it on the chitosan which was stirred. $AgNO_3$ actsed as a precursor to Ag nanoparticles. The solution was produced after the addition of $AgNO_3$, which was originally clear to brownish-yellow and darker with the increase in the concentration of $AgNO_3$, was as shown in Figure 1.

Figure 1. Silver nanoparticles in chitosan solution at various concentrations $AgNO_3$

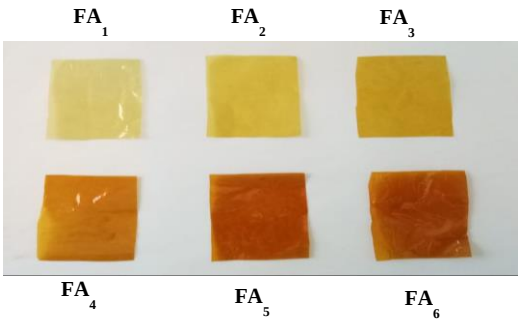

Figure 2. Chitosan Film with Ag nanoparticles at various concentration

Then a film with various concentrations would be produced as the data above which was coded FA_1, FA_2, FA_3, FA_4, FA_5, FA_6. The resulting film from variations in the concentration of AgNO3 with a brownish yellow colour that darkens as the addition of the concentration of AgNO3 in the film can be seen in Figure 2.

2. UV-visible spectroscopy analysis.

The formation of AgNps in chitosan solution can be identified using a UV-Vis spectrophotometer based on the LSPR phenomenon. The appearance of the absorption band at about 400 nm indicates the formation of silver that can be seen in Figure 3.

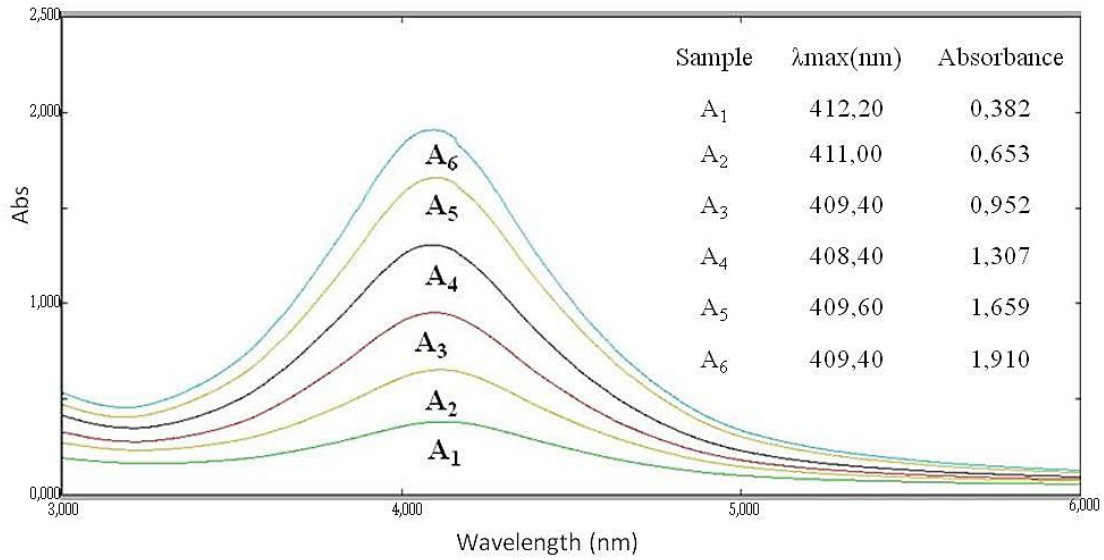

Figure 3. UV-Vis Spectra of silver Nanoparticle Colloidal

Figure 3 can be seen that the absorbance peak of the sample is at a wavelength of 408.40-412.20 nm. This indicates the formation of silver nanoparticles. The number of silver nanoparticles can be identified by UV-VIS because there is a Local Surface Plasmon Resonance phenomenon in the silver nanoparticle system. LSPR is the collective excitation of electrons in the conduction band near the surface of the metal nanoparticle. Electrons are limited to specific modes of vibration by particle size and shape.

Metal nanoparticles have a characteristic optical absorption spectrum in the UV-Vis region. Therefore the LSPR phenomenon can be observed by UV-Vis spectroscopy[20]. The LSPR peak adsorption is in visible light at wavelengths about 400 nm. The concentration of Ag nanoparticles increases in line with the intensity of absorbance. Figure 3 show that the higher the concentration of AgNO3 used, the higher the concentration of Ag nanoparticles produced. The LSPR phenomenon that appears in the 400 nm wavelength range shows that silver nanoparticles is spherical in shape [15].

3. Transmission Electron Microscopy (TEM) analysis.

To analyze the shape and size of the nanoparticles, characterization was carried out using TEM. Figure 4 shows the particle size distribution based on the TEM images for samples A_3 and A_6. Based on Figure 4, the amount of nanoparticle distribution in the A_3 and A_6 samples is also in accordance with the addition of the Ag concentration which will increase the number of nanoparticles produced. The appearance of LSPR single absorption in UV-VIS testing in the wavelength range of 400 nm shows that the particles are spherical, this is consistent with the results of testing using TEM in Figure 4 which shows the particles are spherical. The average the size of silver particles for samples A_3 and A_6 are 6.17 nm and 5.41 nm respectively.

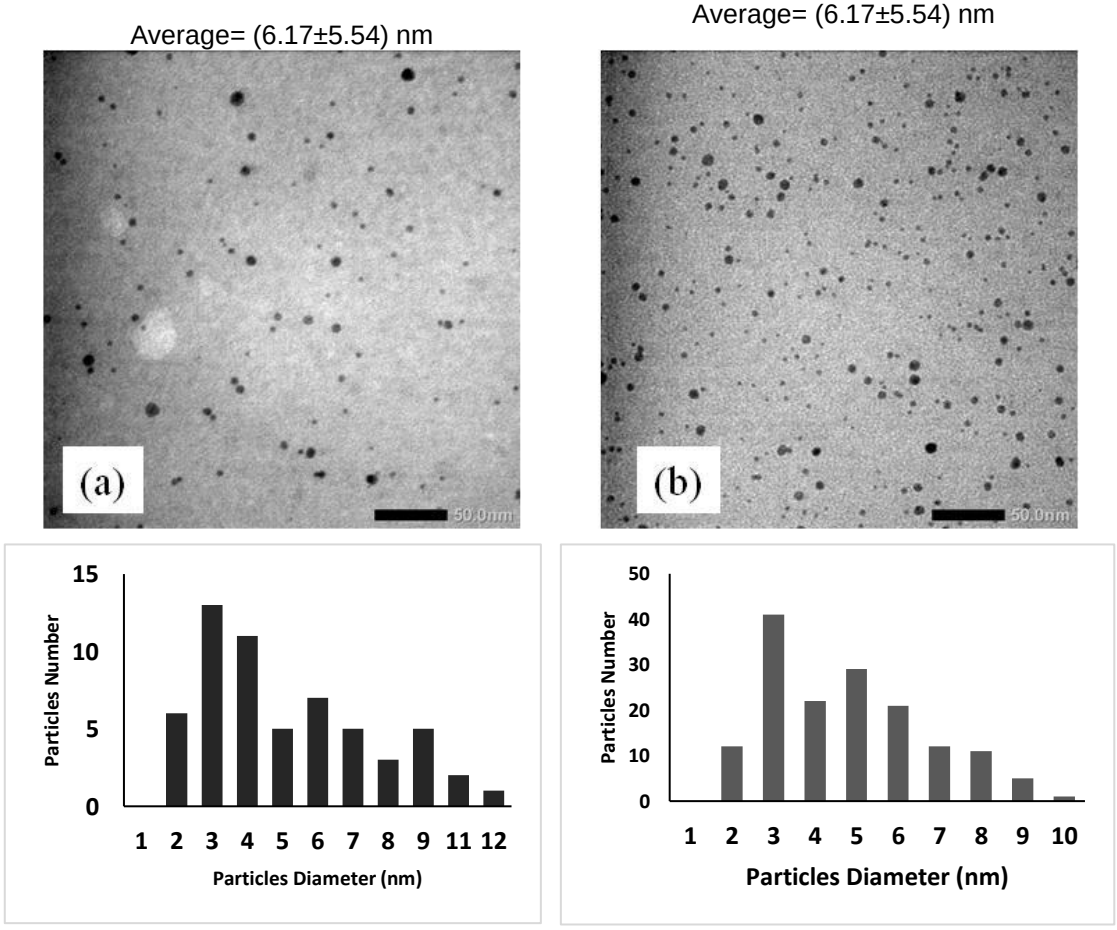

Figure 4. TEM Image and size distribution of silver nanoparticles (a) sample A_3, (b) sample A_6.

4. Scanning Electron Microscope (SEM) analysis

Based on the SEM analysis, it was found that the difference in morphological structure between the FA0 and FA4 samples. Based on the SEM test or the results of the surface morphological analysis carried out with a magnification of fifty to two thousand five hundred times with 2 sample films, including chitosan film and 1 ml chitosan/Ag film. Based on the characterization results, it can be seen that the chitosan film has dense structural characteristics, does not occur cracks, and has a smooth surface. Whereas the addition of Ag made the structure even less flat, there were quite a lot of cracks. The cracks are caused by the expanding polymer chains of chitosan due to interactions or bonds with Ag NPs. However, for the film surface of all samples obtained a film with a relatively smooth and even surface cross-section. On the surface of the film, there are also some disturbing particles or insoluble substances. The results of SEM analysis can be seen in Figure 5.

5. Fourier Transform Infra-Red (FTIR) analysis.

Based on the Fourier Transform Infrared Spectroscopy (FTIR) test that has been carried out, the comparison of the FTIR spectrum in the fingerprint area between chitosan film and chitosan/Ag NPs film was obtained. The FTIR test results can be seen in Figure 6.

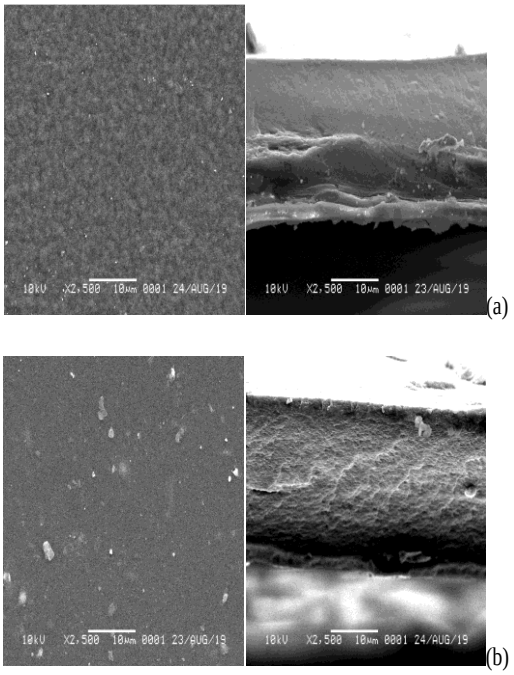

Figure 5. SEM Images (a) FA_0, (b) FA_4

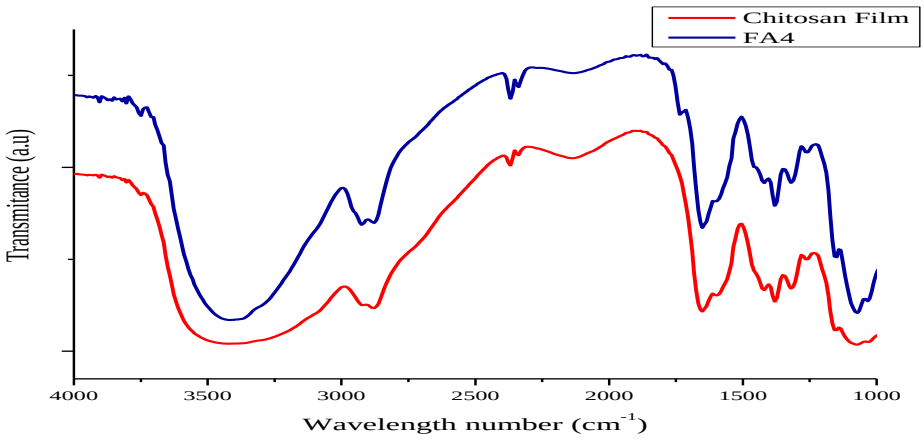

Figure 6. Spectra FTIR of chitosan film and FA4

Based on Figure 6, the characteristics of the chitosan film can be seen from 3417.86 cm-1 which is the overlap of the OH stretch with the NH stretch, at 1651.07 cm-1, there are a carbonyl group C = O vibrations from the amide group and at 1072.42 cm-1 shows the CN vibrational strain of all the amines. Then a band appears at a wavelength of 1597.06 cm-1, which is bending on NH2.

The peak characteristic of the chitosan/Ag NPs film at 3417.86 cm-1 which is the stretching vibration of OH and NH, 1651.07 cm-1 with a higher intensity is the C = O stretching and the bending of NH from the primary amide, 1072, 42 cm-1 represents the stretch of all amines of higher intensity. And the NH2 bending band experienced a shift which was shown at a wavelength of 1604.77 cm-1 due to a reduction in the bond length. This shows that Ag NPs can bind to NH. The presence of a strong band due to O-H and N-H stretching indicates a more even distribution of the O-H group due to the N-H group involved in the binding of Ag NPs. The N-H and O-H groups have a high affinity for the Ag ion. The difference in the electronegativity of O and N plays a role in the deprotonation side which affects the binding of free electrons from silver.

6. Swelling property measurements

Swelling test aims to determine the ability of the material to absorb water which causes swelling. The addition of Ag in the swelling test of chitosan film fluctuated in affecting the ability to absorb water, as shown in Figure 7. However, compared to chitosan film without the addition of Ag NPs, chitosan film with the addition of Ag NPs tended to increase its ability to absorb water. This increase in swelling is caused by the presence of silver which causes the film to be more hydrophobic. Increasing the percent of swelling is also caused by the cavities in the film being filled with Ag particles so that physically it causes fractures as in the SEM image in Figure 7. This causes the film to absorb water more easily.

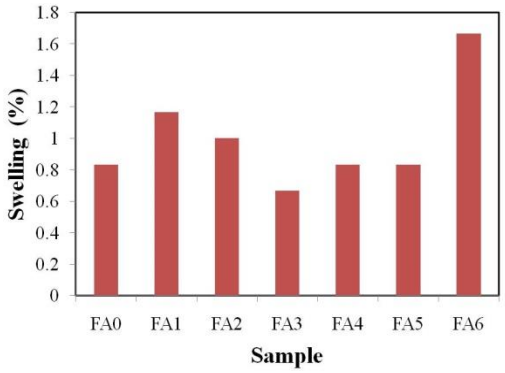

Figure 7. Swelling of Chitosan Film with Variation of Ag Concentration

7. Mechanical properties measurement.

The mechanical strength test results obtained include the tensile strength, elongation and elasticity tests of the film as shown in Figure 8.

Chitosan film with the addition of Ag NPs can increase the tensile strength of the film. The variation in the concentration of Ag NPs on the film gave a fluctuating tensile strength effect but tended to increase with the addition of the Ag NPs concentration in the film. [21].

Meanwhile, for the film elasticity (Modulud Young) from the ratio between tensile strength and film elongation, the addition of Ag NPs increases the elasticity of the film produced. The addition of the Ag NPs concentration to the chitosan film resulted in an increasing film elasticity with the addition of the Ag NPs concentration.

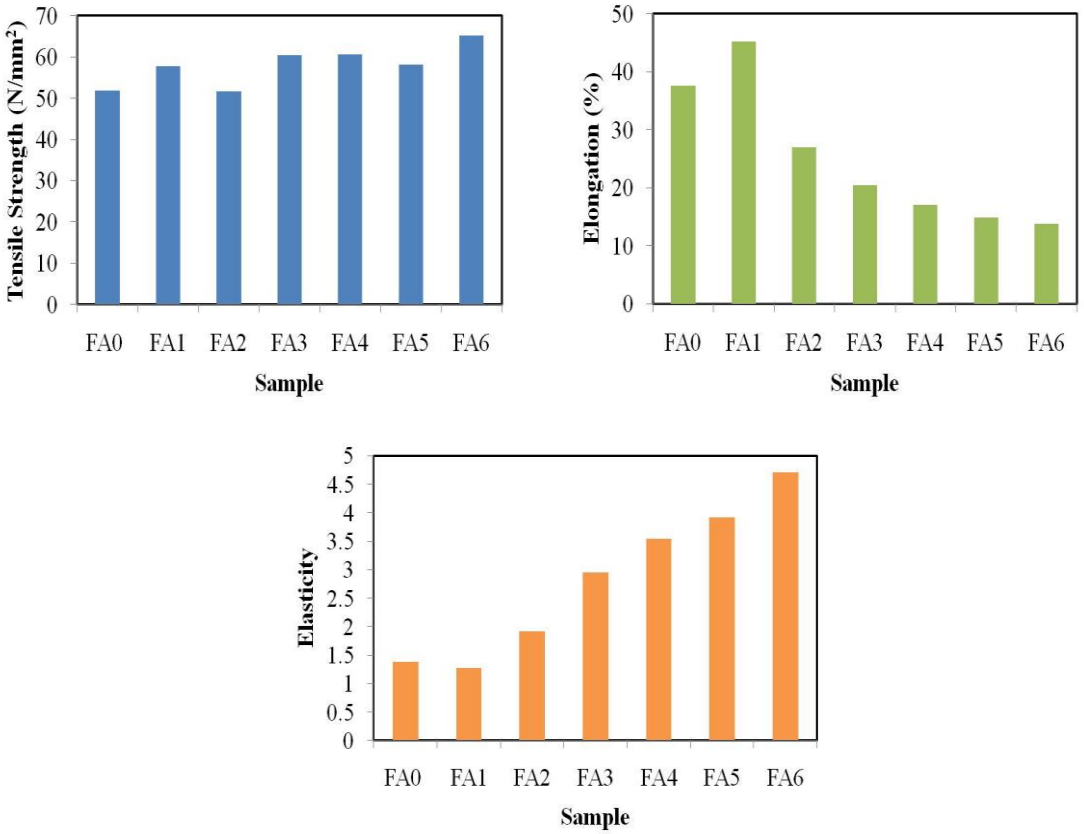

Figure 8. Mechanical properties of Chitosan Silver Nanoparticle Film (a) Tensile Strength, (b) Elongation, (c) Elasticity

8. Antibacterial Activity Test

Based on the antibacterial test, it was found that the presence of Ag nanoparticles would increase the antibacterial properties, as evidenced by the inhibition zone with a solution and film containing Ag NPs. The inhibition zone that appears is a sign of silver release or the release of Ag in the form of ions from the film which can eliminate the replication ability of DNA and turn off cellular proteins due to the binding of protein groups by Ag^+ ions [22]. This can be seen from Figure 9, where chitosan samples without Ag nanoparticles and chitosan samples with the addition of Ag nanoparticles.

Based on the data in the diagram, it can also be seen that the sample solution A0 (chitosan solution without Ag) has no inhibition zone in non-multiresistant bacteria because there is no silver release event. However, in the A0 sample, an inhibition zone appeared in multiresistant bacteria, this is because chitosan itself has antibacterial properties. The antibacterial mechanism of chitosan, among others, is by absorbing the cell surface, then diffusion occurs and disrupts the balance of the cytoplasmic membrane so that the cells become damaged and die. So that the chitosan solution is quite effective for multiresistant bacteria with the appearance of an inhibition zone on the plate.

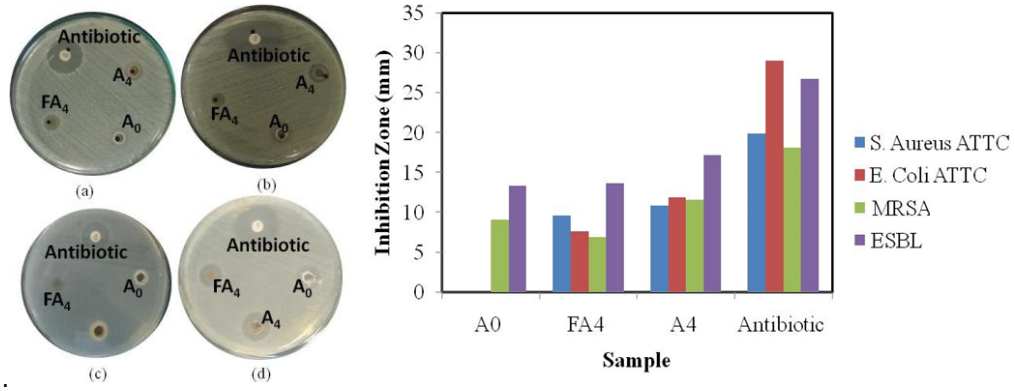

Figure 9. Antibacterial Activity against (a) *S. Aureus* ATTC, (b) *E. Coli* ATCC, (c) MRSA and (d) ESBL

Chitosan film with the addition of Ag NPs with varying concentrations has antibacterial activity against gram-positive bacteria, both non-multiresistant bacteria (S. Aureus ATTC) and multiresistant bacteria (MRSA) shown at Figure 10. The antibacterial activity of chitosan film with the addition of Ag NPs is higher in gram-positive bacteria which are not multiresistant. However, the inhibition zone in the medium containing multiresistant gram-positive bacteria indicates that Chitose film with the addition of Ag NPs is effective as a gram-positive multiresistant antibacterial material.

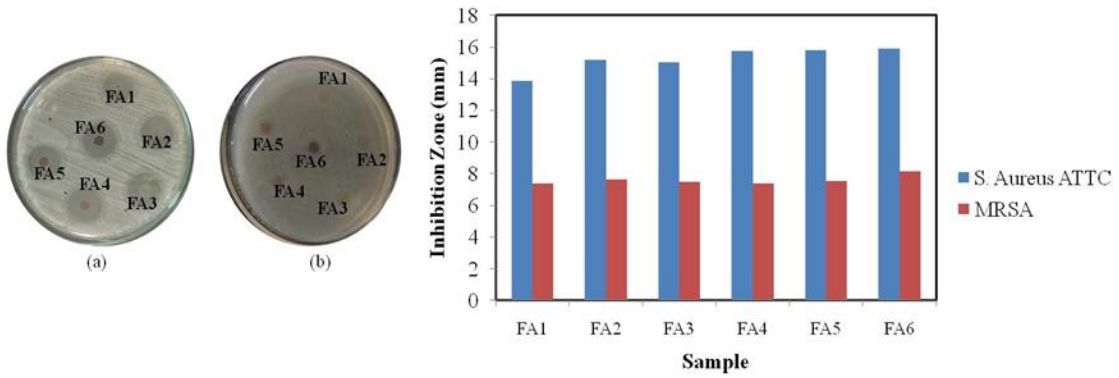

Figure 10. Antibacteria Activity silver-chitosan film against gram positive bacteria (a) *S. Aureus* ATCC, (b) MRSA

Figure 11 showed that chitosan film with the addition of Ag NPs with various concentrations has antibacterial activity against gram-negative bacteria, both non-multiresistant bacteria (*E. Coli* ATCC) and multiresistant bacteria (ESBL). From the histogram data in Figure 11, the anti-bacterial activity of chitosan film with the addition of Ag NPs was higher in multiresistant gram-negative bacteria than the antibacterial activity in non-multiresistant gram-negative bacteria. So, it can be concluded that chitosan film with the addition of Ag NPs is effective as a multiresistant antibacterial material.

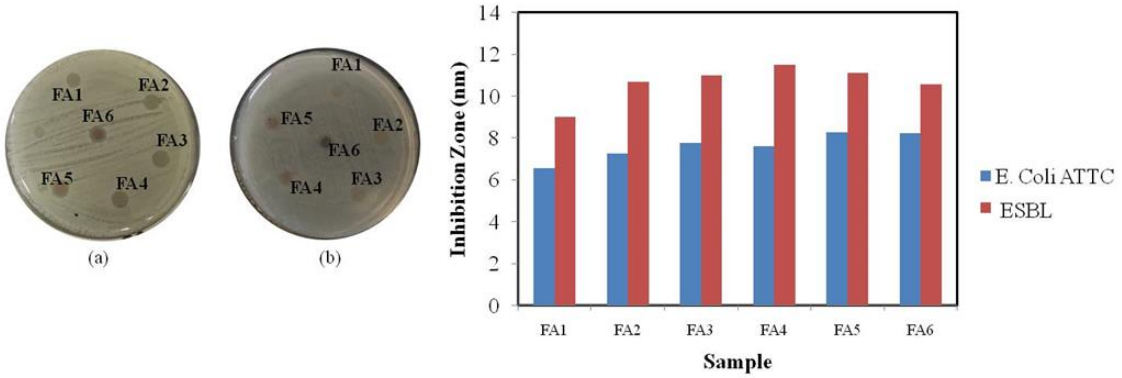

Figure 11. Antibacterial activity of silver-chitosan film against gram-negative bacteria (a) *E. Coli* ATCC, (b) ESBL

CONCLUSION

The results showed that the formation of nanoparticles was indicated by the appearance of the LSPR absorption band at 400-413 nm. The silver nanoparticles were spherical in shape with a size of less than 6 nm. The number of silver particles is influenced by the concentration of $AgNO_3$ as a precursor. The presence of Ag NPs affects increased the swelling properties, tensile strength and elasticity. The films morphology with addition Ag NPs tends to be rough in surface and cross-sectional. The chitosan films with the addition of Ag NP s have antibacterial activity against both standard bacteria (*E. Coli* ATCC and *S. Aureus* ATCC) and multiresistant bacteria including (MRSA and ESBL bacteria). The antibacterial activity of the chitosan film was influenced by the amount of Ag NPs.

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