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Afrizal Lathiful Fadli, Absari Hanifah, Ana Fitriani, Anna Rakhmawati, and Wipsar Sunu Brams Dwandaru

Application of Silver-Chitosan Nanoparticles as a Prevention and Eradication of Nosocomial Infections Due to *Staphylococcus aureus* sp)

Afrizal Lathiful Fadli[1,a)], Absari Hanifah[2,b)], Ana Fitriani[3,c)], Anna Rakhmawati[2,d)], Wipsar Sunu Brams Dwandaru[1,e)]

[1]*Physics Department, Yogyakarta State University, Jl. Colombo No 1 Yogyakarta*
[2]*Biology Department, Yogyakarta State University, Jl. Colombo No 1 Yogyakarta*
[3]*Mathematics Department, Yogyakarta State University, Jl. Colombo No 1 Yogyakarta*

[a)]Corresponding author: afrizallathiful@gmail.com
[b)] absarihanifah@gmail.com
[c)] ana.fitriani2016@student.uny.ac.id
[d)] annna_rakhmawati@uny.ac.id
[e)] wipsarian@uny.ac.id

Abstract. Nosocomial infections in the hospital cause height level of morbidity and mortality. WHO research referring to nosocomial infections is approximately 8.7% of 55 hospitals from 14 countries in Europe, Arabian, and Asia. *Staphylococcus aureus* constitutes bacteria that cause nosocomial infections. Objectives of the research are to know how the step, characteristic, and activity of antibacterial for *S.aureus* from nanoparticle silver-chitosan, and the combination of both materials. The principle of nanosilver particle has antibacterial activity, was proved that silver ions are able to make strong inhibitions on bacteria cell wall. Silver ions will damage and penetrate cell wall bacteria then alternating of DNA bacteria. This condition makes fatality for bacteria. Chitosan was found by chitin deacetylation polyatomic with positive content and pH less than 6,5. Cationic of chitosan can make interaction with the cell wall of bacteria. Material combinations will make interactions and cause the damages on bacteria cell walls and can drag off the growing of bacteria cause of nosocomial infections. The result of the research indicated that silver nanoparticles with spectrums UV-Vis characterization, maximum absorption shows at wavelength 419 nm. The combination of silver nanoparticles and nanochitosan can drag the growth of bacteria *S. aureus*. The even more silver nanoparticle is working better than nano chitosan as antibacterial. As the conclusion, the application of Silver-Chitosan nanoparticles is effective to drag the growth of bacteria 0.62 cm in nutrient agar or medium of *S. aureus* that causes nosocomial infections.

INTRODUCTION

Many nosocomial infections occur with the highest incidence in poor and developing countries. In 2006 WHO has conducted a study of 8.7% of 55 hospitals from 14 countries located in Europe, the Middle East, Southeast Asia and the Asia Pacific with nosocomial infections and 10% in Southeast Asia [1], whereas in the United States there are 20,000 deaths each year from nosocomial infections[2]. Since 2004 Indonesia has researched 11 hospitals in DKI Jakarta and showed that 9.8% of inpatients suffer from nosocomial infections [3]. Nosocomial infections are mostly caused by microorganisms, which in humans previously did not or rarely cause infections in healthy people. The most common bacteria that cause nosocomial infections are *S. aureus*[4]. *S. aureus* is the leading cause of nosocomial infection. Direct contamination between *S. aureus* in open wounds such as post-surgery wounds is one of the causes of nosocomial infection[5]. *S. aureus* is a normal bacteria of the skin, respiratory tract, and food digestive tract in humans. However, it can also be in the air and the environment. Pathogenic aureus is invasive, causing hemolysis, forming coagulase, and dispensing mannitol [5]. *S aureus* is organized into irregular groups

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(colonies) like grapes. The more these colonies are found, the higher the incidence of infection by *S. aureus* [6]. Silver nanoparticles have antibacterial properties; silver anti-bacterial properties have been used in the early 1000 BC to keep water safe. Additionally because of its properties as a silver nanoparticles antibacterial are also used for wound care, catheters, and various household products. The application of antibacterial silver nanoparticles will make sterile and can be used in hospitals to prevent and minimize infection with pathogenic bacteria such as *S. aureus* [7]. Chitosan is one of the compounds used in pharmaceutical as antibacterial. Chitosan can be used as an antibacterial because of its positive charge ability that interacts with the bacterial cell surface negatively charged, thus disrupting the growth of bacterial colonies [8]. Therefore, the authors take the initiative to conduct research: Application of Silver-Chitosan Nanoparticles as a Prevention and Eradication of Nosocomial Infections Due to *Staphylococcus aureus* sp.

RESEARCH METHODS

Silver Nanoparticles

First, made $AgNO_3$ solution with 5 mM concentration with $AgNO_3$ powder as much as 0.17 gram and dissolved into 200 ml aquades with beaker glass and stirred evenly, then took 10 ml of $AgNO_3$ solution into the reaction tube and boiled, then lifted the test tube and add 10 drops of 1% of Trisodium Citrate ($Na_3C_6H_5O_7$) solution, reheated it until the solution was turned into yellow.

Nanochitosan

The first steps to make Nanochitosan solution, was weigh the pure chitosan mass as much as 0.5 gram and add 0.1 M acetic acid as much as 10 ml and stir until dissolved, then shaken until 18 hours.

Antibacterial Test

a. Sterilization tool

The sterilization of the tool begin with prepared all the equipment and wrapped all the tools with a paper umbrella and then sterilized on autoclave for 15 minutes with a temperature of 121 °C.

Then put on the oven to dry the tools to be used.

b. Preparation of NA and NB Liquid Medium

Prepared of 14 grams of NA medium in 500 liters of distilled water. Then prepared the liquid NB medium to renew the *S. aureus* bacteria, weighed 1.95 grams at 150 ml water. Then put into autoclave the liquid NB medium.

c. Isolation of bacteria in liquid NB

Isolation was performed to renew S. aureus bacteria grown on solid NA medium. The isolation was performed sterile by transferring bacteria to solid medium poured liquid NB then planted on NB liquid medium. Then performed a shaker to accelerate the growth of bacteria because S. aureus is aerobic bacteria. Shaker performed for 18 hours with a speed of 160.

d. Solution preparation and Immersion of Paper Discs

Prepared of a solution for immersion of sterile disc paper, carried out simultaneously. The first step is to prepare a small Petri dish and poured a solution each of which is 10 ml. The prepared solution was a sterile distillation solution as a positive control, a solution of chloramphenicol 0.002 gr at 10 ml of aquades, 10 ml of nanosilver solution, 0.5 gram of chitosan nano solution at 10 ml of distilled water, and a 5 ml silver-chitosan nanoparticles solution. Then sterilized in the LAF, ready-to-insert paper and immersed in a solution of about 10 minutes.

e. Planting Bacteria and Paper Discs on Solid Medium

Bacterial planting was done by preparing S. aureus bacteria on the liquid medium of NB then prepared solid NA media. Bacterial sampling was done by micropipette of 1 ml. Then the bacteria was poured in a medium for solid, flattened with Rogalski and then attached each disc paper to the solid media that has been planted bacteria that have been divided into 5 same quadrants. Once planted the Petri dish was closed and incubated at room temperature 25˚C then waited for 24 hours after planting.

f. Measurement of Antibacterial Inhibition

Measurements of antibacterial inhibition were performed to determine the extent of antibacterial effects. Measurements made by measuring inhibition with the slide term. The paper disk diameter is used as a medium that absorbs 0.5 cm long solution. While the inhibition is measured from the paper boundary parallel to the inhibition that occurs. Then the measurement and recording of the inhibition results.

RESULT AND DISCUSSION

Results of Silver-Chitosan Nanoparticles

1. Silver Nanoparticle Results

In this study silver nanoparticles were synthesized using chemical methods through the reduction of $AgNO_3$ powder using trisodium citrate,

$$6AgNO_3 + 2Na_3C_6H_5O_7 + 22H_2O \rightarrow 6Ag + 6NaNO_3 + 15H_2 + 12H_2CO_3$$

Silver nanoparticles which has been synthesized in pale yellow were then characterized by UV-Vis instrument, the result of this characterization was the relationship between wavelength with absorbances. This test showed that the formation of silver nanoparticles was characterized by a typical absorption peak at λmax 419 nm. This was in line with Ahmad et al. 's research [9] where the silver nanoparticles uptake spectra are formed on λ max 415-430 nm. This showed that the synthesis of silver nanoparticles had been synthesized.

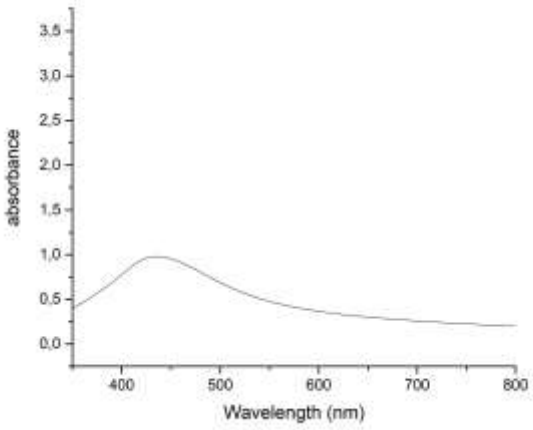

FIGURE 1. Silver nanoparticle test result with UV-Vis

2. Nanochitosan Result

Chitosan was obtained from polyaromatic and positively charged chitin deacetylation with a pH below 6.5. The basic structure of chitosan comprises amino, hydroxyl and thermal groups of group aldehyde ((1-4) -2-amino-deoxy-D-glucose). The cationic nature causes chitosan to interact with the microbial cell membranes; chitosan has the ability to bind metals and formed a chitosan-metal complex [10]. FTIR test showed that there was no absorption in the 1655-1700 cm- region indication the absence of C carbonyl groups and still had an uptake in the OH and NH_2 regions.

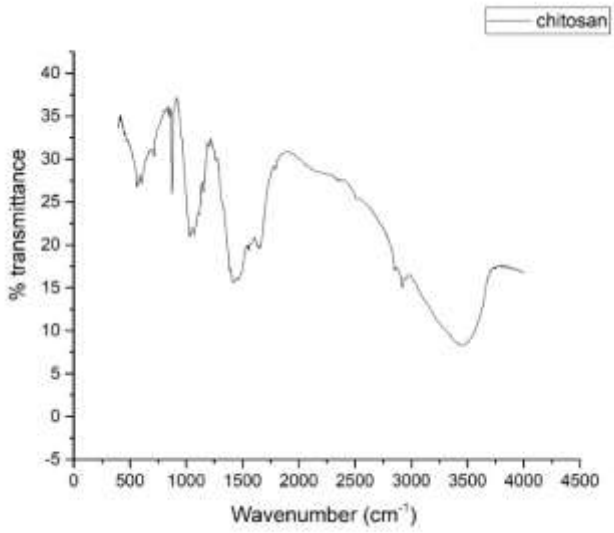

FIGURE 2. The result of silver nanochitosan test with FTIR

3. Mix Silver Nanoparticles with Chitosan

The surface modification provided a considerable tendency to overcome aggregation, mixed silver nanoparticles with nanochitosan could make silver nanoparticles stable, because nanoparticle stability could be done in 2 ways, ie electrostatic stability and steric stabilization. In this study, the stabilization of silver nanoparticles was performed with steric stabilization by chitosan because chitosan has a $–NH_2$ group that could interact with silver.

DISCUSSION

1. Antibacterial activity of silver-chitosan nanoparticles

Antibacterial activity of silver-chitosan nanoparticles can be effective as an anti-bacterial in drawing the growth of S. aureus bacteria as one of the causes of nosocomial infection disease. Staphylococcus aureus is a round-gram-positive bacterium with a diameter of 0.7-1.2 μm, arranged as an irregular grape, anaerobic faculty, does not form spores, and does not move. These bacteria grow at an optimum temperature of 37 ºC but form the best pigment at room temperature (20-25 º C). More than 90% of clinical isolates produce S. aureus with polysaccharide capsules or thin films that play a role in bacterial virulence [6]. Nosocomial infections are caused by pathogenic bacteria such as S. aureus which is a gram-positive bacteria, Pseudomonas aereginosa, Escherichia coli, Klebsiella pneumonia which is a gram-negative bacteria. According to WHO, one of the most prevalent nosocomial infections is postoperative wound infections and the second most common cause of urinary tract infections. Postoperative wound infections are

a major cause of morbidity and mortality [2]. Based on the characteristic of S. aureus bacteria that can cause nosocomial infection, the research done resulted in data inhibition of S. aureus bacteria growth. The antibacterial mechanism of silver nanoparticles according to [11] involves the activity of the silver-chitosan nanoparticles in inhibiting bacterial growth by penetrating the bacterial cell membrane and altering its structural composition by interacting with the bacterial sulfate group, which is the enzyme's active site. Furthermore, silver ions disrupt the essential nature of bacterial survival by blocking some of the bacterial enzymes that regulate energy metabolism and electrolyte transport. This lack of enzyme activity in the end bacteria lack energy, besides this dominant silver ion also blocks the process of bacterial replication by interrupting their DNA backbone. Finally, silver ions bind to bacterial cell walls to weaken the cell's protection and structure, thus creating structural imperfections within the protective cell layer and accelerating the collapse or explosion of bacteria. Therefore, by targeting these three areas, silver ions prevent bacteria from breeding by forming defense systems, slowing bacterial growth, and ultimately killing bacteria.

The results of the antioxidant silver-chitosan nanoparticles testing are as follows:

TABLE 1. The result of measurement of inhibition diameter

Number	Repeat the Sample to	Inhibition diameter (cm)				
		L1	L2	L3	L4	L5
1	A	0	0,62	0,6350	0,36	0,81
2	B	0	0,20	0,4400	0,32	0,60
Average		0	0,41	0,5375	0,34	0,705

Information:
L1: aquadest solution
L2: a silver-chitosan nano solution
L3: silver nano solution
L4: chitosan nano solution
L5: chloramphenicol solution

Based on existing inhibitory data it can be concluded that inhibition of silver-chitosan nanoparticles is more effective than nano chitosan which has an inhibitory diameter of 0.41 cm compared to 0.34 cm. In this study, the use of nano-chitosan is intended as an antibacterial stabilizer. Based on the theory expressed [12]states that the stabilization of nanoparticles can be done in two ways, namely electrostatic stabilization and steric stabilization. Electrostatic stabilization is the ionic adsorption on the surface to form a double layer of the metal and produces a Coulomb repulsion force between individual particles. Steric stabilization is the blanket around the metal center by a sterically bulky material layer, such as surfactants and polymers. While in this study silver nano stabilization is done by steric stabilization by chitosan. Chitosan has a $–NH_2$ group that can interact with Ag. Utilization of chitosan-silver nano modification as a metal ion sensor is carried out using utilization of amine groups present in chitosan.

Therefore, the application of silver-chitosan nanoparticles is effective as an antibacterial for S. aureus bacteria, since it can inhibit its growth with an average inhibition of 0.41 cm diameter and the highest inhibition is 0.62 cm.

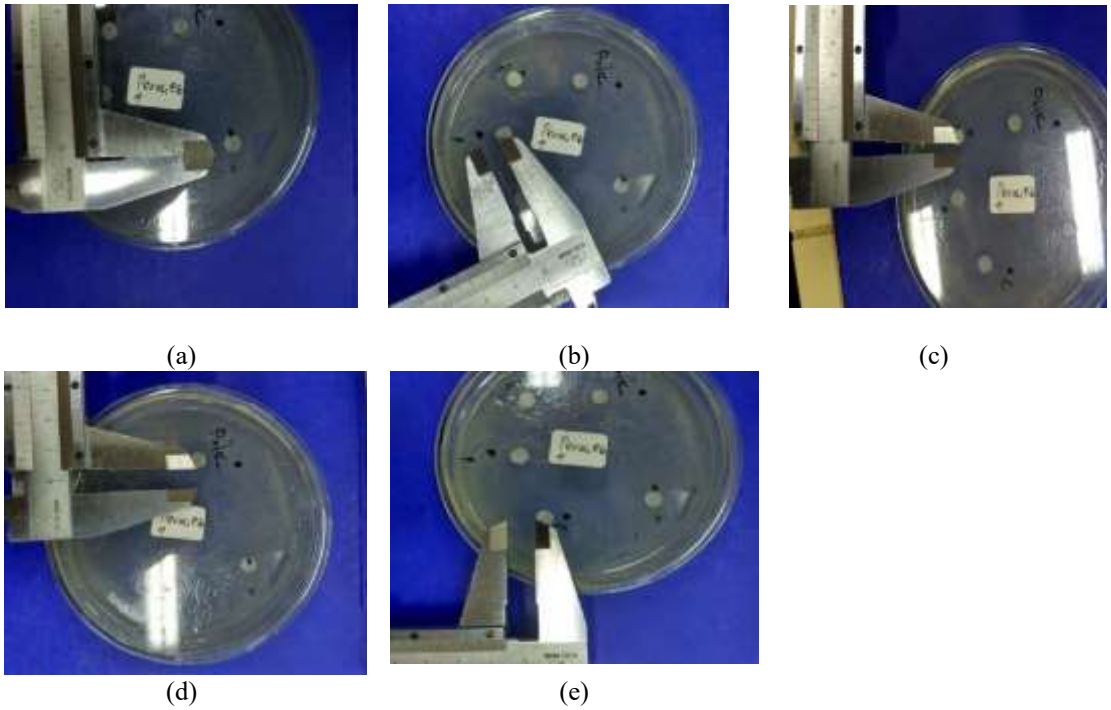

FIGURE 3. The result of measurement of inhibition of S. aureus bacteria diameter. a) Sample A diameter inhibition with aquades, b) Sample A diameter inhibition with silver nano, c) Sample A inhibitory diameter with nano chitosan, d) Sample A inhibitory diameter with the chitosan-silver nanoparticle, e) Sample A inhibitory diameter with chloramphenicol.

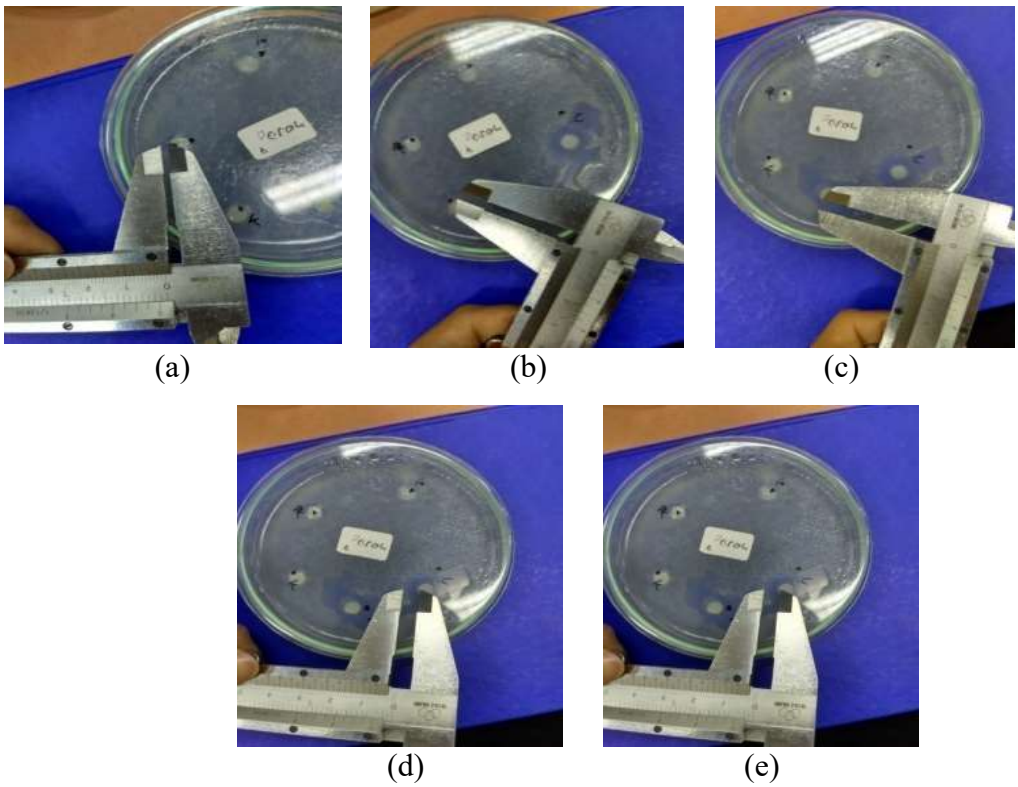

FIGURE 4. The result of measurement of inhibition of S. aureus bacteria diameter a). Sample B inhibition diameter with Silver Chitosan Nano, b). Sample B inhibition diameter with Chitosan Nano, c) Sample B inhibition with Silver Nanoparticle, d). Sample B inhibition diameter with chloramphenicol, e). Sample B diameter inhibition with aquadest

SUMMARY

The silver nanoparticle characterization using UV-Vis spectrometer shows the absorption peak at a wavelength of 4.19 nm, whereas the characterization of nanocitosan with FTIR instrument shows functional group (NH_2). Antibacterial activity of silver-chitosan nanoparticles can be used as an anti-bacterial in inhibiting the growth of S. aureus bacteria as one of the causes of nosocomial infection disease. The inhibition of silver nanoparticle and nanochitosan mixture on the growth of S. aureus bacteria was 0.62 cm on average.

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