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Research Article

The Reductive Synthesis of Silver Nanoparticles Using Sodium Citrate and Their Potential Medical Uses in Skin Diseases

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Abstract:

This study investigates how silver nanoparticles develop and how they might be used as an antibacterial agent to prevent and treat wound infections. The reduction procedure was used to create silver nanoparticles—sodium citrate functions as both a lowering and a stabilizing agent. The properties of the liquid silver nanoparticles were then examined using a UV-Vis Spectrometer, a Particle Size Analyzer (PSA), and a Transmission Electron Microscope (TEM). UV-Vis studies show that nanoparticles produced with 1% sodium citrate are the most stable. PSA and XRD analyses reveal that the smallest silver nanoparticles are approximately 8 nm, with an average size of approximately 30.2nm. According to TEM examination, silver nanoparticles with an FCC crystal structure. In studies, pathogenic microorganisms such as *E. coli, Bacillus subtilis, and Staphylococcus aureus* were employed to assess how successfully the washing process inhibited bacterial growth. The quantitative research demonstrates that increasing the soaking period to 36 hours kills all germs and results in a 100% reduction. The best time to soak wound therapy linen in synthetic silver nanoparticles was 36 hours.

Keywords: Nanoparticles, silver, antibacterial, wound dressing.

Introduction

The field of nanotechnology has recently experienced significant growth and development, and the study of metal nanoparticles (NPs) has attracted much experimental interest. Scholars' interest in and study of nanotechnology has grown significantly in recent years. Nanomaterials have the dimension of synthesis and material manipulation at the nanoscale because of their distinct physical and chemical properties[1]. Nanoparticles of the noble metals silver (Ag) and gold (Au) play important roles in various sectors. Burn wound treatment, dental implant materials, rust-resistant coating materials, cosmetics, and medicinal applications such as anti-cancer, antibacterial, and antioxidant are among the applications of these nanoparticles [2].

Metal nanoparticles have an antimicrobial effect because of their capacity to attach to protein molecules in microbial cells, interrupting microbial metabolic activity and killing bacteria. Silver is the most often used of these metals since it is not harmful to human skin [3]. Many techniques can produce silver nanoparticles, such as chemical reduction, photochemistry, and sonochemistry [2,4]. However, it is a very popular method due to convenience, relatively low cost and the possibility of being produced on a large scale by chemical reduction. Various reducing agents can be used, ranging from weak ones (for example, glucose) and medium reducing agents (for example, formaldehyde) to strong ones (hydrazine and sodium borohydride). One important thing to consider is stabilising the colloidal silver nanoparticles formed so they do not undergo an agglomeration process. The substance commonly used as a colloidal stabilizer for silver nanoparticles is polyvinylpyrrolidone (PVP) and sodium citrate [5-6].

In general, when metal nanoparticles are prepared using the chemical reduction method, the metal ions are reduced by a reducing agent by adding a protective agent to stabilize the nanoparticles. The stability of nanoparticles plays a very important role, especially when the nanoparticles are characterized and applied to a product. Based on its use as an antibacterial agent, the resulting silver nanoparticles will be applied as an antibacterial coating that causes wound infections. The most common microbes that cause infection are *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*[7]. These three bacteria produce toxins that are dangerous to humans and are resistant to antibiotics. To overcome this problem, efforts are needed to control the life of bacteria that cause wound infections. For the reasons mentioned above, chemical reduction synthesised a colloidal solution of silver nanoparticles in this research. Sodium citrate was used as a stabilizer of the resulting silver nanoparticle colloidal solution. The characterization process for the silver nanoparticles produced was carried out using XRD analysis, particle size analyzer (PSA), TEM and a UV-vis spectrophotometer [8].

The problem with this research is that when silver nanoparticles are made, they are often unstable and big. In this study, silver nanoparticles were made using a chemical reduction method and sodium citrate as a stabilizer. The pH, concentration, and storage time were changed to see how they affected the stability and size of the silver nanoparticles. This research aims to

discover how to make silver nanoparticles using the reduction method and how to use them as an antibiotic to treat wound infections caused by bacteria.

Materials and Methods

The equipment used in this research includes a magnetic stirrer (Remi et al. 2-MLH With Hotplate), oven, AUX220 Analytical Balance from Shimadzu, SL-342 Multipurpose UV-visible spectrophotometer (1nm bandwidth), Particle Size Analyzer (Zetasizer Nano ZS 90), and JEOL 1200ex transmission electron microscope (TEM), colony counter, petri dish, incubator, tube needle, autoclave, spirit lamp. XRD analysis was carried out using a Bruker's D8 ENDEAVOR XRD diffractometer at a current of 40 mA and a potential difference of 45 kV with CuKα radiation to confirm the crystal form of silver nanoparticles. The materials for this research include trisodium citrate ($Na_3C_6H_5O_7$, 99%), silver nitrate ($AgNO_3$, 99%), sterile distilled water, wound dressing cloth, Nutrient Broth (NB), Nutrient Agar (NA), suspension of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*.

Synthesis of silver nanoparticles was made by heating 50 mL of $AgNO_3$ with a concentration of 1.0 mM until it boiled in an Erlenmeyer flask. To this solution, add 5mL of 1% $Na_3C_6H_5O_7$ drop by drop. During the heating process, the mixture is stirred using a magnetic stirrer until it turns pale yellow. The pale-yellow colour indicates that silver nanoparticles have been formed. The silver nanoparticles formed were then characterized. In this research, characterization was carried out using a UV-visible spectrophotometer, XRD, PSA, and TEM.

The process of coating silver nanoparticles on wound dressings, namely the method used by Duran.[9,12] The wound dressing is cut into 3x3 cm pieces, then washed clean, sterilized and dried. Clean and sterile wound dressings are soaked in citrate-silver nanoparticle colloid while stirring using a magnetic stirrer for 12 hours, 24 hours and 36 hours, then left for 5 minutes. The fabric that has been coated with citrate-silver nanoparticles is dried again in the oven at a temperature of 70°C for 5 minutes. Antibacterial activity testing in this research was carried out qualitatively and quantitatively. Wahyudi's research procedures[10] carried out qualitative tests, while quantitative tests used the Shake flask method by the procedures carried out by Duran[12].

Qualitative tests were conducted by immersing paper discs in silver nanoparticle colloids and attaching them to the NA surface. NA that had been attached to a paper disc was incubated for 24 hours at 37°C. Qualitative test results can be seen by observing the size of the clear zone formed around the paper disc[11].

Meanwhile, in the quantitative test, wound dressings that have been coated and not coated with silver nanoparticles with a size of 1x1cm are put into a sterile vial. In the sterile vial, 0.8 ml of distilled water was then added and shaken for 10 minutes. After that, 2.2 mL of NB was added as a bacterial growth medium so that the total volume of the mixture became 3 mL. The bacterial suspension, which was previously grown on NB, was added to the mixture in 10 µl. The mixture was then incubated for 24 hours at 37°C. After incubation, 1 mL of the mixture was taken from the vial to be embedded in NA. The bacterial culture in the medium was incubated for 24 hours at a temperature of 37°C, and then the number of colonies was counted using a colony counter.

Antibacterial activity can be determined through the % reduction of bacteria that can survive using the following formula[12]:

$$Reduction\ \% = \frac{B - A}{B} \times 100$$

In this case, A is the number of bacterial colonies after a wound dressing cloth coated with silver nanoparticles, and B is the bacterial colony after being given a wound dressing cloth not coated with silver nanoparticles.

Results

In this research, silver nanoparticles were synthesized by varying the concentration of the reducing agent ($C_6H_5O_7Na_3$) used. The synthesis results show that the addition of sodium citrate at a greater concentration causes the size of the silver nanoparticles formed to become smaller. However, a smaller particle size does not necessarily mean good stability. This is caused by nanoparticles having a tendency to agglomerate. Nanometer-sized particles have a very large specific surface area. On large surface areas, chemical bonds between particles form strong electric dipoles so they can agglomerate. Therefore, stabilizers in the synthesis of silver nanoparticles have a very important role.

Synthesis of silver nanoparticles with reducing agent concentrations of 0.5% and 1.0% produces nanoparticles with a fairly stable particle size distribution. This can be seen in Figures 1 and 2, which show that on day 0 and day 19, the particle size distribution did not show a significant change.

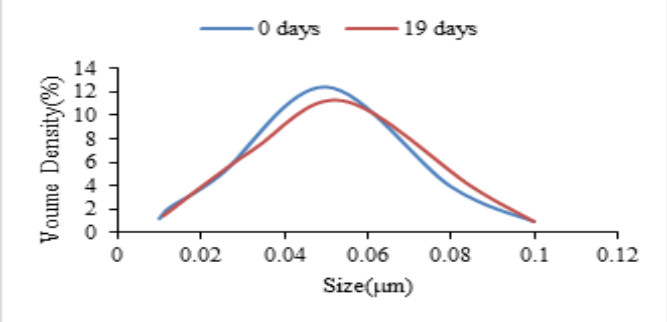

Figure 1. Size Distribution of Silver Nanoparticles with 0.5% Sodium Citrate Concentration at 0 and 19 Days After Synthesis

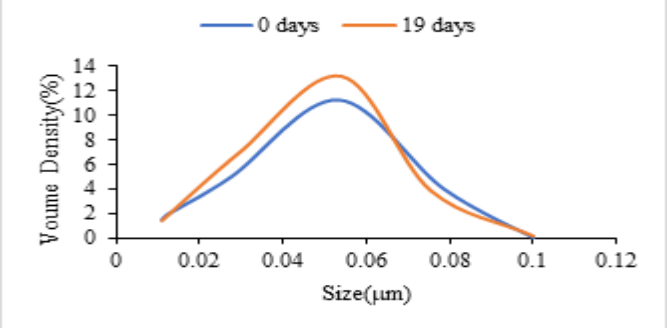

Figure 2. Size Distribution of Silver Nanoparticles with 1% Sodium Citrate Concentration at 0 and 19 Days After Synthesis.

The results showed that the average size of silver nanoparticles on day 0 was larger than the average size of silver nanoparticles on day 19. This was because, on the 19th day, the silver nanoparticles had agglomerated and even formed a shiny layer typical of silver at the bottom of the vial. Therefore, when the silver nanoparticles are to be analyzed with PSA on the 19th day (Figure 3), the average size of the nanoparticles in the colloidal mixture was reported to be between 8 and 31 nm, with the distribution falling somewhere in that range. They are filtered first so that large silver particles do not interfere with the analysis process.

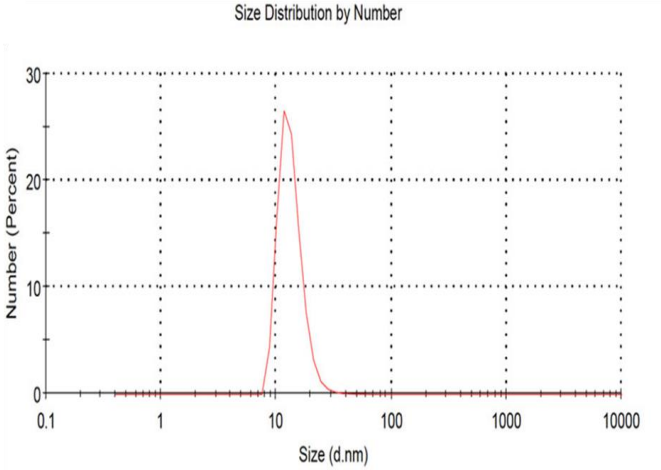

Figure 3: Particle size distribution of AgNPs synthesized with 1.5% sodium citrate on the 19th day

When wound dressings were tested against Escherichia coli bacteria, the highest percentage of bacterial reduction was found in wound dressings that had been soaked in colloidal silver nanoparticles for 36 hours. The bacterial reduction percentage reached 100%. Meanwhile, wound dressings with a soaking duration of 12 and 24 hours in colloidal silver nanoparticles, respectively, had a bacterial reduction percentage of 97.54% and 99.59% (Table 1).

The same thing was also seen in tests of wound dressing cloth against Bacillus subtilis and Staphylococcus aureus bacteria. The highest percentage of bacterial reduction was found in wound dressings that had been soaked in colloidal silver nanoparticles for 36 hours. At 36 hours soaking

It is thought that the distribution of silver nanoparticles is more even and sticks more firmly to the fabric fibres. Therefore, at 36 hours of soaking, the antibacterial activity was greater compared to 12 and 24 hours of soaking.

Monitoring the evolution of the reaction system was carried out by UV-vis spectroscopy. The appearance of absorption bands in the spectrum around 400 to 450 nm wavelengths indicates metallic nanoparticles [13]. These bands are due to resonance absorption of surface plasmons, and the maximum absorbance (Figure 4) was observed at the wavelength of 408 nm, a result that contrasts with the tabulated data that indicate that the 50 nm AgNPs should have their maximum absorbance around 420.9 nm[14]. This deviation may be due to using a low-purity silver salt that produces polydisperse AgNPs compared to those described in the literature. $2x10^{-2}$M $AgNO_3$ and 1% and 1.5% Sodium citrate concentration were used. UV-visible spectra of these colloids present their maximums in the surface plasmon absorption band at 414 nm. A broader peak was observed with a slight shift of λ_{max} at 420 nm for the colloidal solution obtained with 1 % Sodium Citrate (Figure 4) and λ_{max} at 421 nm for 1.5% Sodium Citrate concentration.

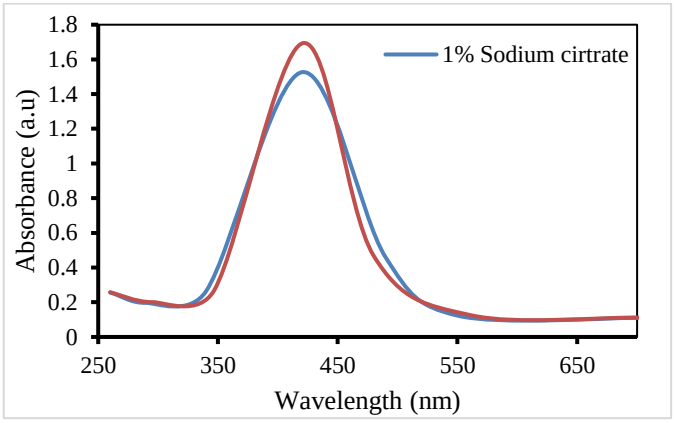

Figure 4: UV-Vis spectrum of the synthesis of AgNPs at two different concentrations of Sodium Citrate

In the TEM images of the mixtures with 1 and 1.5% Sodium Citrate, nanoparticles were observed, mostly spherical, with sizes between 10 and 50 nm. On the other hand, electron diffraction analysis reveals the presence of Ag accompanied by some oxides. When 1% Sodium Citrate was used, silver nanoparticles were obtained, mostly hexagonal (Figure 6). The shape and size of the nanoparticles would be closely related to the shift of the λmax observed in Figure 5. After analyzing the images obtained with the ImageJ program, it was found that the particles have a size of 31 ± 6.14 nm. As can be seen in Figure 6, it was found that the new particles with 1.5% Sodium Citrate have a more homogeneous size and a spherical shape, corroborating that the purity of the silver salt used is a very important factor to take into account for the synthesis of AgNPs. After analyzing the images obtained with the ImageJ program, it was found that its size corresponds to 30 ± 2.10 nm.

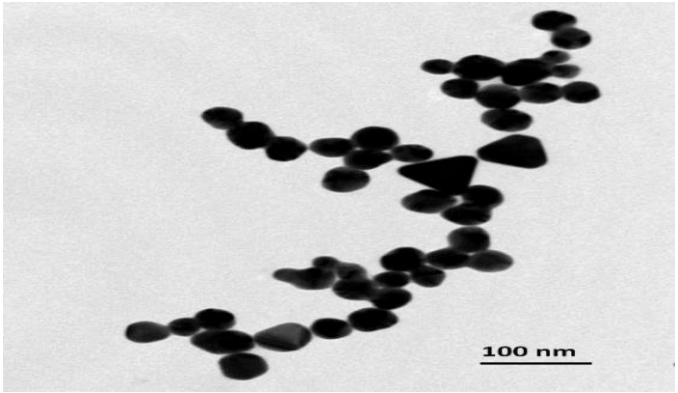

Figure 5: TEM image of AgNPs at 1% Sodium citrate concentration

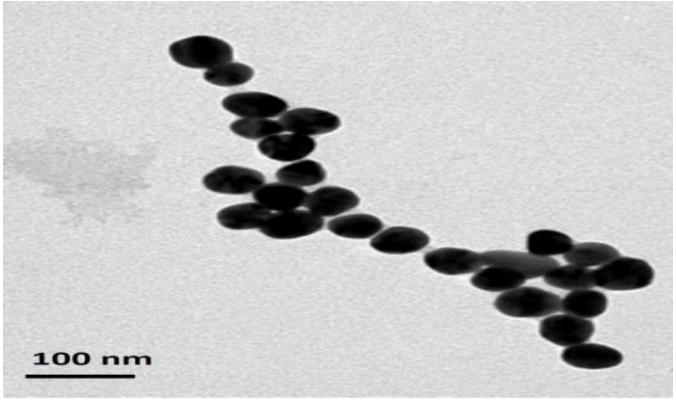

Figure 6: TEM image of AgNPs at 1.2% Sodium citrate concentration

Analysis using XRD:

To confirm the structure of AgNPs, X-ray diffraction (XRD) pattern analysis was carried out. The characteristic peaks observed in the XRD pattern of nanoparticles produced through the reduction of $AgNO_3$ using 1.5% sodium citrate confirmed the presence of silver nanoparticles (Figure 7). The diffraction peaks at 2θ angles 37.79°, 43.97°, 64.25°, and 77.28° correspond to the (111), (200), (202), and (311) planes, respectively. The diffraction pattern with the Miller Index (111) corresponds to the cubic crystal system of AgNP [15]. The relatively sharp peak in the XRD pattern (111) indicates the crystal phase of AgNP. Estimated silver particle size can be calculated from the Debye-Scherer equation[16] by determining the width against the Bragg reflection index (111). Based on calculations using this equation, an estimated particle size of 30.36 nm was obtained (Figure 7).

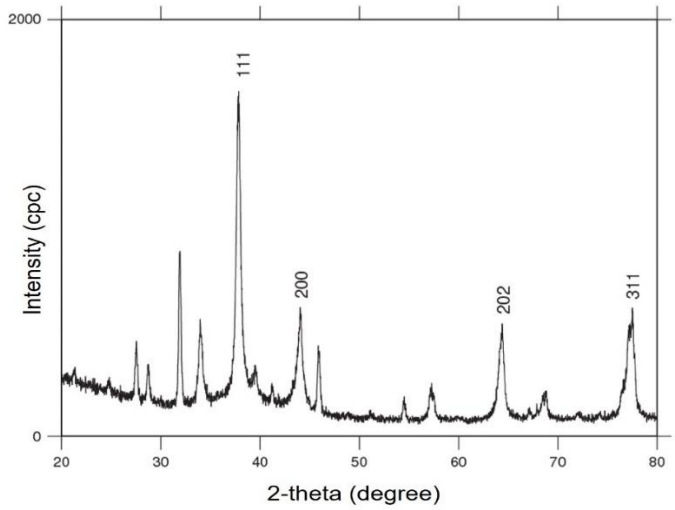

Figure 7: XRD pattern of AgNPs

Table 1. Quantitative Test Results of Antibacterial Activity of Wound Bandage Cloth Coated with Silver Nanoparticles against the Growth of Escherichia coli, Bacillus subtilis, and Staphylococcus aureus bacteria

		B (colony)			A (colony)			% Reduction		
S.No	Soaking Time (hours)	*S. aureus*	*E.coli,*	*B. subtilis,*	*S. aureus*	*E.coli,*	*B. subtilis,*	*S. aureus*	*E.coli,*	*B. subtilis,*
1	12 hours	303	251	263	16	7	26	94.72	97.21	90.11
2	24 hours	303	251	263	5	2	3	98.35	99.2	98.86
3	36 hours	303	251	263	3	0	0	99.01	100	100
Average								**97.36**	**98.80**	**96.32**

Where [A] = Number of bacterial colonies after being given a wound dressing coated with silver nanoparticles

[B] = Number of bacterial colonies after being given a wound dressing that was not coated with silver nanoparticles

Discussion

The principle of colloidal silver nanoparticles used in this research is by adding drop by drop the reducing and stabilizing solution, sodium citrate, to the boiling $AgNO_3$ solution. Chemical reactions that occur:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^o + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2$$

Based on the reduction potential energy, the reaction above can be written as follows:

$$Ag^+ + 1e^- \rightarrow Ag \qquad E^o = 0.799$$
$$4H^+ + O_2 + 4e^- \rightarrow 2H_2O \qquad E^o = 1.224$$

E^oCell = Reduction E^o- Oxidation E^o

E^o cell= 0.779-1.224

E^ocell= -0.445

A negative value of cell potential energy indicates that the reaction above is non-spontaneous. Therefore, this reaction should not be able to take place. However, the reaction between Ag^+ ions and $(C_6H_5O_7)^-$ ions can form → $[Ag^+ \ldots . citrate]$ or $[Ag_3(C_6H_5O_7)_{n+1}]^{3n-}$ complexes, which have a more dominant role in reducing Ag^+ ions becomes Ago slowly so that the reaction can continue. The complex formation reaction is written in the following reaction:

$Ag^+ + 1e^- \rightarrow Ag^o$ (1)

$AgO + Ag^+ \rightarrow Ag^+$ (2)

$Ag^+ + Citrate \rightarrow [Ag^+ \ldots . citrate]$ (3)

When the addition of sodium citrate solution in $AgNO_3$ drop by drop ended, no significant changes were seen. The mixed solution is still clear and colourless. After a time of ±9 minutes from the last drop of sodium citrate, the reaction was a gradual change in the colour of the solution from pale yellow to reddish yellow (Figure 8). This colour is characteristic of colloidal silver nanoparticles[17].

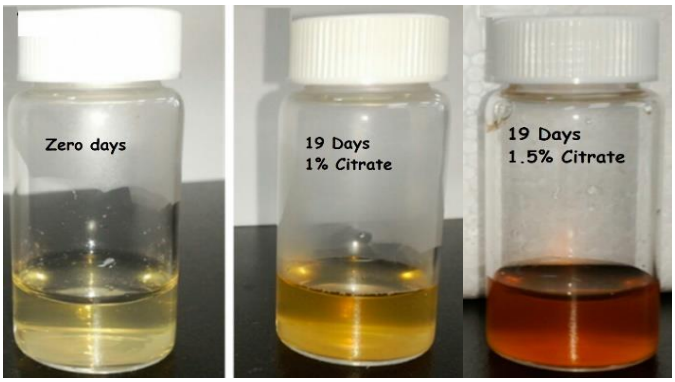

Figure 8: Colour changes of citrate-capped silver nanoparticles at zero and 19 days with 1% and 1.5% citrate solution.

Absorption peak changes can determine the stability of a colloidal silver nanoparticle solution. If the absorption peak changes to a longer wavelength, agglomeration makes the colloidal silver nanoparticle solution unstable. Colloidal silver nanoparticles synthesized with different sodium citrate concentrations were measured for seven days. Silver nanoparticles reduced using 0.5% and 1% sodium citrate coincide with an absorbance peak between 418-419 nm. This situation shows that the synthesized colloid is relatively stable. Sodium citrate, which tends to have a negative charge, is adsorbed by silver nanoparticles, thereby creating a repulsive force between the silver particles and preventing agglomeration. In contrast to silver nanoparticles, which were reduced using 1.5% sodium citrate, the absorbance peak shifted to 420-421nm. This happens because more reduced silver

nanoparticles are produced at the same concentration of silver nitrate. So that collisions between particles occur more frequently and eventually agglomerate[17].

Determination of the size of silver nanoparticles and their distribution was carried out using a Particle Size Analyzer (PSA) and Transmission Electron Microscope (TEM). These two tools produce data that can complement each other. PSA produces data in the form of the size distribution of silver nanoparticles, but the accuracy of PSA is lower than TEM. TEM can show the morphology and diameter of silver nanoparticles with high accuracy, but only in certain sample samples. The silver nanoparticles' size is based on estimates calculations using the Debye-Scherer equation of 30.2 nm with cubic form.

With the same concentration of silver nitrate, sodium citrate with a concentration of 1.5% reduces more Ag^+ to Ag^0. Even though sodium citrate has the ability to act as a stabilizer, the large amount of Ag^0 that is formed makes the collisions between particles more intense, resulting in agglomeration. Meanwhile, synthesising silver nanoparticles using a sodium citrate concentration of 0.5% requires longer to stabilize the silver nanoparticles formed. Therefore, the silver nanoparticles formed experience agglomeration more quickly, so silver nanoparticles tend to have a larger average particle size.

To determine the morphology and diffraction of the synthesized silver nanoparticles, characterization was continued using a TEM [18]. In this study, the samples used for TEM characterization were silver nanoparticles reduced using 1% sodium citrate. These silver nanoparticles were used because, based on UV-Vis and PSA results data, they are the most stable silver nanoparticles compared to silver nanoparticles reduced using 0.5% and 1.5% sodium citrate. The TEM photo results correlate with the size of the silver nanoparticles analyzed using PSA. The average size of silver nanoparticles measured by PSA ranges from 26.4 to 30.2 nm. These results are similar to the TEM results, which show particle sizes reaching 7.36 – 32.68 nm.

Apart from looking at the diameter of silver nanoparticles, TEM characterization is also used to determine the diffraction. Based on the diffraction pattern, the crystal structure of silver nanoparticles can be determined theoretically and experimentally. The m value obtained from the results of data analysis is matched with the crystal structure determination table to determine the arrangement of the crystal structure. Based on this, it can be concluded that the silver nanoparticles in this study have a Face Centered Cubic (FCC) structure [19].

Conclusions and Recommendations

The reducing agent concentration that produces the smallest and most stable silver nanoparticles is 1% sodium citrate. The characterization results using PSA show that the average size distribution of the smallest silver nanoparticles is 8 nm. The characterisation results using TEM show the diameter of the silver nanoparticles based on the morphological image of the silver nanoparticles. The smallest nanoparticle diameter obtained from this analysis was 7.36 nm. Apart from that, using TEM, the diffraction data shows that the silver nanoparticles formed have a face-cantered cubic (fcc) crystal structure. The reduction percentage of *Escherichia coli, Bacillus subtilis, and Staphylococcus aureus* bacteria within a soaking duration of 36 hours, respectively, reached 100%, 100% and 99.01%. It is recommended for future researchers to use different methods to obtain an optimal process.

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