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Tartaric Acid and Sodium Dodecyl Sulphate Driven Silver Nanoparticles Synthesis: Antibacterial Nano-warriors

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Abstract: Silver nanoparticles (AgNPs) have emerged as promising nano-antimicrobials due to their broad-spectrum bactericidal activity. In this paper, we have reported the synthesis of silver nanoparticles (AgNPs) with desired size and high purity using tartaric acid and sodium dodecyl sulphate (SDS). In the synthetic reaction, tartartic acid acts a reducing agent and sodium dodecyl sulphate (SDS) which acts a capping agent and it controls the growth of the particles in the range of nanoparticles. The synthesized AgNPs were characterized by modern method and tools such as UV-Vis. Spectroscopy, dynamic light scattering (DLS), scanning electron microscope (SEM), Energy-dispersive X-ray spectroscopy (EDAX) and transmission electron microscope (TEM). These AgNPs have been used to investigate the anti-bacterial activity study and this study describes that AgNPs gives very good results. IC50 (half-maximal inhibitory concentration) values were found 61.9 μg/ml and 96.7 μg/ml against *Staphylococcus aureus* and *Escherichia coli* bacteria respectively for which silver nanoparticles shows very much potent efficacy against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria, which conclude that these silver nanoparticles are very effective towards broad spectrum antibacterial applications.

KEYWORDS: Silver nanoparticles, SEM, tartaric acid, SDS, anti-bacterial activity

1. INTRODUCTION

A significant area of current research is nanotechnology, which deals with the creation, synthesis, and control of particle formations that range in size from roughly 1 to 100 nm. Silver nanoparticles have been synthesized using a variety of techniques, including chemical reduction, electrochemical reduction, and bio-reduction methods [Agasti etal, Dong et al., Khan et al., Umadevi et al., Arun et al., Shet et al., Vinayagam et al.]. They have also been created in biofilms containing silver nanoparticles [Sambalova et al.], and it has been noted that different sizes of AgNPs are produced by UV-irradiation [Hosny et al.], and the sol-gel method [Wolf et al., Chicea et al., Samani et al.]. AgNPs that are extremely stable are also employed as colorimetric sensors [Le et al.].

Colloidal nanoparticles with higher level form, size, and phase consistency can be processed using a wetted chemical approach that relies on organic surfactants to cap the nanoparticles surfaces in order to prevent aggregation and stabilize the dispersion in the solution. Several stabilizer types, including as organic surfactants, polymers, dendrimers, and ligands, have been utilized between the structured nanomaterials to counteract van der Waals interactions and produce monodispersed AgNPs that would otherwise cause agglomeration. In order to ensure

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long-term stability, researchers use surfactants as stabilizers or templates in synthesis to lower surface energy, control the growth and structure of the nanoparticles, and stop aggregations [Ravindran et al., Rajar et al., Batool et al.].

Health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron transistors, light emitters, nonlinear optical devices, and photo-electrochemical applications are just a few of the many fields in which silver nanoparticles (NPs) find use [Sathishkumar et al., Kim et al., Colvin et al., Wang et al., Schmid et al., Hoffman et al., Hamilton et al., Mansur et al.]. Due to their appealing physicochemical characteristics and biological functionality, such as their high antimicrobial efficiency and comparatively non-toxic, broad range of bactericidal properties [Durán et al., Chen et al., Martínez-Castañón et al.], anticancer properties and other therapeutic abilities, their special capacity to form a variety of nanostructures [Sohn et al., Kumar et al.], and their comparatively low manufacturing cost [Capek et al.], silver nanoparticles are of utmost importance.

The main element that contributes to improved antibacterial effectiveness is AgNPs enormous surface area, which interacts strongly with microorganisms even at lower concentrations. Numerous mechanisms have been proposed to explain how AgNPs kill bacteria by releasing silver ions inside pathogens. These mechanisms include blocking the respiratory chain, preventing protein denaturation due to strong bonding to functional groups, preventing nutrients from being transported to the bacterial cell membrane, preventing the flow of cellular contents by disrupting the cell membrane, and preventing the replication of deoxyribonucleic acid (DNA). Therefore, both Gram-positive and Gram-negative bacteria are effectively killed by AgNPs [Al-Sabri et al., Ameen et al., Bowler et al., Rodrigues et al., Moghaddam et al., Maheen et al., Masood et al., Zafar et al., Sargazi et al., Masood et al.].

Few researchers have reported the synthesis of nanoparticles using tartaric acid as reducing agent [Begum et al., Bunge et al.] where there is lack of clear scientific reaction mechanism and also due to agglomeration the synthesized nanoparticles have not been found in perfect shape. In this work, we have synthesized of silver nanoparticles by reducing the silver ions (Ag^+) present in the solution of silver nitrate by the reaction of tartaric acid which acts as a reducing agent and also we have used SDS which plays role to avoid agglomeration of the nanoparticles. We have given a possible mechanism for the formation of nanoparticles. This method was found to yield faster and stable silver nanoparticles when compared to other methods as the silver nanoparticles thus produced have very narrow size distribution which indicates the maximum nanoparticles are almost equal in size. Initially, the reaction for the synthesis of nanoparticles was monitored by color change of the solution into reddish brown and UV-visible spectroscopy.

2. MATERIALS AND METHODS

2.1. MATERIALS

Tartaric acid, sodium dodecyl sulphate (SDS), silver nitrate ($AgNO_3$), and double-distilled water are employed in this studies. Modern techniques and instruments, including UV-Vis spectroscopy, dynamic light scattering (DLS), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDAX), and transmission electron microscopy (TEM), were used to analyze the synthesized silver nanoparticles AgNPs.

2.2. SYNTHESIS OF SILVER NANOPARTICLES

The sol-gel process was used to synthesize silver nanoparticles. First, a clear solution was prepared by dissolving 0.02 mole of silver nitrate ($AgNO_3$) in 20 ml of double-distilled water while continuously stirring. Separately, 10 ml of double-distilled water were used to dissolve 0.01 mole of tartaric acid, which was then added dropwise to the silver nitrate solution. Subsequently, 10 mL of 0.05 moles sodium dodecyl sulfate (SDS) solution was introduced into the reaction mixture under stirring. SDS acted as a surfactant to control particle size and prevent agglomeration during synthesis. The resulting mixture was continuously stirred at 60-70 °C for 2 hours to ensure uniform distribution of reactants and facilitate the formation of silver nanoparticles. After completion of the reaction, the solution was cooled to room temperature and centrifuged to collect the precipitated silver nanoparticles. To get rid of any remaining surfactants and contaminants,

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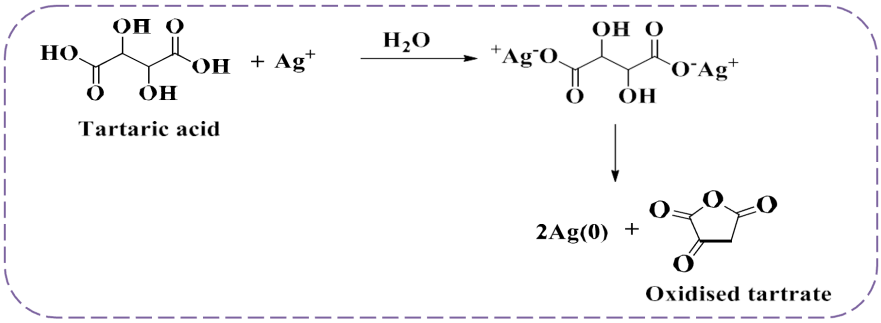
the gathered nanoparticles were repeatedly cleaned using a solution of ethanol and distilled water. The cleaned nanoparticles were then dried in a vacuum oven at 60-80 °C.

3. RESULTS AND DISCUSSION

In this method of preparation, tartaric acid was used as a reducing agent where it donate electron to Ag^+ ions and converts into Ag(0) atoms. Scheme 1 illustrates a potential mechanism in which tartaric acid oxidizes itself and transforms into oxidized tartrate. Agglomeration of the nanoparticles did not happen as a result of the polar head group of SDS depositing on the surface of silver nanoparticles (Figure 1).

Figure 2(a) displays UV-Vis spectroscopy for AgNPs in the nanoparticles aqueous media. The samples UV-Vis spectra showed clear surface plasmon bands. Particle size, shape, contact with the medium, local refractive index, and the degree of charge transfer between the particles and the medium all affect where the SPR band appears in UV-Vis spectra. The absorption peak, which corresponds to the absorption peak for silver nanoparticles, was seen at 415 nm. Therefore, it may be deduced that the resultant solution forms silver nanoparticles at ambient temperature. Figure 2(b) shows the dynamic light scattering (DLS) study of the synthesized silver nanoparticles from which we can know about the particle size distribution. For this experiment we make an aqueous solution of the silver nanoparticles. This DLS image clearly indicates the size range of the nanoparticles is 20-40 nm. Around 80% of the molecules have the size 20-30 nm as a mass product. So we can say this synthesis route is very much productive in terms of size of the particles with in the nano range.



Scheme 1. Reduction of Ag^+ to Ag(0)

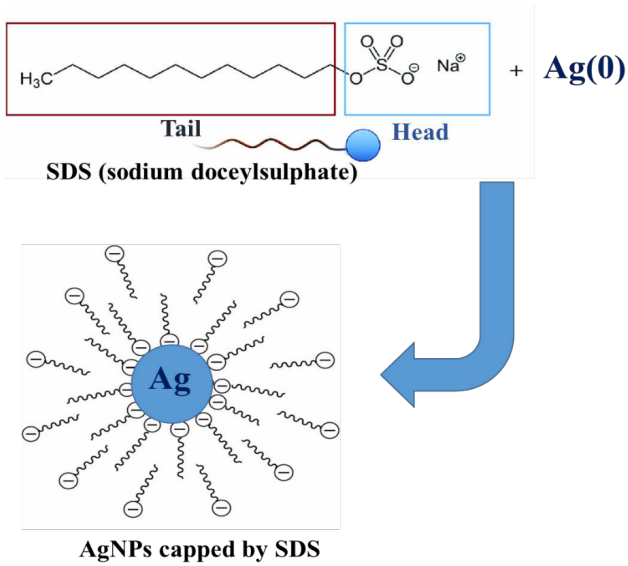


Figure 1. Controlling of AgNPs size by SDS

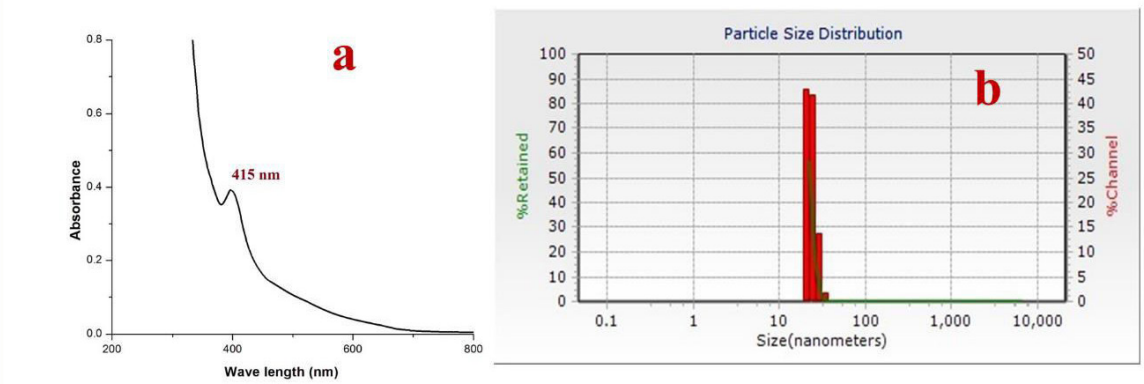


Figure 2. (a) UV-visible spectra of AgNPs & (b) DLS image of AgNPs

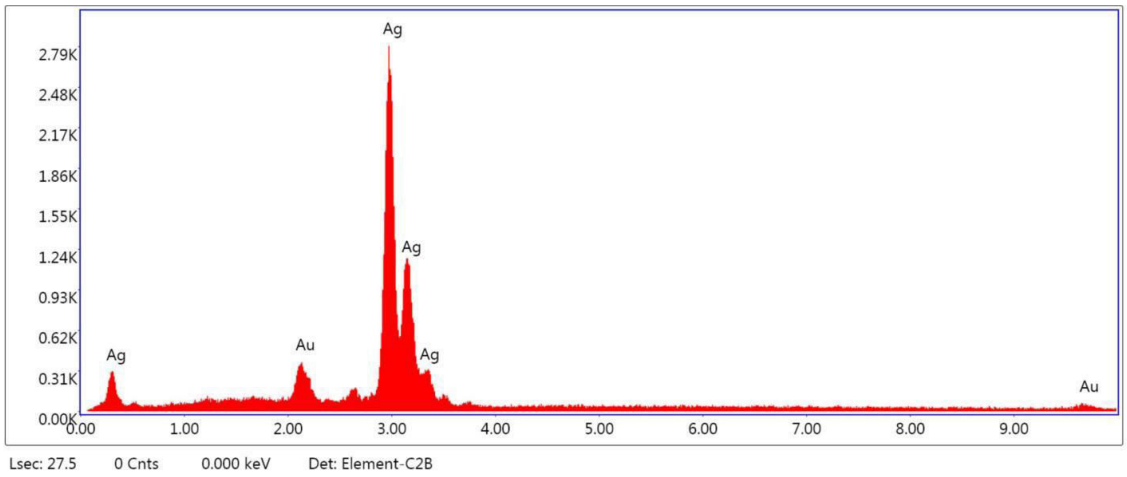


Figure 3. EDAX image of the AgNPs

The EDAX measurement of our synthesized silver nanoparticles has been shown in Figure 3. From this experiment, it becomes very clear to us that our sample contains only silver elements and no other element is there as an impurity. Therefore, it confirms that we have synthesized silver nanoparticles with higher level of purity.

The surface morphology of the produced silver nanoparticles is examined by scanning electron microscopy (SEM) analysis. SEM micrographs of our synthesized silver nanoparticles were shown in Figure 4(a-d). The SEM micrograph analysis showed that there is uniform formation of silver nanoparticles with the size range of (20-40) nm and the shapes of the nanoparticles are spherical in nature. SEM image clearly indicates the nanoparticles are discrete and no agglomeration has been occurred.

In order to analyze the surface morphology of the silver nanoparticles using transmission electron microscopy (TEM), the particles were dispersed in distilled water by sonication for 30 minutes. This caused the aggregated form to break down and transform into a homogenous mixture of nanoparticles in the dispersing media. After that a drop of these well dispersed nanoparticles has been placed onto a copper gird and dried for 10 min. The TEM morphological images of the synthesized AgNPs nanoparticles were shown in Figure 5(a-d) at different area of the copper grid. Figure 5a & 5b shows the well dispersed with the size range of 20-40 nm and the shape of the nanoparticles are very clear. The shape the of the silver nanopartcles are mostly spherical in nature. Figure 5c describes the micrograph of the nanoparticles with more magnification which shows that silver nanoparticles are crystalline in nature with definite crystal planes. Figure 5d is the SEAD (selected area diffraction patterns) pattern of silver nanparticles, which also indicates that the particles are single crystalline and polycrystalline in nature. The SAED pattern

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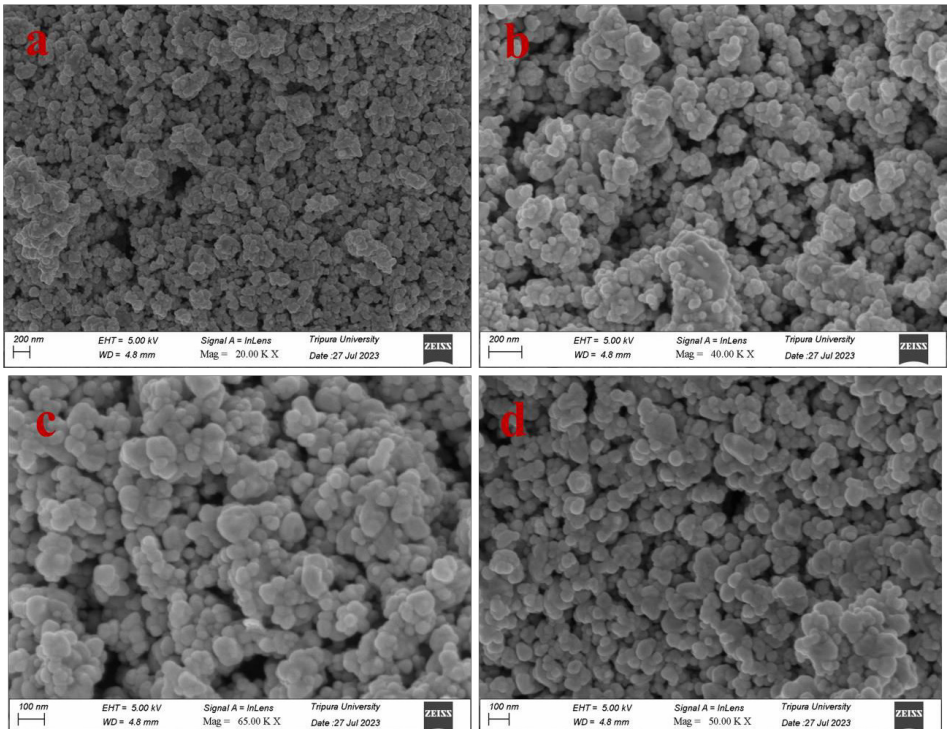

Figure 4. **(a-d)** SEM image of AgNPs

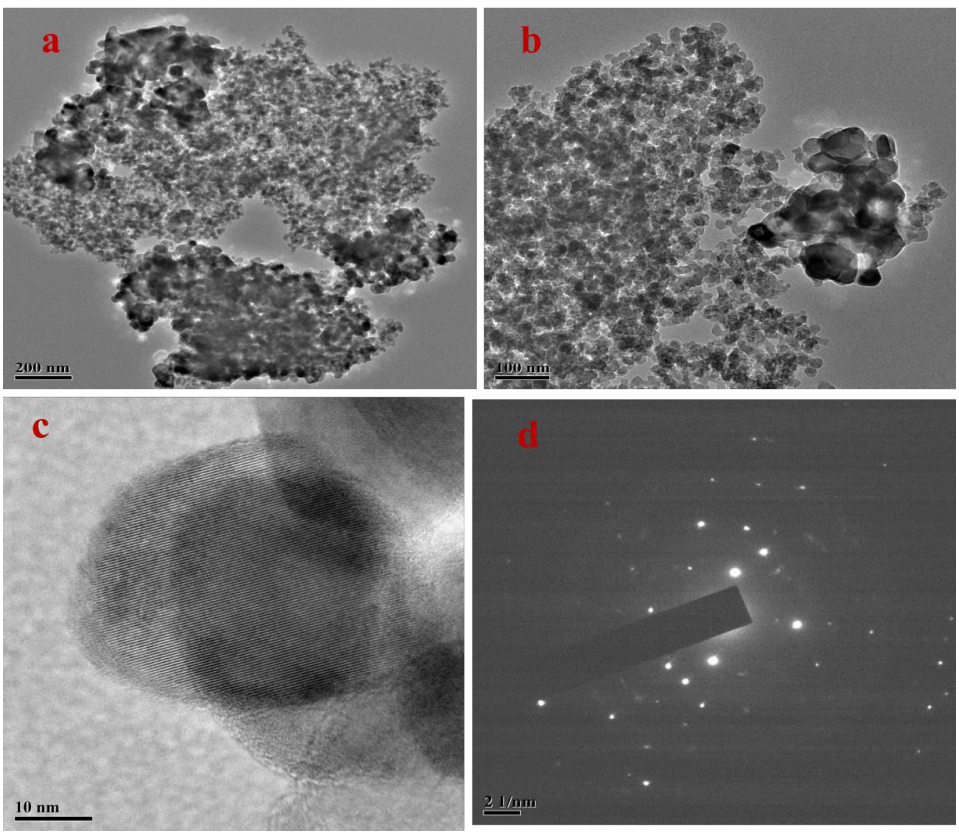

Figure 5. (a, b) TEM, **(c)** HR-TEM and **(d)** SAED pattern images of synthesised AgNPs

actually arises due to the diffraction of a parallel electron beam by the atomic pplanes in the crystal. So, we can conclude that the synthesized silver nanoparticles have good crystalline nature and uniform morphology.

3.1. Antibacterial Study of AgNPs

The anti-bacterial activity study of silver nanoparticles was done with two bacterial strains namely- *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram- negative). In this study, we measured the inhibition zone diameter in the agar well diffusion method. For our anti-bacterial study, we used three different concentrations like 50 µg/ml, 100 µg/ml, and 250 µg/ml of the synthesized silver nanoparticles in water medium along with one standard antibiotic was taken as reference.

Table 1 shows the results in terms of zone of inhibition at different concentration of silver nanoparticles solution with a standard antibiotic. We have also done the comparative study through a bar diagram (shown in Figure 6) between inhibition zone and concentration of AgNPs solution against *S. aureus* and *E. Coli* bacteria. From this study it is very clear that silver nanoparticles show a dose dependent activity on both the bacteria. In case of *Staphylococcus aureus* (Gram-positive) bacteria, when we use concentration of 50 µg/ml, 100 µg/ml and 250 µg/ml solution of silver nanoparticles, the inhibition zone diameter are

11.65 mm, 21.32 mm and 31.73 mm respectively. Where as in case of standard antibiotic sample the inhibition zone diameter is 24.97 mm which indicates our synthesized silver nanoparticles showed stronger efficacy at higher dose (250 µg/ml).

Again, *Escherichia coli* (Gram-negative) bacteria, at different concentration of 50 µg/ml, 100 µg/ml

Table 1. Results of antibacterial study

Antibacterial activity						
Inhibition zone diameter (mm)						
Compound name	S. aureus			E. coli		
	50 µg/ml	100 µg/ml	250 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
Silver nanoparticle s	11.65	21.32	31.73	14.78	22.42	32.12
Standard			24.97			25.56

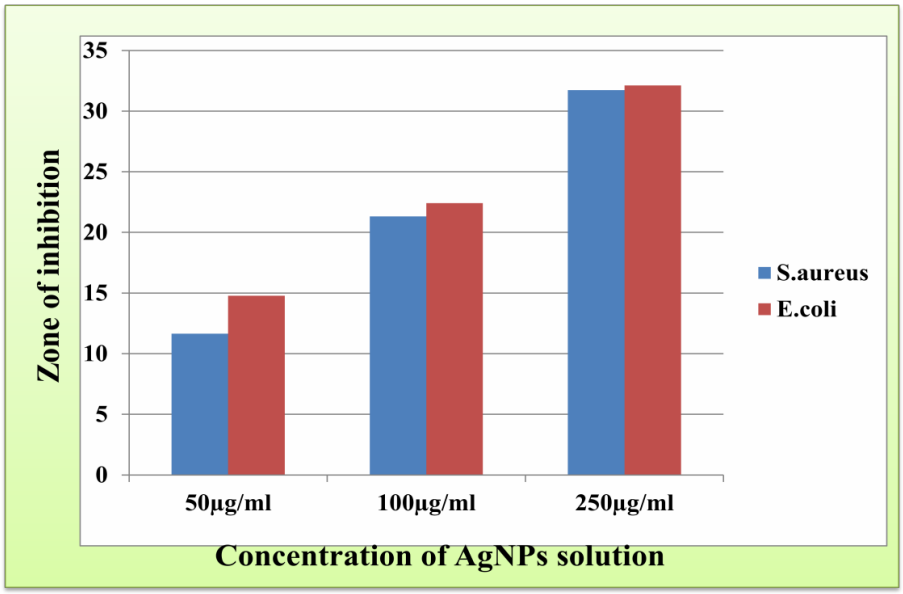

Figure 6. Bar diagram of inhibition zone vs concentration of AgNPs solution against S. *aureus* and E. *coli* bacteria

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and 250 µg/ml solution of silver nanoparticles, the inhibition zone diameter was measured as 14.78 mm, 22.42 mm and 32.12 mm respectively. Where as in case of standard antibiotic sample with concentration of 250 µg/ml, the inhibition zone diameter is 25.56 mm which indicates silver nanoparticles showed more potent antibacterial effect at 250 µg/ml concentration of silver nanoparticles than that of standard sample. Furthermore, at lower concentration of nanoparticles, *Escherichia coli* (Gram-negative) bacteria appears slightly more sensitive than that of *Staphylococcus aureus (Gram-positive) bacteria* (14.78 mm vs 11.65 mm at 50 µg/ml).

So, from this study, we can conclude that silver nanoparticles are very much effective against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria, which indicates these nanoparticles are potential towards broad spectrum antibacterial applications. The mechanism behind this stronger efficacy of these silver nanoparticles, more surface area of nanoparticles is the primary factor that results in better antibacterial activity which arises due to strong interaction with microorganisms even at a lower concentration. Silver nanoparticles releases silver atom inside pathogens which block transport of nutrients to the bacterial cell membrane, flowing out cellular contents by disrupting the cell membrane and destroy the replication of deoxyribonucleic acid (DNA) of the bacteria. Therefore, AgNPs act as a potent killing agent against bacteria, including Gram- negative and Gram-positive.

Figure 7 shows the dose-response curve for silver nanoparticles against both the *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. From the graph the calculated IC_{50} (half-maximal inhibitory concentration) values are 61.9 µg/ml and 96.7 µg/ml against *Staphylococcus aureus* and *Escherichia coli* bacteria respectively. This IC_{50} value indicates that that silver nanoparticles are more effective against *Staphylococcus aureus* than that of *Escherichia coli* bacteria.

4. CONCLUSION

It has always been a challenging for the development of benign method of synthesis of metal nanoparticles as they agglomerate very quickly. Here, we have reported an easy, one pot large scale synthesis of stable AgNPs, using tartaric acid and SDS. In this case, tartaric acid act here as reducing agent and SDS acts as capping agent which help in the controlling of the size of silver nanoparticles. The size of the synthesized silver nanoparticle has been found around 20-50 nm which confirmed from DLS, SEM and TEM analysis of the particles. These silver nanoparticles have shown very effective efficacy against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria, which indicates these nanoparticles are can be used towards broad spectrum antibacterial applications.

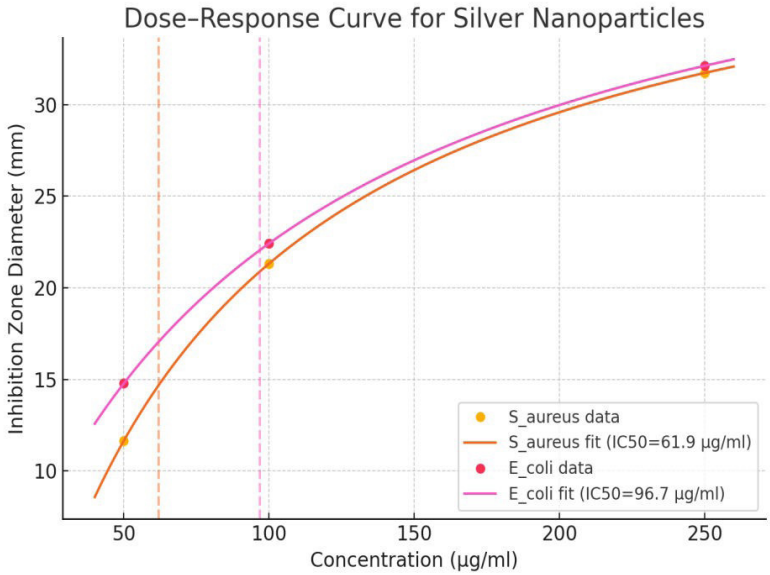

Figure 7. Dose-response curve for silver nanoparticles against S. *aureus* and E. *coli*

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statements

Incorporating a clear Research Data Policy and Data Availability Statement helps ensure the integrity and reproducibility of scientific research. Authors are encouraged to make the data supporting the findings of their research available to others. The datasets generated and/or analyzed during the current study are available in the manuscript.

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