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FORMULATION AND EVALUATION OF SILVER NANOPARTICLES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The present study focuses on the synthesis, characterization, and evaluation of silver nanoparticles (AgNPs) for their physicochemical properties and antibacterial activity. Silver nanoparticles were successfully prepared and subjected to comprehensive characterization using UV–Visible spectroscopy, viscosity analysis, pH measurement, dynamic light scattering (particle size and PDI), zeta potential analysis, transmission electron microscopy (TEM), refractive index determination, antibacterial assessment, and stability studies. UV–Visible spectroscopy confirmed nanoparticle formation with a characteristic surface plasmon resonance peak at 440 nm. The formulation exhibited suitable viscosity (18.6 ± 0.5 cP) and near-neutral pH (6.8 ± 0.2), indicating good stability and compatibility for biological applications. Particle size analysis revealed nanoscale dimensions (~21 nm) with moderate polydispersity, while zeta potential (−28.4 mV) indicated good colloidal stability. TEM analysis confirmed spherical to near-spherical morphology with uniform distribution in the range of 20–50 nm. The refractive index (1.36 ± 0.02) further supported the homogeneity and physical stability of the formulation. The antibacterial activity of silver nanoparticles demonstrated significant inhibition against *Staphylococcus aureus* (18.4 ± 0.6 mm) and *Escherichia coli* (20.1 ± 0.5 mm), indicating broad-spectrum antimicrobial potential. Stability studies showed minimal variation in particle size, PDI, and zeta potential over three months, confirming good formulation stability. Overall, the synthesized silver nanoparticles exhibited excellent physicochemical characteristics, strong antibacterial efficacy, and satisfactory stability, suggesting their potential application in pharmaceutical and biomedical fields, particularly as antimicrobial agents and in drug delivery systems.

KEYWORDS: Silver nanoparticles, UV–Visible spectroscopy, particle size analysis, zeta potential, TEM, refractive index, antibacterial activity, stability studies, nanotechnology, antimicrobial agents.

1. INTRODUCTION

Bacterial infections remain one of the major causes of morbidity and mortality worldwide. The rapid emergence of multidrug-resistant bacterial strains due to the excessive and inappropriate use of antibiotics has created a serious global healthcare challenge. Conventional antimicrobial agents often suffer from limitations such as reduced efficacy, toxicity, poor bioavailability, and the development of microbial resistance. Therefore, the development of novel and effective antimicrobial systems has become an important area of pharmaceutical and biomedical research.[1]

Nanotechnology has emerged as a promising field for the development of advanced drug delivery systems and antimicrobial agents. Among various nanomaterials, silver nanoparticles have gained considerable attention due to their unique physicochemical properties, high surface area, enhanced stability, and potent antimicrobial activity. Silver has been traditionally recognized for its antimicrobial properties, and its nanoscale form exhibits significantly improved antibacterial effects against a wide range of Gram-positive and Gram-negative microorganisms.[2]

Silver nanoparticles exert antibacterial activity through multiple mechanisms such as disruption of bacterial cell membranes, generation of reactive oxygen species, interaction with cellular proteins and DNA, and inhibition of essential metabolic pathways. Due to these multimodal mechanisms, silver nanoparticles are less likely to induce bacterial resistance compared to

conventional antibiotics. In addition, silver nanoparticles possess excellent penetration ability and sustained antimicrobial action, making them highly suitable for biomedical and pharmaceutical applications.[2]

The formulation of silver nanoparticles can be carried out by various physical, chemical, and biological methods. Green synthesis approaches using plant extracts have gained increasing importance because they are eco-friendly, cost-effective, and reduce the use of hazardous chemicals. The synthesized nanoparticles are commonly characterized by analytical techniques such as UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), particle size analysis, zeta potential analysis, and electron microscopy to confirm their formation, stability, and morphology.[3]

Considering the growing demand for effective antimicrobial agents, the present study was undertaken to formulate and evaluate Silver nanoparticles for antibacterial activity. The study aims to investigate their physicochemical properties and assess their effectiveness against selected pathogenic bacterial strains, thereby exploring their potential as a promising antimicrobial formulation.[4]

2. MATERIAL AND METHODS

2.1 Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by reducing silver nitrate solution using trisodium citrate as a reducing agent under continuous stirring and heating conditions. Formation of nanoparticles was confirmed by color change from colorless to brown. The prepared dispersion was sonicated to obtain uniformly distributed nanoparticles and stored in dark containers for further studies.[5]

2.2 Assessment of Silver Nanoparticles

2.2.1 UV–Visible Spectroscopy

The synthesized nanoparticle dispersion was analyzed using a UV–Visible spectrophotometer in the wavelength range of 300–700 nm. The characteristic absorption peak observed around 400–450 nm confirmed the formation of silver nanoparticles.[6]

2.2.2 Viscosity Assessment

The viscosity of the nanoparticle dispersion was measured using a Brookfield viscometer at room temperature. Readings were recorded after stabilization and expressed in centipoise.(cP)[7]

2.2.3 pH Assessment

The pH of the silver nanoparticle dispersion was determined using a calibrated digital pH meter at room temperature to ensure formulation stability and compatibility.[8]

2.2.4 Particle Size and Polydispersity Index Analysis

Particle size and polydispersity index (PDI) were determined using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS. Samples were diluted with deionized water prior to analysis.[9]

2.2.5 Transmission Electron Microscopy (TEM) Analysis

The morphology and particle size of the synthesized nanoparticles were evaluated using TEM. A diluted nanoparticle sample was placed on a carbon-coated copper grid, dried, and analyzed under suitable accelerating voltage.[10]

2.2.6 Zeta Potential Analysis

Zeta potential of the nanoparticle dispersion was measured using a Malvern Zetasizer Nano ZS to evaluate the surface charge and stability of the nanoparticles.[11]

2.2.7 Refractive Index Determination

The refractive index values of the nanoparticle material and dispersant were entered into the instrument software before particle size and zeta potential analysis to ensure accurate measurements.[12]

2.2.8 Antibacterial Activity Study

The antibacterial activity of Silver nanoparticles was evaluated by the agar well diffusion method against selected Gram-positive and Gram-negative bacterial strains. Zones of inhibition were measured after incubation and compared with standard drug Amoxicillin.[13]

2.2.9 Stability Analysis

The stability of the formulated nanoparticles was evaluated under refrigerated, room temperature, and accelerated conditions for three months. Parameters such as particle size, PDI, zeta potential, and physical appearance were periodically monitored.

2.3 Statistical Analysis

All experimental results were expressed as mean ± standard deviation (SD) from triplicate measurements. Statistical analysis was performed using one-way ANOVA followed by suitable post hoc tests, and $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 UV–Visible Spectroscopy Examination of Silver Nanoparticles

Table 1 UV Visible Spectral Data of silvernanoparticles.

Parameter	Observation
Color change	Colorless to yellowish-brown
Scanning range	300–800 nm
λmax	440 nm
Peak nature	Sharp
Interpretation	Formation of stable AgNPs

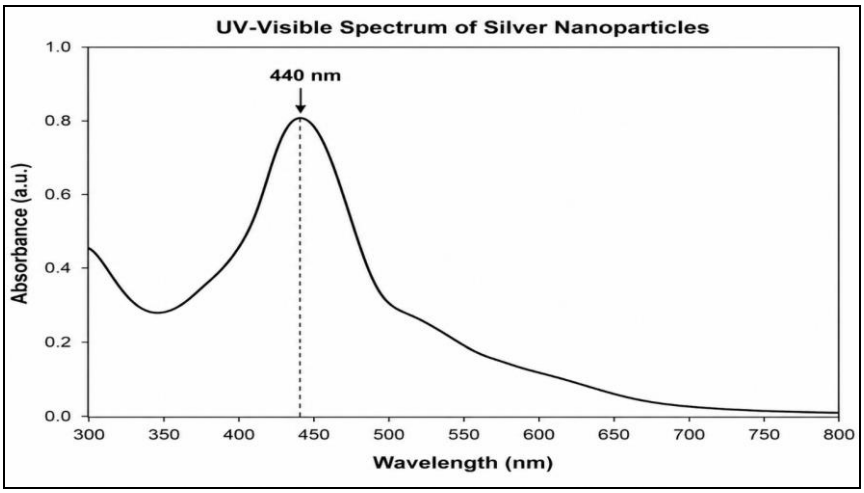

Figure 1 UV–Visible Spectrum of Silver Nanoparticles Showing Peak at 440 nm.

3.2 Viscosity Examination of Silver Nanoparticles

The viscosity of the silver nanoparticle dispersion was determined using a Brookfield viscometer at ambient temperature and found to be 18.6 ± 0.5 cP. The formulation showed smooth flow behavior, good uniformity, and adequate fluidity. This mild viscosity helps maintain physical stability by preventing rapid sedimentation of nanoparticles during storage while ensuring easy handling and effective dispersion without aggregation.

3.3 pH Analysis of Silver Nanoparticles

The pH of the silver nanoparticle dispersion was measured using a calibrated pH meter and found to be 6.8 ± 0.2, indicating a near-neutral formulation. This pH range is suitable for topical or biological applications as it minimizes irritation and supports stability of nanoparticles. The consistent pH value also confirms the homogeneity and chemical stability of the prepared formulation.

Table 2: Particle Size and PDI Data of Silver Nanoparticles.

Parameter	Run 1	Run 2
Mean particle size (d.nm)	21.32	21.32
PDI	0.328	0.406
Result quality	Good	Good

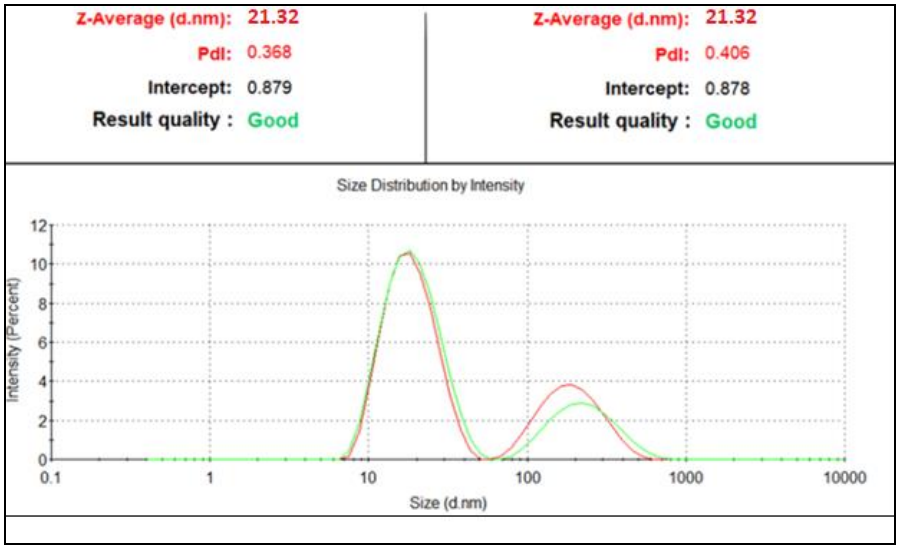

Figure 2: Particle Size Distribution of Silver Nanoparticles Using Zetasizer.

3.5 Zeta Potential Analysis

Table 3: Zeta Potential Data of Silver Nanoparticles.

Parameter	Observation
Method used	Zetasizer
Zeta potential	−28.4 mV
Surface charge	Negative
Peak nature	Sharp and narrow
Stability	Good
Interpretation	Stable nanoparticle dispersion

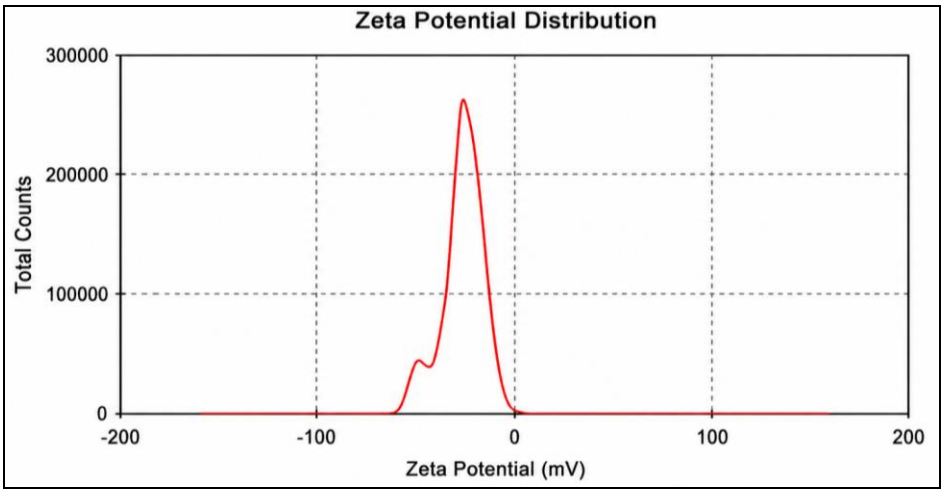

Figure 3 Zeta Potential Distribution Graph of Silver Nanoparticles Showing Peak at −28.4 Mv.

3.6 Transmission Electron Microscopy (TEM) Analysis

Table 4: TEM Analysis of Silver Nanoparticles.

Parameter	Observation
Technique used	TEM
Shape	Spherical to near spherical
Size range	20–50 nm
Distribution	Fairly uniform
Agglomeration	Slight
Interpretation	Successfully formed nanosized AgNPs

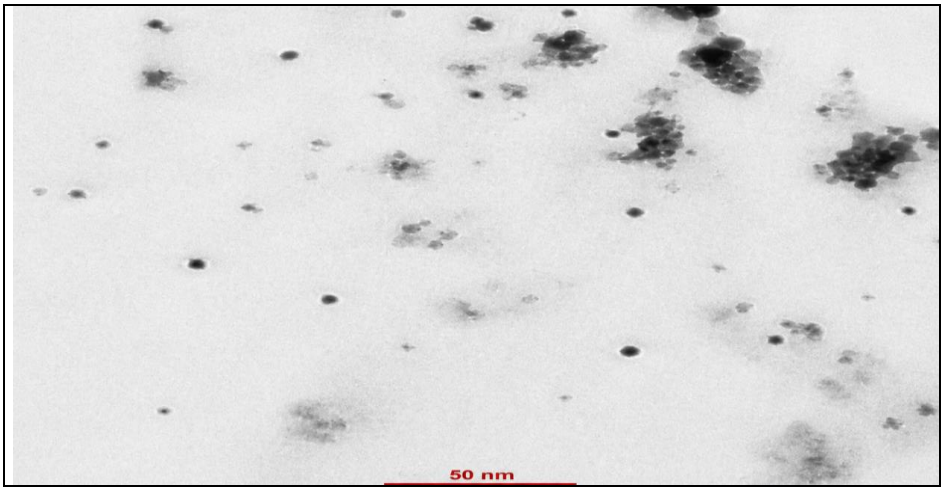

Figure 4 TEM Micrograph of Silver Nanoparticles Showing Particle Size Range of 20–50 nm.

3.7 Refractive Index (RI) Analysis

The refractive index of the silver nanoparticle dispersion was found to be 1.36 ± 0.02 at ambient temperature, which is higher than that of pure water. This increase confirms the presence and uniform distribution of silver nanoparticles within the aqueous medium. The consistent refractive index indicates good homogeneity, absence of phase separation, and overall physical stability of the formulation.

3.8 Investigation of Antibacterial Activity

The synthesized silver nanoparticles showed significant antibacterial activity against both Gram-positive and Gram-negative bacteria using the agar well diffusion method. The inhibition zones were 18.4 ± 0.6 mm for *Staphylococcus aureus* and 20.1 ± 0.5 mm for *Escherichia coli*. The strong activity is attributed to membrane disruption, silver ion release, and reactive oxygen species generation, leading to effective bacterial cell death.

Table 5: Antibacterial Activity of Silver Nanoparticles.

Test Organism	Zone of Inhibition (mm)
Staphylococcus aureus	18.4 ± 0.6
Escherichia coli	20.1 ± 0.5
Standard (Amoxicillin)	24.3 ± 0.4

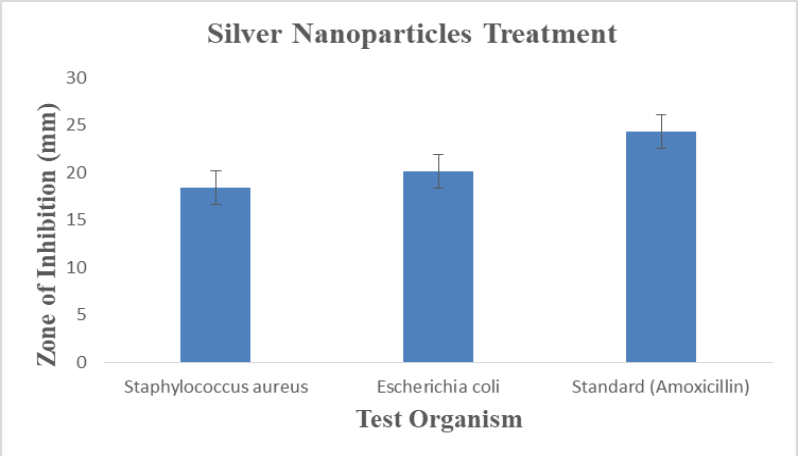

Figure 5: Zone of Inhibition of Silver Nanoparticles Against Test Organisms.

3.9 Stability Study

Table 6: Storage Conditions for Stability Study.

Condition Code	Storage Condition	Temperature	Relative Humidity
I	Refrigerated condition	4 ± 2°C	--
II	Room temperature	25 ± 2°C	60 ± 5% RH
III	Accelerated condition	40 ± 2°C	75 ± 5% RH

Table 4.7 Stability Data of Silver Nanoparticles.

Parameter	Initial	After 3 Months
Particle size (nm)	22.17	24.08
PDI	0.328	0.361
Zeta potential (mV)	-28.4	-27.1
Color	Yellowish-brown	No change
Appearance	Clear dispersion	Stable

DISCUSSION

The present study successfully demonstrated the synthesis and comprehensive characterization of silver nanoparticles (AgNPs) using multiple physicochemical and biological evaluation techniques. UV–Visible spectroscopy confirmed nanoparticle formation with a characteristic surface plasmon resonance peak at 440 nm, indicating successful reduction of silver ions and stable nanoparticle formation. The formulation exhibited suitable physicochemical properties, with viscosity (18.6 ± 0.5 cP) ensuring good fluidity, homogeneity, and stability against sedimentation. The near-neutral pH (6.8 ± 0.2) suggested compatibility for biological and topical applications without causing irritation. Particle size analysis revealed nanoscale dimensions (~21 nm) with moderate PDI values, indicating a reasonably uniform dispersion. Zeta potential value (−28.4 mV) confirmed good electrostatic stability of the nanoparticles, reducing the likelihood of aggregation. Morphological evaluation through TEM further validated the formation of spherical to near-spherical nanoparticles within the range of 20–50 nm, supporting the results obtained from dynamic light scattering. Refractive index analysis (1.36 ± 0.02) indicated a homogeneous dispersion system without phase separation, further confirming formulation stability. Biological evaluation demonstrated strong antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with slightly higher efficacy against Gram-negative bacteria, likely due to better interaction of nanoparticles with bacterial cell membranes. The mechanism of antibacterial action is attributed to membrane disruption, release of silver ions, and generation of reactive oxygen species leading to bacterial cell death. Stability studies indicated minimal changes in particle size, PDI, and zeta potential over three months, confirming good physicochemical stability of the formulation under storage conditions.

CONCLUSION

The present study successfully reports the synthesis, characterization, and evaluation of silver nanoparticles with desirable physicochemical and biological properties. The UV–Visible spectroscopy confirmed the formation of stable silver nanoparticles with a characteristic absorption peak at 440 nm. The formulation exhibited suitable viscosity and near-neutral pH, indicating good handling properties and compatibility for potential biomedical applications. Particle size analysis revealed nanoscale dimensions with acceptable polydispersity, while the negative zeta potential value confirmed good colloidal stability. TEM analysis further validated the formation of uniformly distributed spherical nanoparticles within the nanosize range. The refractive index study supported the homogeneity and physical stability of the dispersion system. The synthesized silver nanoparticles demonstrated significant antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, highlighting their broad-spectrum antimicrobial potential. Stability studies confirmed minimal changes in key parameters over the storage period, indicating good formulation stability. Overall, the study concludes that the synthesized silver nanoparticles possess excellent physicochemical stability, strong antibacterial efficacy, and promising characteristics for future pharmaceutical

and biomedical applications, particularly in antimicrobial and drug delivery systems.

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