

Characterization of Silver Nanoparticles Synthesized using Chemical Method and its Antibacterial Property

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Abstract: Metal nanoparticles are gaining importance nowadays in nanoscience. The nanoparticle had better physical and chemical properties compared with solid particles due to their large surface area. The silver nanoparticles are employed mostly in medical and electrical applications having outstanding conductivity and antimicrobial activity. In the present investigation, NaBH₄ and ethanol were used as a reductant and stabilizer agent from silver nitrate salt as a precursor. The silver nanoparticles obtained were characterized using Fourier-transform infrared spectroscopy (FT-IR), X-Ray diffraction (XRD) and high-resolution transmission electron microscopy (HRTEM) to determine their morphology and size. In XRD analysis, the average particle size was found to be 18.31 nm. The TEM analysis shows crystalline morphology with a face-centered cubic structure. The antibacterial activity was tested against two bacterial cultures, namely *Bacillus subtilis* and *Pseudomonas aeruginosa*. The inhibition zones of 19mm and 17mm were observed against *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively.

Keywords: Chemical synthesis; Silver nanoparticle; Antibacterial; NaBH₄.

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1. Introduction

The development of nanoparticle in recent years had evolved various methods to synthesize nanoparticle of various sizes and shapes of the metal. Metal nanoparticles possess unique chemical and physical properties from their bulk materials due to their high surface area to volume ratio [1].

In recent medical applications, the use of metal nanoparticles has made its standard. It has been observed that the use of silver nanoparticles is extensively used for medical purposes due to its better chemical stability and good antimicrobial activity [2]. The silver nanoparticles were well known for the antibacterial property, which makes use of surgical prostheses and dental implants. In general, there are two approaches employed in synthesis nanoparticles, first is the top-down approach (Physical methods), and second is the bottom-down (chemical and biological methods) [3].

The physical method employs machining and etching to produce nanoscale structures whereas, other method employs chemicals such as sodium borohydride, ethanol, ethylene glycol, etc. as reducing agents [4]. The chemical method is commonly used for producing silver particles. This is because of the simple equipment needed and convenient to operate. Badi'ah

et al. synthesized silver nanoparticles using AgNO_3 as a source of Ag and NaBH_4 as a reducing agent. They observed the change of color in the solution, which confirms the formation of the silver particle [5].

The first phase for the formation of particles involves the reduction of Ag^+ to Ag^0 atoms takes place forming clusters. In the second phase, the clusters coalesce to form nanoparticles of 2-3nm. The next phase is the metastable state takes place within 5-10 min maintaining the size. The last phase is the coalescence phase, which results in the formation of AgNPs of an average size of 5-8nm within 30-60 s [6]. Stabilizers are the compound which helps in the favors the formation of silver nanoparticle also helps in preventing it from aggregation [7].

For producing spherical particles, the biological method is useful in some cases despite being cheaper and eco-friendly [8]. Chemical synthesis of silver nanoparticle (AgNPs) was carried out by employing polyvinylpyrrolidone and di-sodium succinate hexahydrate and trisodium citrate dihydrate to form spherical to triangular nanocrystals in 300 seconds of reaction time at room temperature [9]. Sodium borohydride and polyvinylpyrrolidone used to synthesize the AgNPs from the aqueous silver nitrate solution. The morphology of the particle also influences the antimicrobial property.

It was reported that a higher concentration of spherical AgNPs has high antibacterial activity against *Escherichia coli* bacteria [10]. In another investigation, sodium nitrate was used as a precursor salt with sodium borohydride in talc as a reductant to obtain AgNPs. The particle size of AgNPs was of the range of 7.60nm to 13.11 nm and spherical shape [4]. The size of the AgNPs makes them good absorbing and scattering of the light particles [11]. TEM images reveal that the molar ratio of AgNPs $\text{NaBH}_4/\text{AgNO}_3$, which decides the degree of aggregation.

With the increase of NaBH_4 concentration, the aggregation was reduced considerably [12]. Some researchers had reported triangular shape silver particles in their study [13]. The silver nanoparticle has been reported to be the most effective against microbial activities [14]. Many researchers have reported the use of silver and silver nanoparticles in medical applications like medial health care and dental materials [15].

However, many bacteria have developed resistance against most of the medicine, which results in lower efficiency. Hence, in this study, silver nanoparticle was synthesized using sodium borohydride as reductant ethanol as the stabilizer. The antibacterial potential of the AgNPs against the *Bacillus subtilis* and *Pseudomonas aeruginosa* was evaluated as there is no report concerning the synthesis of AgNPs followed by antimicrobial activity with the above bacteria.

2. Materials and Methods

The chemical synthesis of silver particles was carried out using silver nitrate (AgNO_3), sodium borohydride (NaBH_4), and ethanol with analytical grade. Sodium borohydride was used as a reducing agent and ethanol as a stabilizer. To produce silver particles, 500 mg of silver nitrate is dissolved with 20 ml of ethanol and magnetically stirred to obtain a uniform solution. Then 500 mg of sodium borohydride was added drop by drop to this solution. Color change from colorless to black color of the solution implies the formation of AgNPs. The aqueous solution poured in the Petri dish at room temperature and dried to get solid particles. Figure 1 shows photographs of chemical synthesis. Fourier transform infrared spectroscopy (FTIR Spectrophotometer-1 Raffinity, Shimadzu, Japan) studies were performed for analysis of chemical bonding and functional group. X-ray diffraction (XRD, D8 Advance, Bruker) using

Cu-K α radiation was used for the identification of phase transformations of the AgNPs. The XRD pattern was recorded in the 2θ range between 10° to 80° at a scan speed of $2^\circ/\text{min}$. The silver nanoparticles film was placed on carbon-coated TEM grids and analyzed by high-resolution transmission electron microscopy (Model FEI-Technai T20 Twin 200 KV instrument). The antimicrobial properties for silver nanoparticles were tested against *Bacillus subtilis* and *Pseudomonas aeruginosa*. The disc diffusion method was used to analyze antimicrobial properties. *Bacillus subtilis* and *Pseudomonas aeruginosa*, inoculums were prepared by using nutrient broth media. Silver nanoparticles synthesized were placed on sterile discs. Discs were dried aseptically under laminar airflow to remove solvents. Dried discs placed on the surface of culture inoculated Mueller Hinton agar plates and plates incubated at 37°C for 24hr. Antibacterial activity was evaluated by using the Himedia zone reader [16].

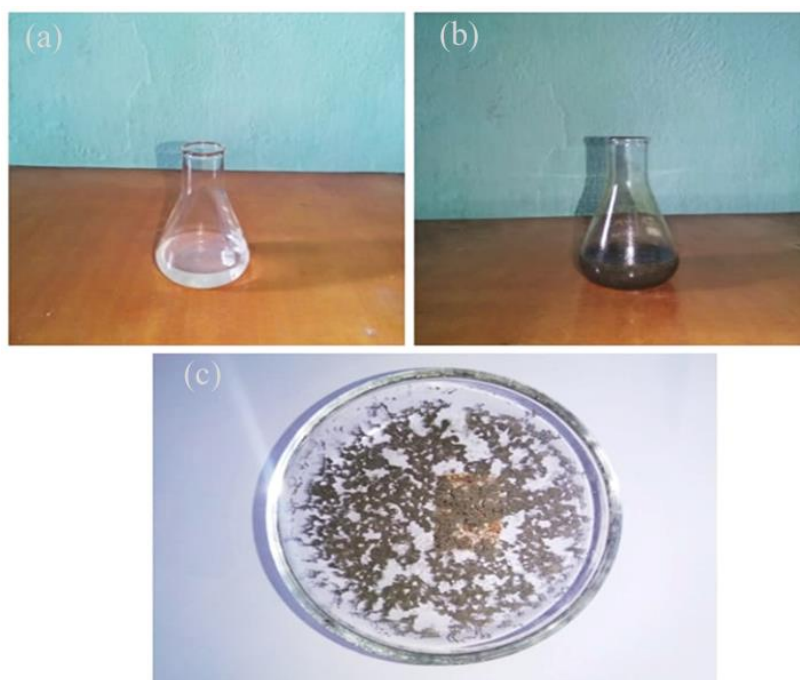


Figure 1. Photograph of chemical synthesis (a) AgNO_3 dissolved in ethanol (b) AgNO_3 solution after adding sodium borohydride and (c) silver nanoparticles.

3. Results and Discussion

3.1. FTIR.

The FTIR spectrum shows various functional groups present at different positions, as shown in Figure 2. The peaks in the region between 3490 cm^{-1} to 2850 cm^{-1} were assigned to O-H stretching of alcohol compounds and aldehyde-C-H- stretching of alkanes. The peaks 1625 cm^{-1} correspond to C-N stretch vibration medium of arenes, 1379 cm^{-1} to 1068 cm^{-1} correspond to primary and secondary amides of N-H (bond) of and -C-N- stretching vibration of amines and peaks between 806 cm^{-1} , and 761 cm^{-1} were assigned to O-H (H-bonded), usually weak bending vibrations of alcohol. After the reaction, the shift in the peak from 3489.23 to 3205.69 , 1408.04 to 1350.17 , 1122.57 to 1068.56 , and 806.25 to 761.88 is indicating that carboxyl, hydroxyl and amide groups may be participating in the process of nanoparticle synthesis[17]. This analysis shows the dual behavior of molecules which may be responsible for the reduction and stabilizing of silver nanoparticle [18].

3.2. X-ray diffraction spectroscopy.

Figure 3 shows the XRD peaks of the silver nanoparticle. The peaks were observed at 2θ values of 38.45° (Ag₂O), 44.67° (Ag/AgO), 64° (Ag₂O), 77° (Ag), and 81.88° (Ag) correspond to Ag (111), (200), (220), (311), and (222). High intensity at 38.45° reflection indicates that the crystallites are mainly orient in this plane. Dorobantu et al. reported that the formation of Ag from AgO is mainly due to lower temperatures below 30°C [19]. The reflections are broader, revealing that smaller size of AgNP crystals. The size of crystals (t) was determined using the Scherrer formula as given in equation 1 [20].

$$t = \frac{0.9\lambda}{\beta \cos\theta} \tag{1}$$

Where λ is the wavelength of the X-ray used (1.5418 \AA), β is the full width at half maximum (FWHM) in radians and θ is the angle of the reflection. Crystalline sizes calculated, and the values are listed in Table 1. The average particle size of AgNPs was observed to be 18.31 nm . Similar particle size was found by Gulbagca et al. of 19.75 nm [21]. The highest peak at $2\theta = 38.5^\circ$ matching to spherical nanoparticles crystallized in the FCC structure with (111) lattice plane [22].

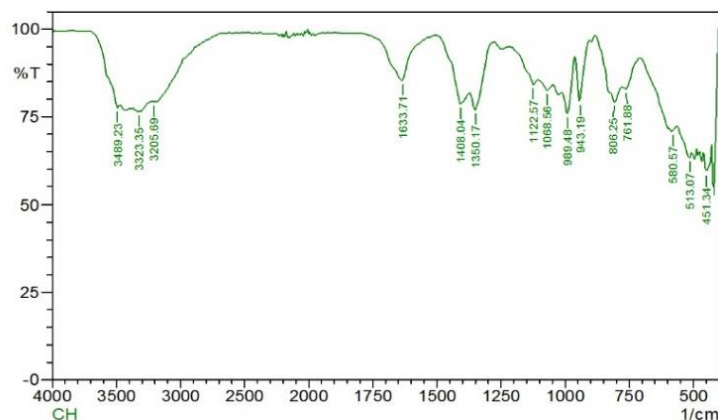


Figure 2. FTIR of dried silver nanoparticles.

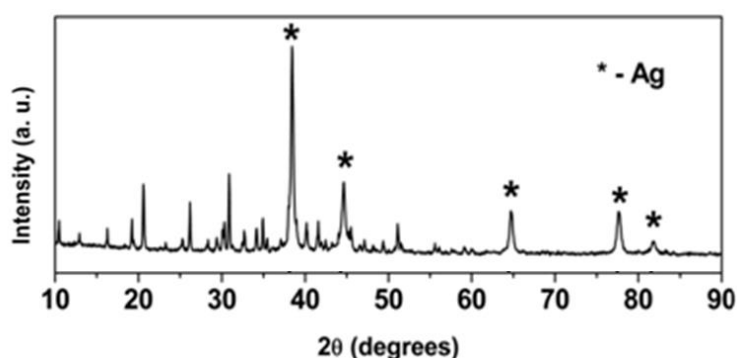


Figure 3. XRD image of dried silver nanoparticles.

Table 1. Calculation of particle size of silver nanoparticles.

S.No	2θ	d (ang)	FWHM (ang)	D(nm)
1	38.451	2.339	0.345	24.40
2	44.676	2.027	0.433	22.05
3	64.762	1.438	0.534	17.62
4	77.699	1.228	0.580	17.60
5	81.881	1.1715	1.062	9.91
The average value of the crystalline size of nanoparticles of silver				18.31

3.3. TEM and EDS analysis.

Figure 4 (a and b) shows the morphology and size of the particles analyzed using a high-resolution transmission electron microscope.

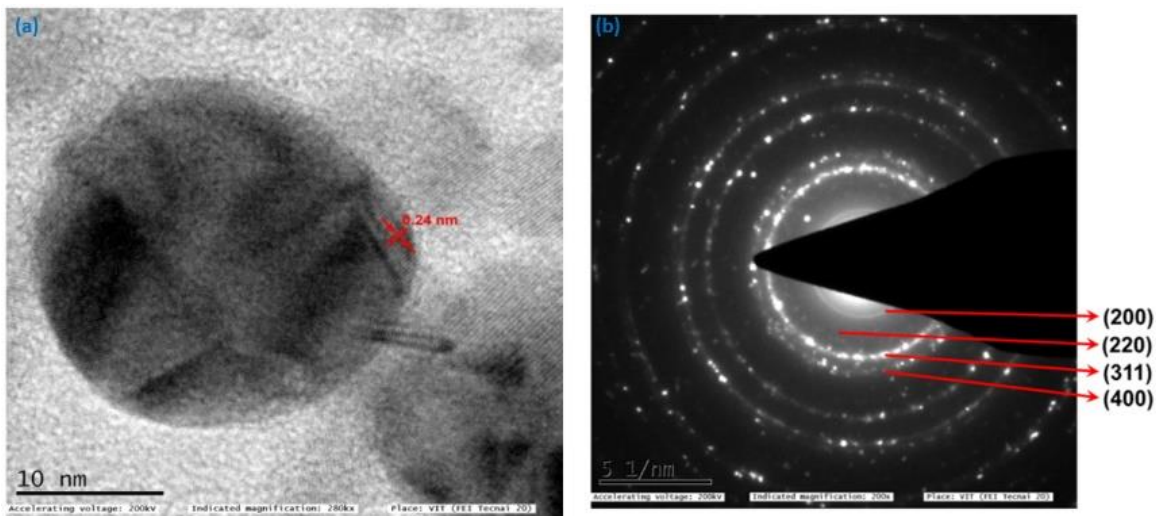


Figure 4. HRTEM Micrograph (a) Image showing d spacing (b) SAED pattern of Ag nanoparticle.

Table 2. Zones of Inhibition at various concentration.

S.No	Name of the Microorganism	Zone of inhibition (mm in diameter)				
		PositiveControl (Tetracycline 30 µg)	Samples (µg)			
			25	50	75	100
1	Bacillus subtilis	21±5	2±5mm	5±5mm	10±5mm	14±5mm
2	Pseudomonas aeruginosa	16±5	R	5±5mm	8±5mm	12±5mm

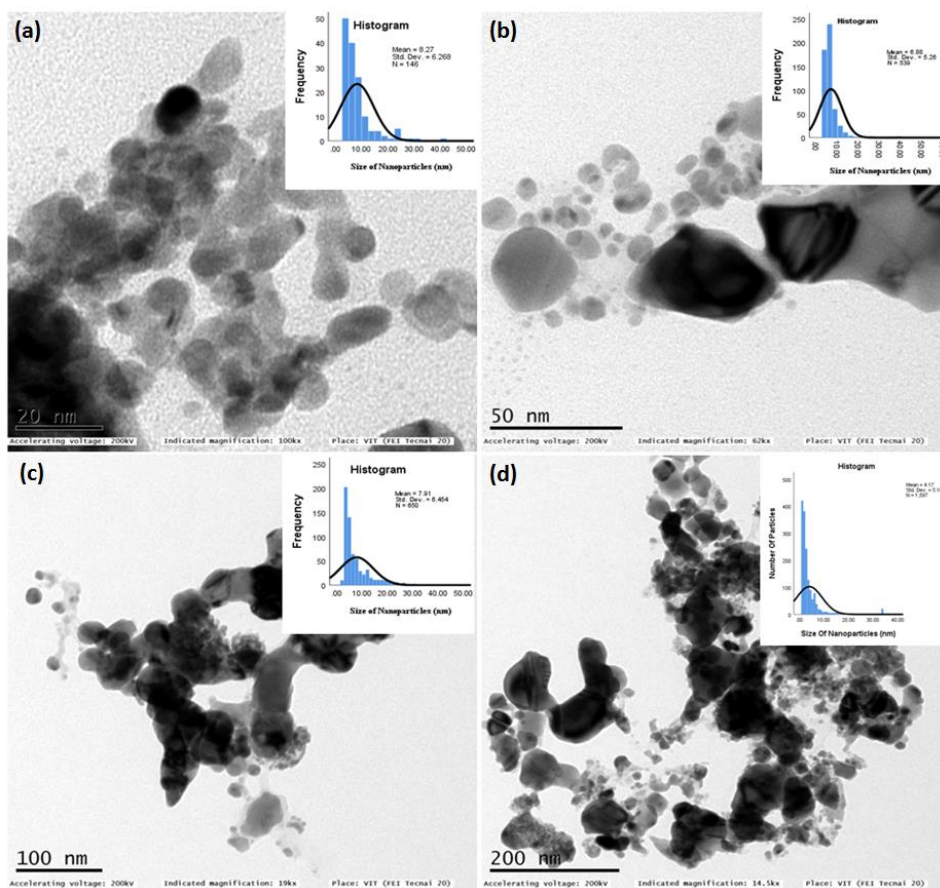


Figure 5. TEM micrograph of silver nanoparticle.

The selected area electron diffraction (SAED) pattern shows the ring-like diffraction pattern, which indicates that the particles are crystalline. The diffraction rings may be indexed based on the fcc structure of silver. The four rings formed due to reflections from (200), (220), (311) and (400) lattice planes of fcc silver, respectively. A similar SAED pattern was obtained with silver nanoparticles synthesized by song et al. [6]. Figure 5 (a to d) shows TEM micrograph of silver nanoparticles at 20 nm, 50 nm, 100nm, 200nm. It was observed that the particle size with variable shape. However, most of them showed spherical shape in nature with some triangular morphology. The majority of the nanoparticles were observed to be scattered with few of them showing aggregates of different sizes as observed under TEM micrograph. Image J software was used to estimate the particle sizes. The data obtained were used to draw the frequency distribution histogram using IBM statistics software. The particles are nearly spherical nature with a particle size of 8 ± 3.05 nm. The HRTEM image used to determine the fringe width or interplanar spacing and was found to be 0.221 nm. EDS analysis confirms the formation of the silver particle, as shown in Figure 6.

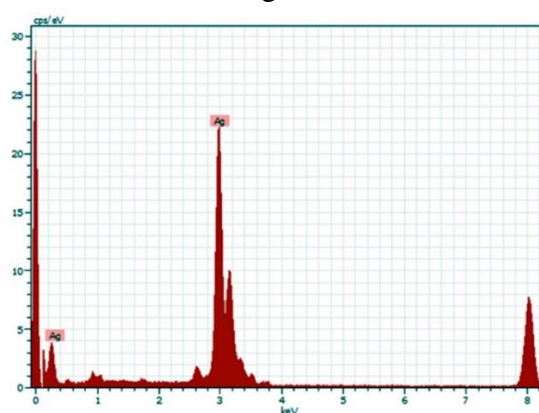


Figure 6. EDS analysis of silver particle.

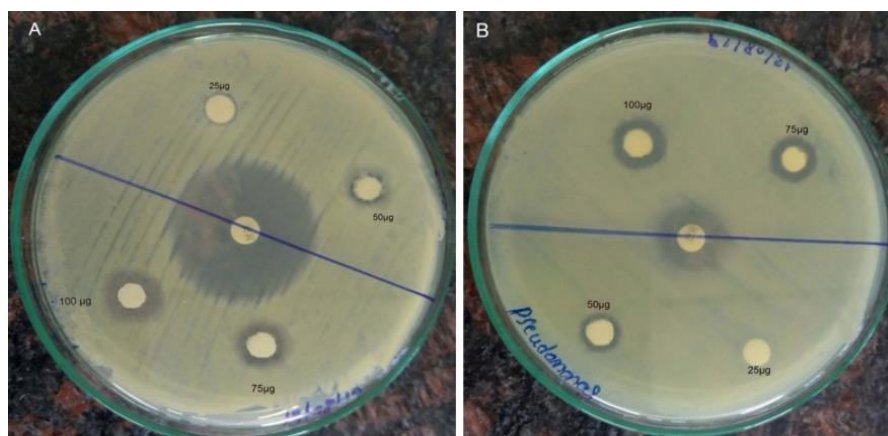


Figure.7. AgNPs showing antibacterial activity against human pathogenic: (a) *Bacillus subtilis*; (b) *Pseudomonas aeruginosa*.

3.4. Antibacterial activity.

Figure 7 shows the disc diffusion method confirms the antibacterial activity of the AgNPs. The inhibition zones found out to be of a maximum value of 19 mm and 17 mm against the bacteria's *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. The values tabulated in Table 2. The agar well diffusion method conforms to the antibacterial activity of the AgNPs synthesized using NaBH_4 against the bacterial strains [23]. Guzman et al. reported the size of silver nanoparticles also affects the inhibition to a greater extent [1]. It was observed that the

decrease in particle size shows lower resistance against the microbial activity. The spherical shape of nanoparticle observed in TEM analysis has influenced the antimicrobial properties. In this analysis, *Pseudomonas aeruginosa* shows better antimicrobial properties as compared to *Bacillus subtilis*. Similar results were observed by Xuan et al. In his observation AgNPs exhibited a zone of inhibition of 10mm against *Escherichia coli* and 7 mm against *Staphylococcus aureus* bacteria [24].

4. Conclusions

In this study, the AgNPs were successfully synthesized by using the chemical method. The characterization of the synthesized silver nanoparticles carried out by employing XRD, HRTEM, FTIR, and EDX, X-Ray diffraction, and HRTEM revealed the amorphous and crystalline nature of the AgNPs. The X-Ray diffraction implied the average crystalline size of 18.31 nm as calculated by employing the Debye-Scherrer formula. HRTEM also implied the formation of AgNPs particle size range of $8 \text{ nm} \pm 3.05 \text{ nm}$. EDX analysis confirms the Ag peak. AgNPs here exhibited acceptable zones of inhibition against *Bacillus subtilis* and *Pseudomonas aeruginosa* bacteria. Thus the research implies that chemical synthesis of AgNPs using Sodium borohydride and ethanol can be used as methods for preparing silver nanoparticles.

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Conflicts of Interest

The authors declare no conflict of interest.

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