

Optical and antibacterial properties of synthesised silver nanoparticles

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In the present study, tri-sodium citrate has been used as both the reducing agent and stabiliser for the synthesis of silver nanoparticles (AgNPs). Sodium borohydride (NaBH₄) was used in various concentrations. The absorbance of AgNP solution was measured after the addition of NaBH₄. The samples were characterised by ultraviolet-visible spectroscopy after 5, 10, 15, 25, 60 and 120 min of synthesis to investigate the stability of AgNPs for different concentrations of NaBH₄. Transmission electron microscopy revealed the nanostructure of the prepared samples with average sizes as 18, 20 and 29 nm. Photoluminescence spectra of these AgNPs showed maximum emission at wavelengths 473, 470 and 469 nm, respectively, when excited at 350 nm. Further, Disk diffusion test showed that all the prepared samples are good candidates for antibacterial activity while AgNPs of average size 18 nm were found most effective against the tested Gram-positive and Gram-negative bacteria.

1. Introduction: Among the noble metals, silver nanoparticles (AgNPs) have become very popular among the researchers due to their analytical, electrical, magnetic and catalytic properties [1–4]. These properties depend on their size, shape and surroundings [5, 6]. Hence controlled synthesis of AgNPs with specific size and morphology is very important and a challenging task. Various methods for the preparation of metallic nanoparticles have been adopted such as reverse micelles process [7], microwave dielectric heating reduction [8], chemical route using citrate etc. to synthesise nanoparticles of desired size and shape. Among all these, citrate reduction method has become very popular due to the higher solubility of reactant in various solvents. The study of Pillai and Kamat [9] revealed that citrate plays an important role in determining the growth of the particle and helps to reduce the metal cation and stabilising the nanoparticles for a long time. They investigated the effects of citrate on controlling the size and shape. Van Hyning and Zukoski first elucidated the mechanism of AgNP formation by sodium borohydride (NaBH₄) as reducing agent [10] (Table 1). Researchers also found that NaBH₄ acts as strong reducing agent which partially oxidises the metal nano-particle surface by forming silver oxides and these oxides finally decreases the electrostatic attraction to stabilise the nanoparticle for a long time. In this report, tri-sodium citrate and NaBH₄ have been employed as reducing and capping agents to control the formation of AgNPs and stabilise them. The prepared samples were then characterised by using UV-vis absorption spectroscopy and Transmission electron microscopy (TEM) to study the optical and morphological properties of nanoparticles.

With the continuous emergence and increase of microbial organisms resistant to various antibiotics, AgNPs have received considerable attention as antimicrobial agents and have shown the potential in broad spectrum bacterial killing [11]. As they are cost effective and at the same time possess low toxicity to human cells, they can be considered as better replacement of antibiotics. It is believed that the antibacterial effect takes place through its binding and penetration through the bacterial cell wall. Once inside the cell, AgNPs bring structural changes in the nuclear membrane leading to cell distortion and death of the bacteria. It has been found that silver also binds to the bacterial DNA and RNA causing denaturing and inhibition of the bacterial cell replications [11]. Based on the above fact, we have synthesised different sized AgNPs by a

single step protocol and studied their antibacterial properties on Gram-positive (*Staphylococcus aureus* ATCC25922) and Gram-negative (*Escherichia coli* ATCC25922) bacteria.

2. Material and methods: Silver nitrate (AgNO₃), tri-sodium citrate (C₆H₅Na₃O₇·2H₂O), NaBH₄ and deionised water were used in the synthesis of AgNP. C₆H₅Na₃O₇·2H₂O has been used as both the reducing agent and stabiliser for the synthesis of AgNPs. NaBH₄ was used in various concentrations to investigate their effect in the synthesis process. All solutions of the reacting materials were prepared in the deionised water. Three different solutions of AgNO₃, C₆H₅Na₃O₇·2H₂O and NaBH₄ were prepared separately. 0.0075 g of AgNO₃, 0.0014 g of C₆H₅Na₃O₇·2H₂O and 0.0014 g of NaBH₄ were dissolved separately in 20 ml deionised water. Three solutions were prepared separately using these stock solutions. In each of these, 3.5 ml of AgNO₃ and 0.2 ml of C₆H₅Na₃O₇·2H₂O were mixed together and x μ l of NaBH₄ was added drop wise one drop per second. The value of x is 25, 50 and 75 μ l for the three solutions. All the prepared samples were found to be light yellow in colour in different shades, which is the evidence of the formation of silver colloidal nanoparticle. The prepared samples were then subjected to ultraviolet-visible spectrometer (UV-Vis) study (Perkin-Elmer, model Lambda-25) at 25°C. In order to determine the kinetics of formation of AgNPs, the UV-visible absorption spectra were measured for various maturation times after the addition of NaBH₄ for 25, 50 and 75 μ l concentration, respectively.

3. Results and discussions

3.1. UV-vis study: UV-vis absorption spectroscopy is an analytical technique used to determine the optical properties of the nanoparticles. The technique is based upon the interaction of electromagnetic radiation with matter. As electromagnetic wave interacts with nanomaterial surface, oscillation of the free electrons in the conduction band occurs. When frequency of the oscillation becomes resonant with the incoming electromagnetic radiation, a strong absorption at specific wavelength occurs which is the origin of the appearance of different colour. The phenomenon is known as surface plasmon resonance (SPR). However, SPR of the nanoparticles is influenced by the shapes, size and interparticle interaction, electron density of the nanoparticles along with its surrounding medium. For the smaller

Table 1 Variation of concentration of NaBH₄ with sizes of AgNP

Quantity of NaBH ₄ , μ l	SPR, nm	FWHM, nm	Particle size, nm	Sample name
25	392	106.45	18	S1
50	395	103.01	20	S2
75	395	98.84	29	S3

sized nano particles, SPR shows a shorter wavelength, while for the larger or more asymmetrical nanoparticles SPR is found at longer wavelengths [12]. Here, the absorbance of AgNP solution was measured at specific time intervals after the addition of NaBH₄. The spectra were taken at 5, 10, 15, 25, 30, 60 and 120 min, respectively, after the addition of NaBH₄ to investigate the stability of AgNP. It was found that there was no change in SPR peak position at 392 nm (Fig. 1a) for 25 μ l NaBH₄ for various maturation times, which indicates that the particle did not aggregate. This may be due to the fact that the reduction of Ag²⁺ to Ag⁰ at this concentration is completed and the reaction gets saturated. The saturation of reaction may results the saturation of electron injections in AgNPs. Further, for 50 and 75 μ l NaBH₄, variation in intensity and bandwidth can be seen, which may be due to the presence of different particle size and size distributions of the nanoparticles formed due to aggregation. The UV-visible spectra of AgNP (25–75 μ l) presented in Fig. 2 with various concentrations of NaBH₄ for fixed maturation time (5 min and 2 h) showed the red shift of absorption peak after the addition of NaBH₄, which is the evidence of increase in particle size. The increasing integrated peak area of the band spectrum indicates decrease in interparticle spacing, which is the evidence of aggregation [13]. Here NaBH₄ and trisodium citrate played as first and second reductant in the synthesis process. The initial reduction was performed by NaBH₄ at room temperature, which induced the generation of a larger number of AgNPs. AgNPs formed at initial stages subsequently participated in the growth process. Citrate played a crucial role in this process. Since the Ag colloids possess negative charge due to the absorbed citrate ions, a repulsive force work on the particles preventing their agglomeration and thus ensuring stability [13].

3.2. TEM analysis: TEM images of different sized AgNPs at various concentration of NaBH₄ are presented in Fig. 3. The analysis revealed the morphology and structure of the prepared samples. Fig. 3a shows the nearly spherical shaped AgNPs with an average size 18 nm. Fig. 3b shows a number of AgNPs with diameter between 5 to 30 nm. The averages size was found as 20 nm. Nanoparticles with some smaller and larger sizes ranging from 10 to 80 nm can be seen in Fig. 3c. Larger nanoparticles, with diameter >50 nm were found to be surrounded by some smaller AgNPs of ~10 nm. It can be seen that the larger nanoparticle formed did not adopt spherical shape, rather elongated shapes are observed. Formation of these longer shaped particles may be due to the aggregation of two or more particles together. This finding is supported by UV-vis spectra (Fig. 2) showing a broad area of the band. The average sizes obtained from TEM images were found as 18, 20 and 29 nm for the concentration of 25, 50 and 75 μ l of NaBH₄, respectively (Table 1), and coded as S1, S2 and S3.

3.3. PL study: Visible photoluminescence (PL) observed in nanoparticles is due to the inter-band transition between sp band and valance band. In silver, out of six bands, five bands lie below the Fermi levels are called d or valance and the single band which lies above the Fermi level are denoted as sp or conduction band [14]. The transition between the sp and d band is known as

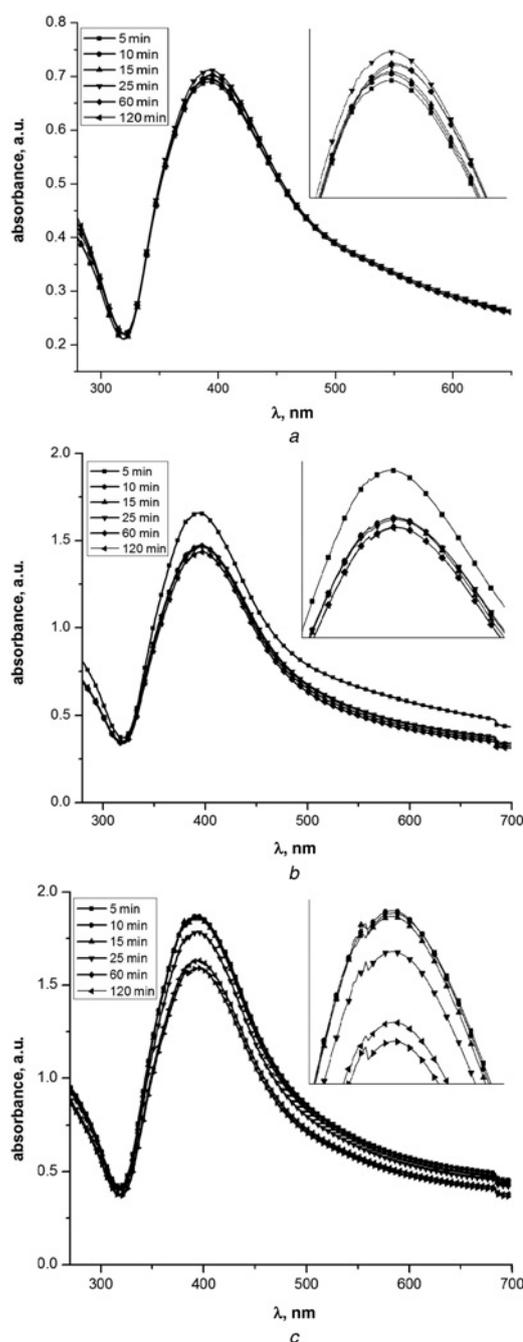


Fig. 1 UV-vis spectra of colloidal AgNPs for various maturation times at the concentration of
a 25 μ l
b 50 μ l
c 75 μ l NaBH₄

interband transition. Here (Fig. 4) for S1, the emission wavelength was found at 473 and 492 nm, for S2 470 and 486 nm, while for S3 emission observed at wavelength 469 and 500 nm, respectively, for an excitation wavelength 350 nm. The luminescence observed were attributed to interband transition while the damping of PL spectra with the increase in size may be attributed to the radiative damping effect that causes the decrease in local electric field inside the particle [15].

3.4. Antibacterial study: To examine the antibacterial effects of different sized AgNPs, we performed disk diffusion test against selected bacteria. The experiments were carried out with one

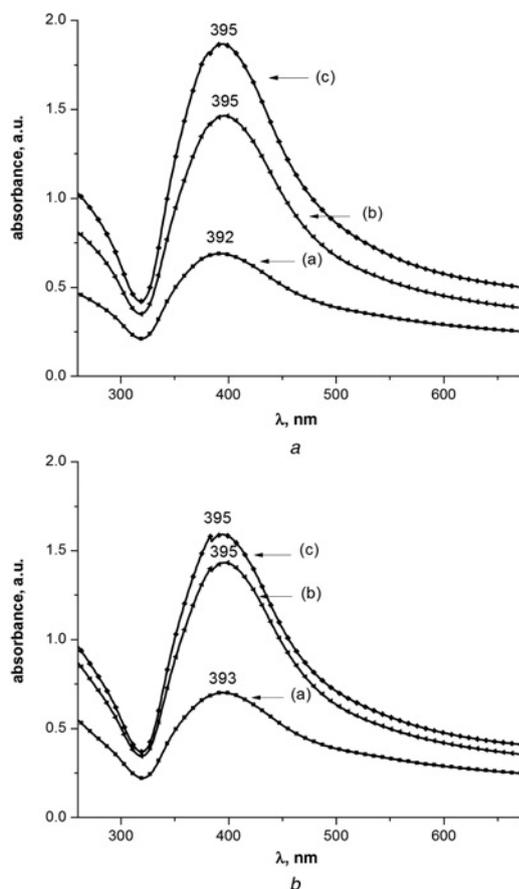


Fig. 2 UV-Vis spectra for different concentrations ($a = 25 \mu\text{l}$, $b = 50 \mu\text{l}$ and $c = 75 \mu\text{l}$) of NaBH_4 after
 a 5 min of precipitation of AgNP
 b 2 h of precipitation of AgNP

Gram-negative (*E. coli* ATCC25922) and one Gram-positive bacteria (*S. aureus* ATCC25923). Layers of bacterial suspension were applied uniformly on the surface of nutrient agar plate. Immediately afterwards small disks of uniform size (5 mm diameter) containing 228, 223, 217 and 207 $\mu\text{g}/\text{ml}$ designated as C1, C2, C3 and C4, respectively, were placed carefully on the plates and incubated at 37°C for 24 h. Three sets of plates S1, S2 and S3 were prepared in this way. S1 contained AgNPs of size 18 nm, S2 20 nm and S3 29 nm, respectively. Each set had two plates, one for *E. coli* and the other for *Staphylococcus aureus*. The zones of inhibition were measured by a standard ruler (Fig. 5). The test revealed that the different sized AgNPs were not equally effective against two bacteria as variation in zone of inhibition were observed. For all the four doses (C1-C4) of samples S2 and S3, lesser antibacterial effect has been observed for both *E. coli* and *S. aureus*, while S1 showed greater antibacterial effects against two bacteria. Moreover, C2 and C3

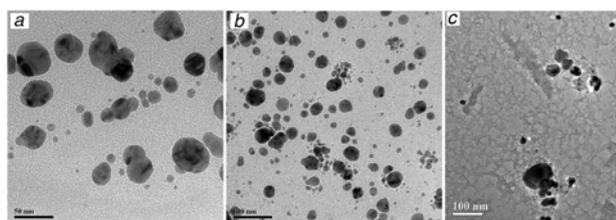


Fig. 3 TEM images of AgNPs for
 a $25 \mu\text{l}$
 b $50 \mu\text{l}$
 c $75 \mu\text{l}$ NaBH_4 concentrations

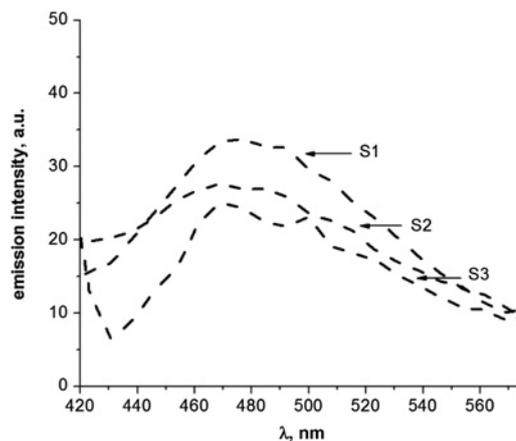


Fig. 4 PL spectra of AgNPs in aqueous solution

were the effective dose found against *E. coli* and *S. aureus* (Table 2). This implies that smaller sized AgNPs synthesised using our approach have promising antibacterial potential against tested microorganism. The formation of zone of inhibition is an indication of bactericidal potential of S1 (18 nm) where the tested bacteria were incapable of surviving in this zone. The result found in our case is in good agreement with others [16, 17]. It is believed that obtained AgNPs $<100 \text{ nm}$ size shows greater antibacterial effects. Because for smaller sized nanoparticles,

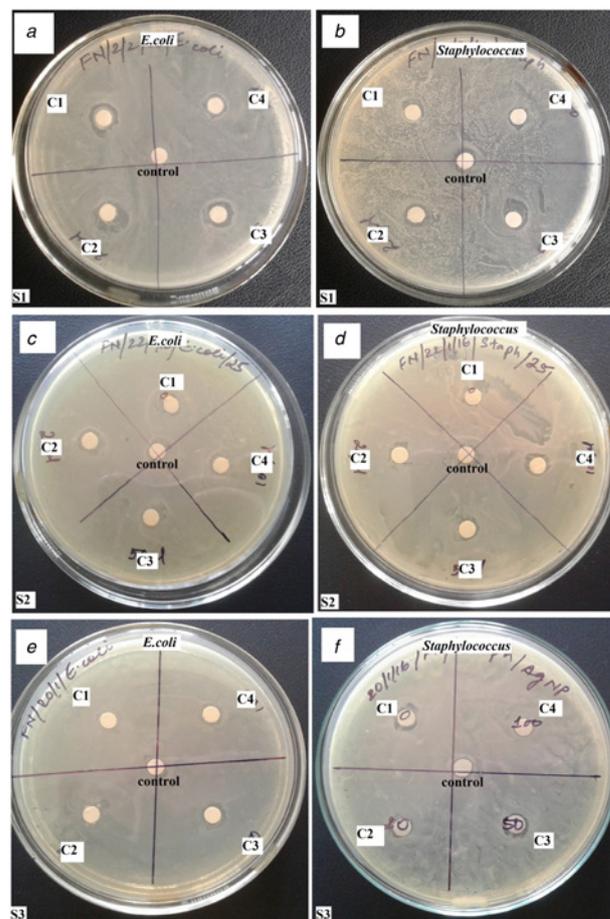


Fig. 5 Representative images of disk diffusion test of AgNPs different conc. (C1, C2, C3 and C4) against *E. coli* and *Staphylococcus* for different sizes (S1, S2 and S3)

Table 2 Zone of inhibition against various bacteria at different concentration of AgNPs

AgNP Conc., µg/ml	<i>E. coli</i>			Staphylococcus		
	S1	S2	S3	S1	S2	S3
C1 = 228	10	no zone	no zone	7.5	no zone	no zone
C2 = 223	11.2	9	no zone	8.9	8	9
C3 = 217	9.4	5	9	10.8	9	7
C4 = 207	7.3	7	8	7	8	no zone

surface to volume ratio increases thus probable mode of interaction with bacterial cell wall also increases results the greater antibacterial effects [18]. As Gram-positive and Gram-negative bacteria has different membrane structure, hence they show different response against AgNPs. Several researchers have reported different antibacterial activities against different bacteria though actual mechanism is still unknown. Sondi–Salopek reported that antibacterial activity of AgNPs on Gram-negative bacteria was depended upon concentration of AgNP. It was observed that AgNPs interact with the bacterial membrane and causes cell damages. Further, TEM and EDAX study confirmed the incorporation of AgNPs into the membrane, which was recognised by formation of pits on the cell surface [19]. Panacek *et al.* [16] reported a single step protocol for synthesis of smaller sized AgNP. They found higher antibacterial activity of AgNPs against Gram-positive and Gram-negative bacteria at low concentration.

Shrivastava *et al.* [17] in 2007 reported synthesis of AgNPs having size range 10–15 nm and studied the dose depended effect on the Gram-positive and Gram-negative organism.

Similar to previous work, we found our result is consistent with other studies where smaller sized AgNPs showed greater antibacterial activity and the activity were attributed to the maximum contact area with nanoparticles and bacterial surface. We also assume that silver ion released in the bacterial suspension or the direct surface contact with bacterial cell wall is responsible for the antibacterial effect; particularly for our case 18 nm size range is attributed to the contact mode killing mechanism [18].

4. Conclusion: We have shown a single step protocol to synthesise AgNPs by chemical reduction of silver nitrate with trisodium citrate and NaBH₄ as reducing agents and we could achieve AgNP with sizes 18, 20 and 29 nm, respectively for 25, 50 and 75 µl concentration of NaBH₄, respectively. It was found that the concentration of reducing agent (NaBH₄) increases the absorption band and also increases the particle size. The first step involves the formation of Ag⁰ atoms from Ag⁺ ions. These atoms form cluster in later by coalescing small particles. This stage is called metastable state, where the particles maintain their size for few minutes. After this period, the formation of final AgNP rendered. The dependence of the bandwidth upon particle size i.e. $\omega \sim 1/R$ shows the decrease of the band width with the increase of particle size (Table 1). PL study shows that the prepared samples were optically active and inferred the possibility of AgNPs to be used in ‘nano laser’ with optical pumping. Further, disk diffusion test revealed that AgNPs having size 18 nm have greater antibacterial effect. The enhanced antibacterial effects of smaller sized nanoparticles were attributed to the higher surface to volume ratio

which makes a probable contact mode with bacterial surface and ultimately cell death. However, there is need of detailed study such as how the surface area of nanoparticles influences its killing activity, the exact killing mechanism etc. for better understanding of antibacterial activity of AgNPs.

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6 References

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