Conference paper

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Green synthetic approach for starch capped silver nanoparticles and their antibacterial activity

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Abstract: In this work silver nanoparticles (AgNPs) have been prepared from silver nitrate ($AgNO_3$) precursor using a green synthesis method at room temperature. Starch with its abundance of hydroxyl groups and its biocompatibility was used as a capping and reducing agent. The formation of AgNPs was confirmed by absorption spectroscopy with the surface plasmon resonance peak at 400 nm. The sharp reflection at (111), (200), (220) and (311) was observed by powder X-ray diffraction (XRD), which indicated the presence of cubic phase AgNPs. Transmission electron microscopy (TEM) revealed that the average size of AgNPs were between 0.5 and 4 nm with a spherical shape under optimum conditions. The nanoparticles showed a decrease in size with an increase in precursor concentration as well as the increase in capping agent concentration. The nanoparticles also showed to be bactericidal towards the tested Gram-negative *(Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria.

Keywords: antibacterial activity; antimicrobial activity; chemical synthesis; Green Chemistry V; green synthesis; nanoparticles; silver; silver nanoparticles; starch.

Introduction

Metal nanoparticles have gained attention in recent years as a result of their wide variety of applications in biomedical field. Silver nanoparticles (AgNps) have drawn much research interest due to its antimicrobial properties [1]. Chemical reduction of metal salt is the most commonly used method for the preparation of nanoparticles. Although this method is easy, simple and convenient for metal nanoparticles preparation in nanometer range, most of the reducing and capping agents used are environmentally and biologically hazardous. For this reason there is a growing interest in using green chemistry principles for the synthesis of nanoparticles where the use of chemicals that are hazardous to human health and the environment will be eliminated [2, 3]. The physico-chemical properties, stability, size and morphology of the metal nanoparticles are strongly affected by experimental conditions, the interaction of metal ions with the reducing agents as well as the absorption process of the stabilizing agent with the metal nanoparticles. The concentration of the metal salt, reducing agent and solution temperature has a great effect on the particle size [4, 5]. For this reason, the effect of precursor concentration and capping agent concentration were investigated in this work. In this work, AgNPs have been synthesized at room temperature in distiled water by the reduction of silver

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nitrate ($AgNO_3$) with starch used as the reducing agent and the capping agent. This method was used as the form of green chemistry in order to produce nanoparticles that are environmental and biological friendly. Starch was used because it contains hydroxyl groups in its template that protect nanoparticles from aggregation and it is environmental friendly and biocompatible.

Methodology

Chemicals

$AgNO_3$ (99.8 %) and soluble starch (99.9 %) were purchased from Merk (Germany). Acetone (99.8 %) was purchased from Sigma Aldrich (South Africa). Muller–Hinton broth and Muller–Hinton agar were purchased from Neogen (Michigan). *Escherichia coli* and *Staphylococcus aureus* bacterial cells were bought from Anatech (South Africa). All chemicals were used as purchased, without any further purification.

Synthesis of starch capped AgNPs: Effect of precursor and starch concentrations

Starch capped AgNPs were prepared at room temperature by adding various concentrations of $AgNO_3$ solution (0.05, 0.1, 0.2 M) to various starch concentrations (0.5, 1, 2 % (w/v)) in a round-bottom flask. The mixture was stirred for an hour under inert atmosphere. The resultant solution was centrifuged and extracted with acetone to obtain AgNPs precipitate. The precipitate was washed and dried at room temperature. The samples were then characterized with UV-Vis spectroscopy, Fourier transform infared (FTIR) spectroscopy, thermogravimetric analyzer (TGA), transmission electron microscopy (TEM) and X-ray diffraction (XRD) techniques.

Characterization

A UV-Vis spectrophotometer (Perkin Elmer Lamda 25) was used to carry out optical analysis. The measurements were taken in room temperature at 200–1100 nm wavelength range. The samples were placed in quartz cuvettes cell of 1 cm path length to take the measurements, a beam was passed through and the spectrum was recorded. FT-IR spectral studies were carried out using a Perkin Elmer spectrum 400 FT-IR-NIR Spectrometer equipped with a universal ATR sampling accessory. The nanoparticles microstructure was studied using a JEOL JEM-2100 TEM operating at 200 kV. The TEM was coupled with an energy dispersive X-ray (EDX) detector which was used to determine the elemental composition of the synthesized nanoparticles. The samples were prepared by placing an aliquot solution of the water soluble nanocrystalline material onto an amorphous carbon substrate supported on a copper grid and then the solvent was allowed to dry at room temperature. The X-ray diffraction patterns were recorded using a Bruker D2 diffractometer at 40 kV and 50 mA and a secondary graphite monochromated Co Kα radiation ($l = 1.7902$ Å). The measurements were taken at high angle 2θ in a range of 5°–90° with a scan speed of $0.01°\ 2\theta\ s^{-1}$. Perkin Elmer thermogravimetric analyzer (TGA 4000) was used for thermal analysis. The samples were heated under nitrogen and the heating range was between 25 and 900 °C at a heating rate of 5°/min.

Antibacterial activity of AgNPs

The antibacterial activity of AgNPs was evaluated with *S. aureus* and *E. coli* bacteria species. One hundred microliters of actively grown broth cultures of the test bacteria was spread on the Mueller–Hinton agar plates.

Sterile discs loaded with different concentrations of AgNPs (6.0, 3.0, 1.5, 0.75, 0.37 mg/mL) were placed on the inoculated plates in triplicates. The plates were incubated for 24 h at 37± 2°C. The inhibition zone around the nanoparticles loaded discs was measured and expressed in millimeters (mm).

Minimum inhibitory concentration (MIC) of AgNPs

The MIC of the prepared starch capped AgNPs was further investigated using a 96-well microtiter plates for Resazurin Based Microtiter Dilution assay under sterile conditions. AgNPs (100 µL) in sterile water was filled on the first raw of the microtiter plates. Nutrient broth (100 µL) was also filled in all the wells of the microtiter plates. Two-fold serial dilutions were prepared by transferring 100 µL of the nanoparticle from the first row to the following wells in the next row of the same column so that each well contains 100 µL of nanoparticles in serially descending concentrations. Resazurin solution (10 µL) was added in each as well as the indicator. A 10 µL volume of the bacterial suspension was added to each well to achieve a final concentration of 5×10^6 CFU/mL. Each plate was then wrapped lightly with foil in order to avoid dehydration of bacterial culture. Each microtiter plate had a set of three controls, (i) a column with a positive control (amoxycilin), (ii) a column with all the solutions except the nanoparticles and (iii) a column with all solutions except bacterial solution which was replaced by 10 µL of nutrient broth. The plates were then incubated for 24 at 37 °C for 24 h. The change in color in the wells was visually observed. A color change observed from purple to pink or colorless was taken as positive. The lowest concentration of the nanoparticles at which color change occurred was recorded as the MIC value. The MIC was evaluated using the following concentrations of nanoparticles (0.37, 0.18, 0.093, 0.046, 0.023 mg/mL). The experiments were done in triplicates.

Results and discussion

This work reported the characterization of starch capped AgNPs as well as the effect of starch and precursor concentrations on size and shape of particles. The characterization and the effects of the concentration was done using FTIR spectroscopy, UV-Vis spectroscopy, TEM, TGA and XRD. The starch and precursor concentrations have been investigated because they control the size and morphology of the nanoparticles. The antibacterial activity of the synthesized AgNPs on *S. aureus* and *E. coli* bacterial cells is also discussed.

Optical and structural analysis

UV-Vis spectroscopy is a very useful technique for studying metal nanoparticles because the peak position and shapes are sensitive to particle size [6]. The effect of the capping agent concentration on the optical properties of the synthesized AgNPs is shown in Fig. 1. The 0.5 % (w/v) starch did not show any absorption peak. The 1.0 % and 2.0 % (w/v) starch showed broad surface plasmon peak at 414 nm (2.99 eV) and 409 nm (3.03 eV), with broad plasmon and sharp intense, respectively. The spectrum showed a shift in peak absorbance to lower wavelength as the starch capping agent concentration increases [1.0–2.0 % (w/v)]. This means that at higher concentration of starch smaller size of AgNPs were produced. The broadness of the peaks also showed a decreased size with an increase in precursor concentration. This means that the nanoparticle size distribution became narrower as the capping agent concentration was increased.

The FT-IR spectroscopy was used to investigate the interactions between the functional groups of starch capping agent and AgNPs. Figure 2 shows the FT-IR spectra of starch capped AgNPs at different

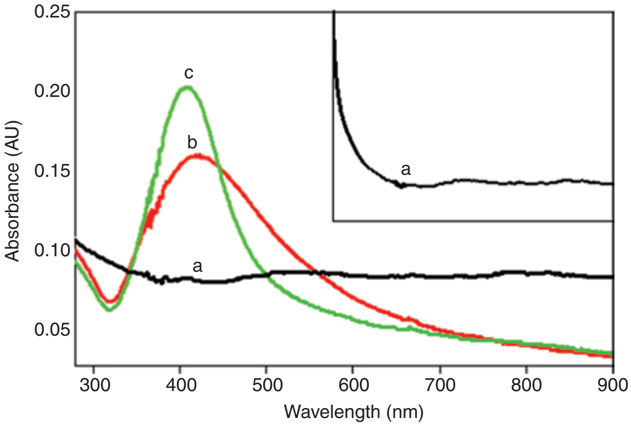

Fig. 1: Absorption spectra for starch capped AgNPs with (a) 0.5 % (w/v), (b) 1.0 % (w/v) and (c) 2.0 % (w/v) starch.

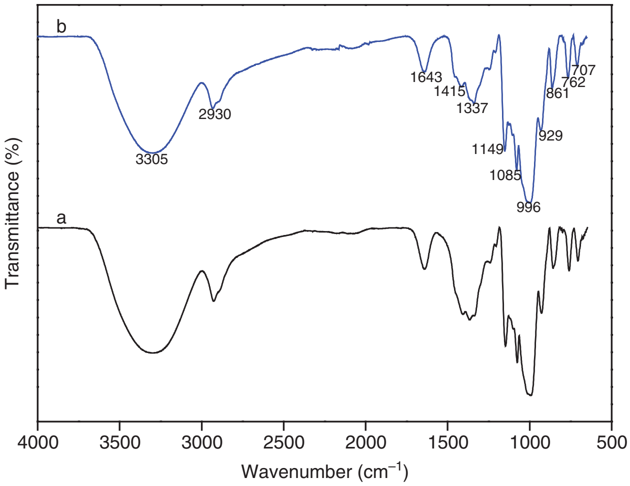

Fig. 2: FTIR spectra of (a) starch capped AgNPs, (b) pure starch.

precursor concentrations, synthesized with 1.0 % (w/v) of starch. A strong band observed at 3305 cm^{-1} was due to the O–H stretching of starch. An asymmetric stretching of C–H band was observed at 2930 cm^{-1}, and the band at 1643 cm^{-1} was attributed to water adsorbed in the amorphous region of starch. The observed characteristic band at 1085 cm^{-1} was due to C–O–H bending of starch. The shift at 3305 and 1085 cm^{-1} band to 3295 and 1076 cm^{-1}, respectively, was observed in the presence of nanoparticles. The peaks were also broader and longer in the starch capped nanoparticles as compared to starch alone, which might be due to inter and intra-molecular hydrogen bonding. These starch characteristic bands observed in the synthesized nanoparticles confirmed the successful binding of the OH group of starch capping agent to the AgNPs [7].

Morphological analysis

Figure 3 shows the TEM micrographs and size distribution of the synthesized AgNPs with different starch capping agent concentrations. The 0.5 % (w/v) starch gave bigger nanoparticles due to insufficient amount of capping molecule to control the nucleation and growth of particles. The 1.0 and 2.0 % (w/v) starch gave well-dispersed nanoparticles without any agglomeration. The results showed that the nanoparticles size decreased with an increase in capping agent concentration [0.5–1.0 % (w/v)] which indicates that higher

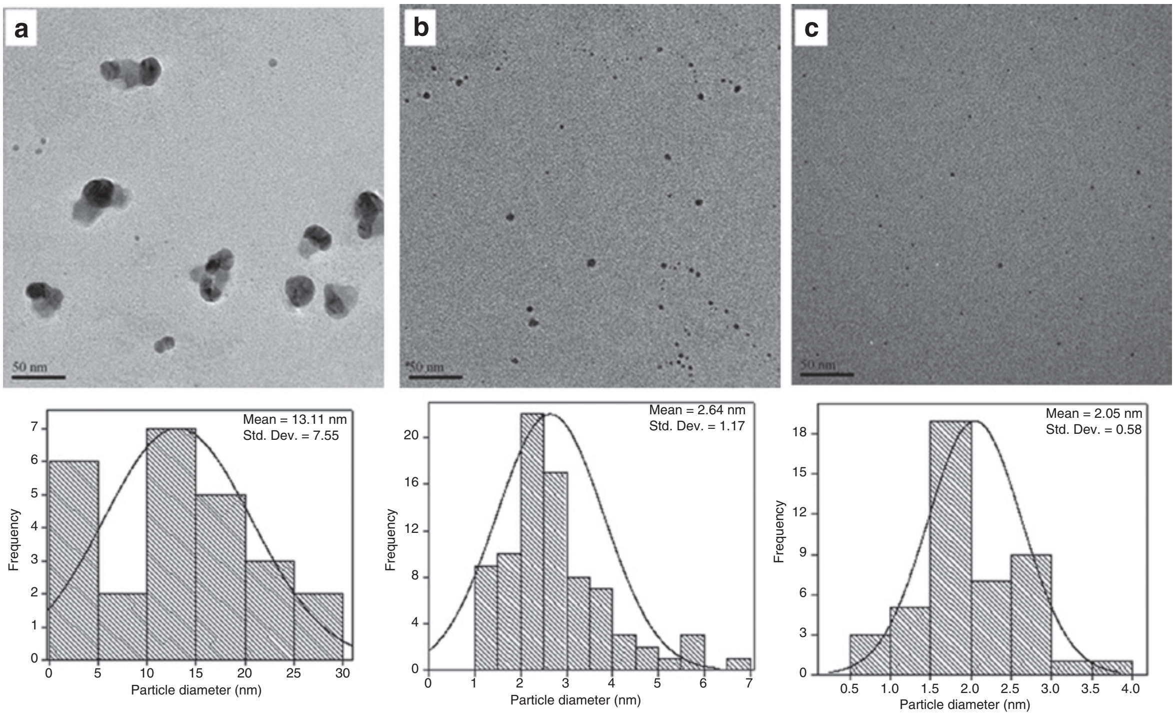

Fig. 3: TEM image and size histograms for starch capped AgNPs with (a) 0.5 % (w/v), (b) 1.0 % (w/v), (c) 2.0 % (w/v) starch.

capping agent concentration led to a more effective capping capability and thus smaller AgNPs were produced. Increasing the precursor concentration to 2.0 % (w/v) did not show any major change in the nanoparticles size. The average particle sizes as calculated are 13.11 nm for 0.5 % (w/v) starch, 2.64 nm for 1.0 % (w/v) starch and 2.05 nm for 2.0 % (w/v) starch, all with a spherical shape. The decrease in particle size with an increase in capping agent concentration has also been observed by Shen et al. [8] on the AgNPs stabilized by polyvinylpyrrolidone (PVP). Nanoparticles obtained with 0.1 M $AgNO_3$ and 2.0 % starch gave the smallest particle size with narrow distribution range, hence these conditions were taken as optimum for the preparation of AgNPs. Li et al. [9] also used starch as a capping agent for cadmium selenide nanoparticles and spherical nanoparticles with an average size of 3 nm were obtained. Wei et al. [10] capped cadmium sulfide nanoparticles with starch and obtained spherical nanoparticles with an average size of 4 nm. The starch capped AgNPs have also been prepared by Mohan et al. [11] and Hebeish et al. [12]. The nanoparticles obtained were spherical in shape with an average size of 6 nm and 7 nm, respectively. The nanoparticles size for the literature results is slightly bigger than the average size (2 nm) obtained in this work under optimum conditions, while the nanoparticles shape is similar. Khan et al. [13] also synthesized AgNPs capped with *Raphanus sativus* extract, starch and CTAB. The nanoparticles shape and average size were found to be irregular (6–20 nm), triangular nanoplates with nanorods (50–450 nm), and spherical (5–30 nm), for extract, starch and CTAB-capped AgNPs, respectively.

The TEM micrographs and size distribution of the synthesized AgNPs with different precursor concentrations are shown in Fig. 4. Lower concentration of both capping agents and the precursor yielded particles with larger diameters as compared to higher concentrations. For 0.05 M concentration well-dispersed, bigger nanoparticles which could be attributed to rapid nucleation and growth of particles of the small amount of the precursor. The 0.1 and 0.2 M gave small with a decreased polydispersity of spherical shaped nanoparticles. The results showed a decrease in nanoparticle size with an increase in precursor concentration (0.05–0.1 M). There was no major change observed on increasing the precursor concentration to 0.2 M. This indicate that similar amount of capping agent used was enough to effectively passivate nanoparticles at these higher precursor concentration which then led to the production of smaller nanoparticles. The average particle sizes as calculated are 11.95, 2.64 and 2.59 nm for 0.05 M, 0.1 M and 0.2 M, respectively. This

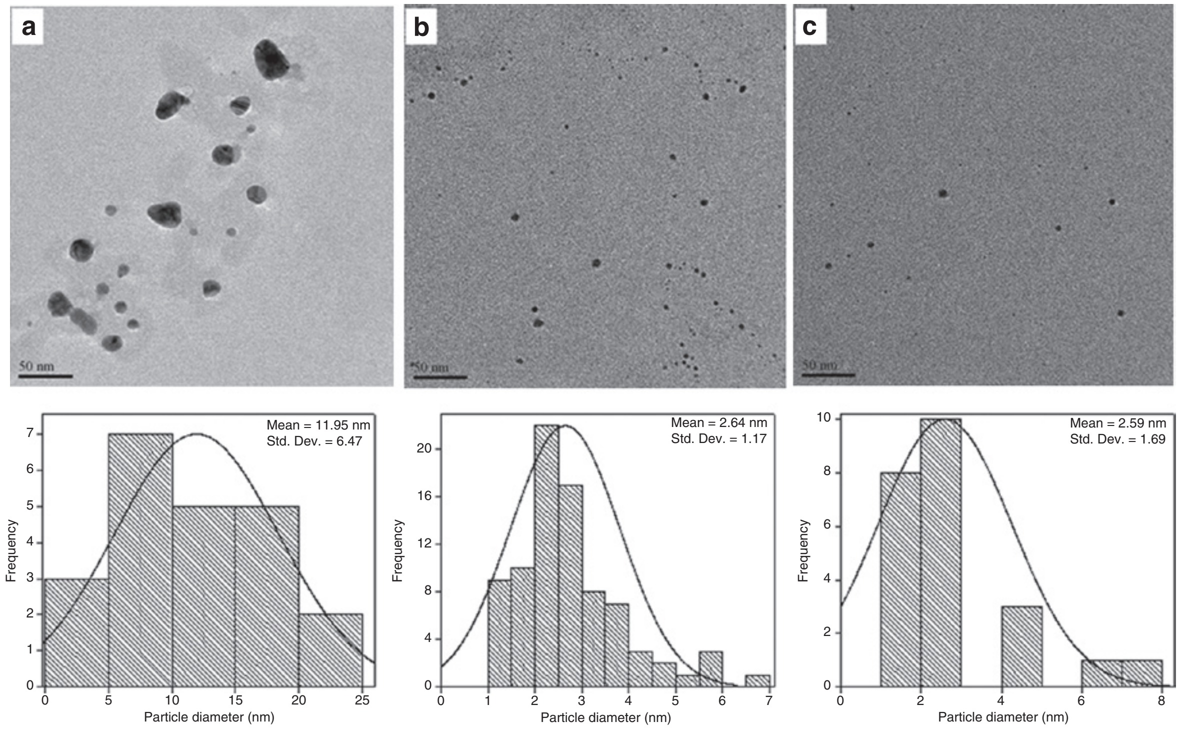

Fig. 4: TEM image and size histograms for starch capped AgNPs with (a) 0.05 M, (b) 0.1 M, (c) 0.2 M.

trend was also observed by Umadevi et al. [14] on their AgNPs using $AgNO_3$ as a precursor and sodium borohydride as a reducing agent. The EDX profile also confirmed the presence of elemental Ag in the synthesized nanoparticles.

The XRD patterns (Fig. 5) showed the sharp reflection at (111), (200), (220) and (311) which indicates the presence of AgNPs (JCPDS No. 04-0783) with a face-centered cubic phase [10]. The XRD showed high intense peaks which indicate high degree of crystallinity of the synthesized AgNPs. On the other hand, the diffraction peaks are broad which indicate that the crystallite size is very small as shown in Figs. 3 and 4.

Figure 6a and b shows the TGA thermographs and derivative curves of pure starch and starch capped AgNPs. The TGA thermographs of pure starch and starch capped AgNPs showed two decomposition steps. The degradation step observed around 100 °C is due to water desorption from the starch and nanoparticles. The weight loss around 290 °C for pure starch and 298 °C for starch capped nanoparticles is the

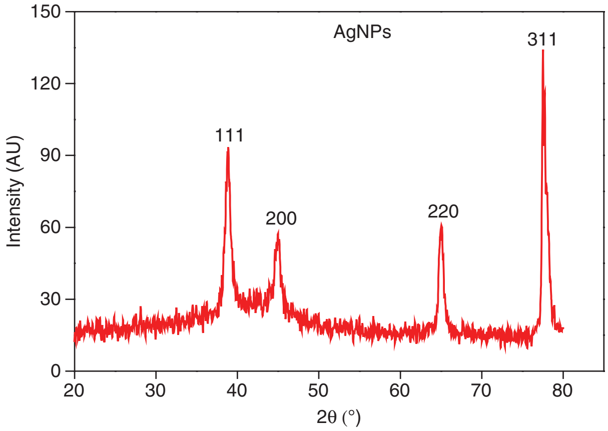

Fig. 5: XRD patterns of starch capped AgNPs.

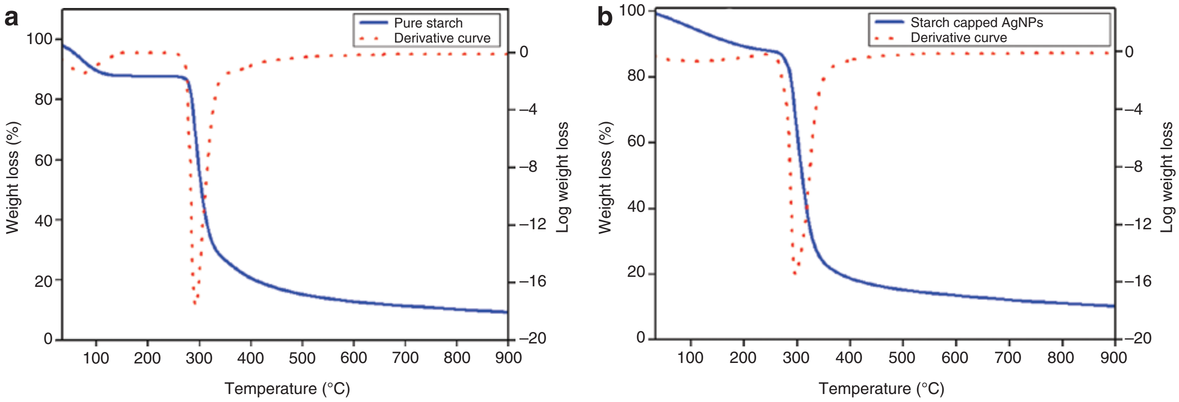

Fig. 6: TGA and derivative thermograph of (a) pure, (b) starch capped AgNPs.

degradation of starch capping agent leaving the AgNPs behind. The TGA thermograph showed no further weight loss above 298 °C. The relative percentage of the residue is 18 %, which represent the amount of AgNPs remaining after thermolysis. Starch capped AgNPs showed a decomposition profile at higher temperature than starch alone, which indicate an improved thermal stability of starch due to the presence of AgNPs.

Antibacterial activity

Escherichia coli and *S. aureus* bacteria species were used in order to investigate the antibacterial activity of the synthesized AgNPs. The antibacterial studies results are shown in Table 1. A positive response was observed towards both bacterial species in all the concentrations studied (6.0–0.37 mg/mL). AgNPs showed the maximum inhibition zone of 13±0.16 mm diameter against *E. coli* and 13 ± 0.11 mm diameter for *S. aureus*. The inhibition zones obtained increase slightly with the increase in concentration, this could mean that the antibacterial activity of the nanoparticles is dependent of precursor concentration used. The smaller particle sizes also result in increased inhibition zones. The reason for this could be the similarity in shape of the

Table 1: Antibacterial activity of nanoparticles against bacteria.

Nanoparticles	Concentration (mg/mL)	Zone inhibition (mm)	
		Escherichia coli	*Staphylococcus aureus*
0.05 M Ag	6.0	9±0.34	10±0.23
	3.0	9±0.25	10±0.33
	1.5	9±0.24	9±0.25
	0.75	8±0.28	8±0.14
	0.37	8±0.33	8±0.2
0.1 M Ag	6.0	12±0.15	12±0.16
	3.0	12±0.39	12±0.19
	1.5	11±0.34	12±0.32
	0.75	11±0.15	11±0.14
	0.37	10±0.22	11±0.25
0.2 M Ag	6.0	13±0.19	13±0.24
	3.0	13±0.16	13±0.11
	1.5	12±0.19	13±0.19
	0.75	12±0.29	12±0.17
	0.37	11±0.11	12±0.13

Table 2: MIC of nanoparticles against bacteria.

Nanoparticles	Concentration (mg/mL)	
	Escherichia coli	*Staphylococcus aureus*
0.05 M Ag	0.046	0.023
0.1 M Ag	0.023	0.023
0.2 M Ag	–	–

(–) Means no minimum inhibitory concentration was found throughout the concentrations used.

nanoparticles synthesized in different precursor concentrations. The MIC was then further investigated using 0.37–0.023 mg/mL of nanoparticles, the results are presented in Table 2. The MIC results showed that 0.05 M AgNPs have MIC of 0.046 mg/mL against *E. coli* and 0.023 mg/mL against *S. aureus* and 0.023 mg/mL for 0.1 M Ag against both bacteria species. No MIC was observed throughout the concentrations studied at 0.2 M which could be due to higher concentration of precursor. The results indicated that the synthesized nanoparticles are susceptible towards both bacteria species and it is more sensitive towards *E. coli*. The reason behind the obtained results is that the antimicrobial activity of Ag is related to the quantity of Ag and the rate at which Ag is released. Ag binds to the bacterial DNA and RNA by denaturing and consequently prevents the replication of bacteria [11]. The antibacterial activity of starch capped AgNPs has also been investigated by Mohan et al. [11], the MIC obtained were between 0.011 and 0.021 mg/mL for *E. coli*. Khan et al. [13] also investigated extract capped AgNPs and obtained MIC of 0.031–0.05 mg/mL for *E. coli* and 0.0078–0.023 mg/mL for *S. aureus*. These results are in agreement with those obtained in this work.

Conclusion

The starch capped AgNPs were successfully prepared and the effect of both precursor and capping agent investigated. The XRD study confirmed that the synthesized AgNPs have face centered cubic phase (fcc). TEM analysis showed that the nanoparticles were spherical in shape with a size of 0.5–4 nm under optimum conditions. The precursor and capping agent concentrations showed to have an influence on the size of the nanoparticles as the nanoparticle size decreased with an increase in both concentrations. The antibacterial activity showed that the prepared starch capped AgNPs has the antibacterial effect towards *S. aureus* and *E. coli* bacteria species. These nanoparticles can therefore be used as growth inhibitors of the investigated bacteria species. They can also be used in biological systems as they have been synthesized using a green synthesis method.

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