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Synthesis and Antibacterial Activities of Silver Nanoparticles

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Silver nanoparticles (AgNPs) were synthesized from silver nitrate and sodium borohydride and the structures were characterized using ultraviolet-visible, fourier transforms infrared and energy-dispersive X-ray (EDS) spectroscopy. Zeta potential measurements and scanning electron microscopy were also used to measure the dimensions of silver nanoparticles. The particle radii were in the range of 50 to 80 nm with typical surface plasmon absorption maxima at 428 nm. The synthesized AgNPs showed antibacterial activity against Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration (MIC) of AgNPs was achieved at 5 µg/mL for *Staphylococcus aureus* and *Yersinia* spp. and at 1 µg/mL for *Acinetobacter* spp., *Streptococcus pyogenes*, *Salmonella typhi* and *Vibrio cholera*. *Escherichia coli* isolate showed a visible inhibitory effect compared to the positive control, but the highest studied concentration of AgNPs (5 µg/mL) did not inhibit the bacterial growth completely. These results suggested that silver nanoparticles can be used as an effective antibacterial agent.

Keywords: Silver nanoparticles, Antibacterial activities.

INTRODUCTION

Silver nanoparticles (AgNPs) have potential applications in various medical fields and could, in theory, serve as antimicrobials [1-4], anti-inflammatories [5,6] and even in drug delivery systems [6,7]. In addition, they can be used as photosensitive components, optical receptors for bio-labeling and spectrally selective coatings for solar cells [8-11]. The diameter (1-100 nm) and shape (rods, cubes, and spheres) of AgNPs can be controlled by adjusting the reaction conditions such as temperature and reagents proportion [12]. Moreover, the types of reducing reagents and reaction time can have significant effects on the size of AgNPs [13].

Various synthesis methods (*e.g.* chemical, physical, photochemical and biological) are known for the production of AgNPs. The chemical approach is the most common process for the AgNPs formation. Such approach involves the use of various reducing agents (organic and inorganic), physicochemical reduction, electrochemical processes and radiolysis. The chemical synthesis of AgNPs usually involves a metal precursor (*e.g.* silver nitrate), a reducing reagent (*e.g.* sodium borohydride) and a stabilizing/capping reagent (*e.g.* sodium oleate) [14-18]. However, there are several problems (*e.g.* stability and aggregation of nanoparticles) associated with the methods used for the production of nanoparticles. Therefore, the current methods used for the production of nanoparticles require further development and optimization.

The emerged resistances of pathogenic bacteria for conventional antibiotic encourage researchers from industry and/or academia to devise effective new antibacterial agents. Silver nanoparticles are an effective inhibitor and showed various antibacterial activities [19-21]. Based on the values of minimum bactericidal concentration (MBC) and the minimal inhibitory concentration (MIC), AgNPs present a potential alternative for the treatment of bacterial infections [22,23]. In the current work, AgNPs were synthesized using a simple chemical method in which citrate ions have been used as a reducing and stabilizing reagent. The resultant nanoparticles were analyzed and characterized using various spectroscopic techniques including UV, FTIR, XRD, SEM and EDX. The antimicrobial activity of silver nanoparticles against *Streptococcus aureus* and *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli*,

Salmonella typhi, *Acinetobacter* spp., *Vibrio cholera* and *Yersinia* spp. (Gram-negative bacteria) has been investigated. The results showed that the synthesized silver nanoparticles are effective against the tested microorganisms.

EXPERIMENTAL

The synthesized silver nanoparticles were characterized by various standard spectroscopic techniques such as FTIR and UV-visible spectroscopy. The FTIR spectra (4000-400 cm^{-1}) were recorded on a Bruker tensor 27 FTIR spectrometer with RTDLATGS detector using KBr pellet method at room temperature. The UV spectra (190-1100 nm) were detected on Shimadzu UV-Visible spectrophotometer, Model 1800 in which distilled water was used as a blank. The SEM and EDX analysis were measured on Hitachi S-340N. A small quantity of AgNPs sample was allowed to dry on an aluminum plate under a mercury lamp for 5 min to produce a thin film.

Synthesis of silver nanoparticles: Silver nitrate (16 mg) was dissolved in distilled water (100 mL) to produce 1 mM solution. Similarly, sodium borohydride (37.8 mg) was dissolved in distilled water (500 mL) to produce 1 mM solution. Colloidal silver was made by the addition of an excess of sodium borohydride (reducing and stabilizer reagent) to silver nitrate as shown in eqn. 1:

$$AgNO_3 + NaBH_4 \longrightarrow Ag + 0.5\,H_2 + 0.5\,B_2H_6 + NaNO_3 \quad (1)$$

The chilled sodium borohydride (at about 0 ºC) and silver nitrate solution (1 mM, 10 mL) was added in a dropwise manner to a stirred solution of $NaBH_4$ (1 mM, 30 mL) using a dropper (one drop/sec).

Determination of silver nanoparticles concentration: The concentration of silver nanoparticles was determined using a reported procedure [24]. Eqn. 2 can be used to calculate the number of atoms per nanoparticles (N).

$$N = \frac{\pi \rho D^3}{6M} N_A \quad (2)$$

where $\pi = 3.14$, ρ is the density of silver in the face-centered cubic unit cell (10.5 g/cm^3), D is the average diameter of nanoparticles (70 nm), M is the atomic mass of silver (107.868 g/mol) and N_A is the number of atoms per mole (Avogadro's number; 6.023×10^{23}). Based on the assumption that the reduction of Ag^+ to Ag^0 was complete, ρ should be converted to g/nm^3 unit (*i.e.* 1.05×10^{-20} g/nm^3). Therefore, N should be 10519567.58, as shown in eqn 3:

$$N = \frac{3.14 \times 1.05 \times 10^{-20} \times (70)^3}{6 \times 107.86} 6.02 \times 10^{23} = 10519567.58 \quad (3)$$

The total number of silver atoms added as $AgNO_3$ (N_T) was calculated using eqn 4:

$$N_T = 20\ mL \times \left(\frac{1 \times 10^{-3} L}{1\ mL} \times \frac{0.001\ AgNO_3}{1\ L}\right)\left(\frac{1\ mol\ Ag}{1\ mol\ AgNO_3} \times \frac{6.023 \times 10^{23} Ag}{1\ mol\ Ag}\right)$$

$$= 1.20 \times 10^{19}\ atm \quad (4)$$

The molar concentration of nanoparticles (C) in solution was calculated using eqn 4 and V is the volume of reaction mixture in L.

$$C = \frac{N_T}{NVN_A} = \frac{1.20 \times 10^{19}}{10519567.58 \times 0.02 \times 6.023 \times 10^{23}} = 9.49 \times 10^{-19} M/L = 10.24\ \mu g/mL \quad (5)$$

Determination of silver nanoparticles minimum inhibitory concentration (MIC): The MIC dilution assay was carried out as previously reported [25]. Seven pathologic isolates were employed in the current assay and provided by Al-Sadar Medical City, Al-Najaf province, Iraq. To prepare the inoculation solution, a normal saline suspension of active cultures from the isolates was made to be the same as the intensity of 0.5 McFarland solutions. The suspension was diluted 1000 times by Molar-Hinton broth (HiMedia Laboratories, India) to give about 10^5 CFU/mL. Then inoculation solution (1 mL) was added to a test-tube which contained Molar-Hinton broth (4 mL) and AgNPs. The concentrations of AgNPs were 5, 1, 0.2, 0.04 and 0.008 µg/mL. Positive and negative control tubes were included for each isolate. The tubes were incubated at 37 ºC for 20 h. The lowest concentration that prevents the visible growth of microorganisms was considered as the MIC.

RESULTS AND DISCUSSION

Silver nanoparticles were synthesized from the reaction of silver nitrate and sodium borohydride. A light yellow colour slowly appeared as the silver nitrate was being added to sodium borohydride aqueous solution. The appearance of light yellow colour indicates the formation of AgNPs. However, the development of a greyish and turbid appearance could be due to the mixing rate, duration and stirring. Such factors can affect the stability and size distribution of the colloidal silver.

UV-visible absorbance analysis: The formation of AgNPs was recognized when the colour changes from colourless to yellowish as a result of excitation of surface plasmon resonance bands (UV-visible region). The maximum absorption wavelength (λ_{max}) is generally associated with the average particles size. While particle dispersion can be measured from the spectrum full width at $\lambda_{max}/2$ (FWHM). Silver nanoparticles showed absorbance at 428 nm (Fig. 1), which is typical for silver nanoparticles with FWHM of 140-150 nm.

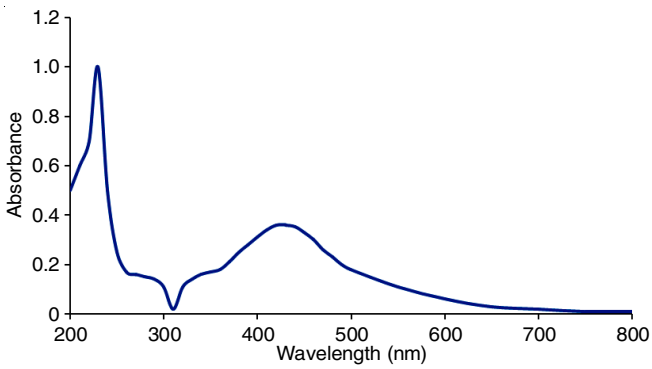

Fig. 1. UV-visible spectrum of AgNPs

The correlation between FWHM and particles diameter can be used to characterize AgNPs [26,27]. The plasmon resonance produces a peak near 430 nm with a peak width half maximum (PWHM) of 144 nm. Evidently, synthesized silver nanoparticles

are spherical and nanometer-sized [28]. However, the nanoparticle size affects the peak position. The optical absorption spectrum with Mie theory showed that the spectrum maximum position corresponds to particles between 50 and 80 nm sizes which are in agreement with SEM. However, such calculations can only be applied in case of the spherical particles. The absorbance spectrum shows one peak only in which shorter wavelengths peaks originated from the initial compounds were ignored. Again, it has been concluded that synthesized AgNPs are spherical. The change in absorbance led to a change of absorbing species in solution [29].

FTIR analysis: The interpretation of infrared spectrum involves the correlation of absorption bands in the spectrum with that for the desired sample. The FTIR for silver nitrate shows a strong symmetrical stretch band that resonates within 1400-1200 cm^{-1} region. The major peak resonates at 1390-1380 cm^{-1} region can be assigned to the symmetrical stretching vibration for O-N-O bond in nitrate radical [30].

Zeta potential measurement of silver nanoparticles: Zeta potential value gives an indication for the nanoparticles stability. The zeta potential value for the synthesized AgNPs was found to be 35.6 mV (Fig. 2). Evidently, such zeta potential value indicates that AgNPs is stable with a narrow size distribution. In addition, zeta potential value provides satisfactory evidence that AgNPs have little tendency towards aggregation [31].

Scanning electron microscope (SEM) and energy dispersive X-Ray (EDX) analysis: The morphology and size of AgNPs were studied by SEM (Fig. 3). The synthesized silver nanoparticles after solvent evaporation, displayed a high degree of nanoparticle aggregation with irregular and spherical shapes. The SEM images show that the aggregated particle diameters were in the range of 50 to 80 nm with an average diameter of 70 nm. Also, similar results for the size distribution measurements are obtained (Fig. 4).

Furthermore, elemental composition of the samples was also determined by EDX analysis. The EDX spectrum (Fig. 5)

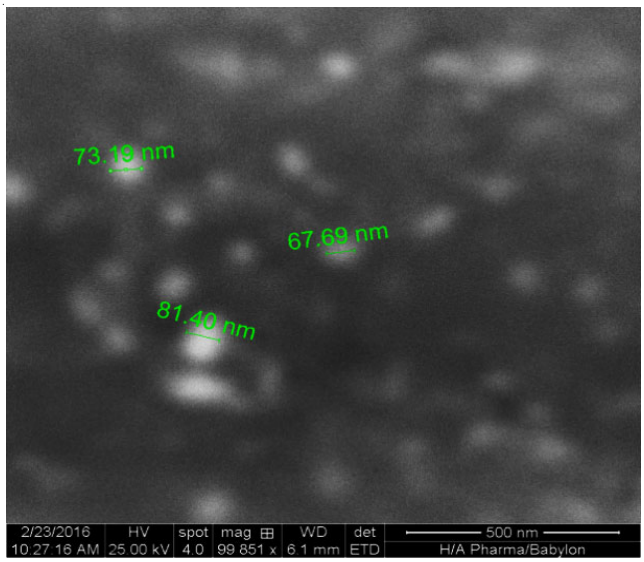

Fig. 3. SEM images of AgNPs

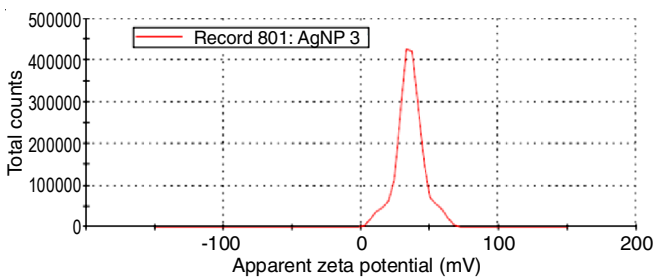

Fig. 2. Zeta potential of AgNPs

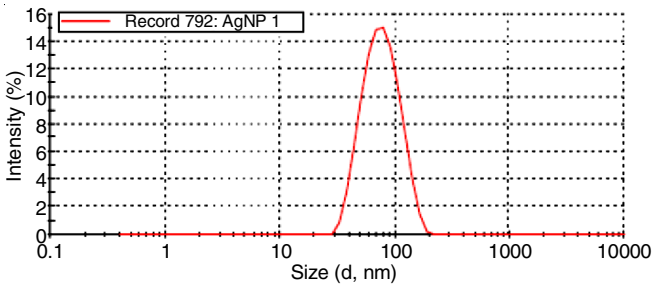

Fig. 4. Size distribution measurements

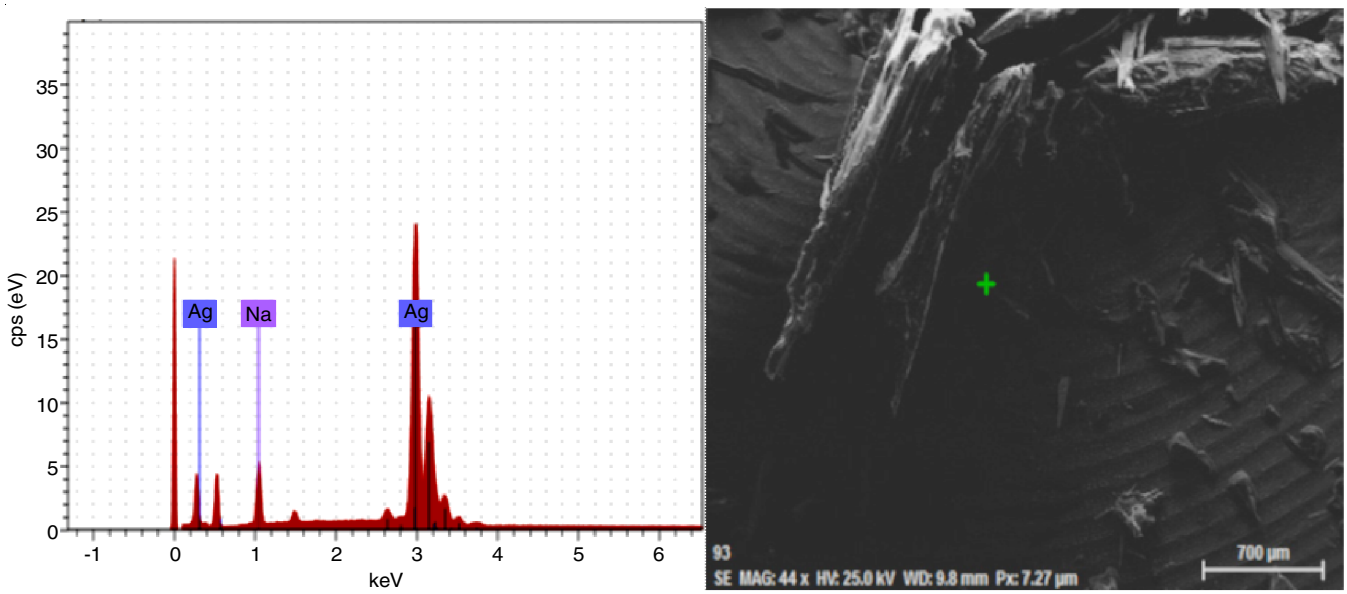

Fig. 5. Energy dispersive spectroscopy spectrums of AgNPs

shows a high intense signal at 3 kV which indicates that the sample is pure and contains silver as the major element. The EDX confirmed the existence of both silver and $NaHB_4$ that covers AgNPs. The EDX spectrum suggests the presence of both O and Na. Moreover, it proves that silver nanoparticles are in the metallic form with no Ag_2O or other impurities.

Minimum inhibitory concentration (MIC) of silver nanoparticles: To determine the antibacterial activity of AgNPs, the MIC was assayed against the pathogenic isolates of *S. aureus* and *Streptococcus pyogenes* (Gram-positive), *Escherichia coli*, *Salmonella typhi*, *Yersinia* spp., *Acinetobacter* spp. and *Vibrio cholera* (Gram-negative). The results (Table-1) showed that MIC for AgNPs against *Staphylococcus aureus* and *Yersinia* spp. were observed at 5 µg/mL, while *Vibrio cholera*, *Streptococcus pyogenes*, *Salmonella typhi* and *Acinetobacter* spp. were more sensitive (MIC = 1 µg/mL) towards AgNPs. On the other hand, *Escherichia coli* showed a visible inhibitory effect at 5 µg/mL compared to the positive control. However, the highest concentration used did not inhibit the growth of tested microorganism completely. Table-1 clearly shows that the AgNPs which synthesized by this method produced MIC values equal to or better than the majority of other studies.

TABLE-1
MIC VALUES (µg/mL) OF AgNPs FOR THE INVESTIGATED BACTERIAL ISOLATES OF THIS STUDY COMPARED TO OTHER STUDIED

Bacterial strain	Reference				This study
	[32]	[33]	[34]	[35]	
Acinetobacter spp.	–	–	–	83.3	1
Escherichia coli	3.12	3.3-6.6	12.5	–	> 5
Salmonella typhi	3.12	–	–	–	1
Staphylococcus aureus	–	33	12.5	83.3	5
Streptococcus pyogenes	–	–	–	29.2	1
Vibrio cholerae	3.12	–	–	–	1
Yersinia spp.	–	–	–	–	5

Silver ion antimicrobial activity is well known and has been employed to control the bacterial growth and infection in clinical applications [36,37]. Silver ions inhibited the microbial growth by interacting with specific targets such as thiol groups and hydrogen bonding [38,39]. However, the antimicrobial activity of silver nanoparticles has not been revealed clearly. Several studies suggested that AgNPs can cause a lethal morphological change in the bacterial membrane which can lead to an increase in membrane permeability and cause bacterial death as a consequence [40]. The membrane morphological change can be due to the direct anchoring of nanoparticles [40] or by free radicals releasing [41,42]. Other studies proposed that the antimicrobial activity of silver nanoparticles could be due to the direct antimicrobial effect of silver ions which could be released from the nanoparticles [43].

Conclusion

Silver nanoparticles were synthesized using the reduction method from silver nitrate and sodium borohydride. Various spectroscopic techniques have been used to characterize the synthesized AgNPs also exhibited an excellent antibacterial activity against *Staphylococcus aureus*, *Yersinia* spp., *Acinetobacter* spp., *Streptococcus pyogenes*, *Salmonella typhi*, *Vibrio cholera* and *Escherichia coli*. The minimum inhibitory concentration of AgNPs was achieved at 1-5 µg/mL.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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