



EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

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ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)



Silver Nanoparticles Synthesis in A Trial to Treat the Multi-Drug Resistant Bacteria: A Preliminary Prospective Study

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ARTICLE INFO

Article History

Received:23/8/2022

Accepted:16/10/2022

Available:22/10/2022

Keywords:

AgNPs, XRD,
TEM. Multi-
Drug.

ABSTRACT

The primary reason behind the failure/setback of significant antibiotics is the bacterial resistance to antibiotics and their entry into the cell. Incorporating metallic nanoparticles with antibacterial characteristics, nanotechnology provides a dynamic strategy for creating novel formulations. Therefore, the goal of this study was to design the silver nanoparticles (AgNPs) as the first step in our study rationale, and by then the second step will be used as an efficient alternative treatment to obliterate the multi-resistant bacteria. The synthesized materials were characterized using X-ray powder diffraction (XRD) and transmission electron microscope (TEM). The XRD studies revealed that the synthesized AgNPs have face-centered cubic. TEM showed AgNPs with spherical regular smooth shapes. AgNPs have a zeta average of 152.9 nm as confirmed also by TEM. This study could be postulated that the higher NPs concentrations significantly increase antibacterial efficacy. AgNPs, however, can be investigated as a significant opportunity for more study since they have a lot of potential as an antibacterial agent, according to the verdicts of the current work.

INTRODUCTION

It has always been extremely concerning how often pathogenic microbes cause mortality (Casadevall 2017). Even with our growing understanding of pathophysiology, the number of diseases and fatalities brought on by microbes continues to rise (Serwecińska 2020). One factor is that pathogenic bacteria are developing resistance as a result of the treatments' indiscriminate usage. The creation of new antibiotics is currently not enough to stop the spread of antibiotic resistance (Bungau *et al.*, 2021). For a variety of reasons, the ongoing rise in the formation of drug-resistant strains is incompatible with the availability of novel treatment variables (Zhu *et al.*, 2022).

Above all, certain antimicrobials are difficult to pass through cell membranes due to their size and might lose their effectiveness in cells. Antibiotics might be hazardous to healthy tissues, severely restricting their usage (Brandelli 2020). Due to these problems, new strategies and antimicrobials derived from both natural and synthetic materials are needed to create an army of new medications to combat microbial infestations (Nicoletti 2020).

The discovery of novel antimicrobial compounds as well as modifications to therapy methods and existing antimicrobials are therefore essential in the current situation (Mantravadi *et al.*, 2019).

The primary reason behind the failure/setback of significant antibiotics is the bacterial resistance to antibiotics and their entry into the cell. Incorporating metallic nanoparticles with antibacterial characteristics, nanotechnology provides a dynamic strategy for creating novel formulations (Niño-Martínez *et al.*, 2019). Nanotechnology represents a modern approach involving the development of new formulations based on metallic nanoparticles with antimicrobial properties (Khandel *et al.*, 2018). According to a few studies, combining antimicrobial drugs with nanoparticles might increase their effectiveness against many infections (Raza *et al.*, 2019; Alabdali *et al.* 2022), such as *Staphylococcus aureus* (Surwade *et al.*, 2019), *Pseudomonas aeruginosa*, *Escherichia coli*, and others. For instance, AgNPs have proven to be effective agents for multidrug-resistant bacteria (MDR) like ampicillin-resistant *Escherichia coli*, *Pseudomonas aeruginosa* (Ahmed *et al.* 2020), and *Streptococcus pyogenes* (Kadhum and Hussein 2020), erythromycin-resistant and methicillin-resistant *Staphylococcus* (MRSA) (Yang *et al.*, 2018).

The chief goal of combination treatment is to provide a synergistic impact, meaning that the result is greater than the combined effects of the individual antibiotics utilized (Naqvi *et al.*, 2013). Combination treatment can aid in the recovery of some antibiotics that were formerly successful, such as some penicillins, as well as combat the rising antibiotic resistance, such as that vancomycin (Allahverdiyev *et al.*, 2011). In addition, combination therapies of nanoparticles and antibiotics or other antimicrobial agents lessen the potential for human toxicity of both substances (Gold *et al.*, 2018). Given that nanoparticles are non-toxic and could be a synergistic impact with key antibiotics; they can be employed in

combination treatment to treat microbial diseases.

Therefore, the goal of the current research was to study the synthesis of AgNPs to be used along with antibiotics in the treatment of MDR-bacteria (the second objective of this study).

MATERIALS AND METHODS

Synthesis of Nanoparticles:

AgNPs Biosynthesis:

AgNPs were prepared by reducing silver nitrate with sodium borohydride in aqueous sodium citrate. Silver nitrate (AgNO_3 : 2×5 -10 mol) dissolved in water (79.5 cm^3) at ordinary temperature. A solution containing the equivalent of 1 mole of sodium citrate (2×10^{-5} mol) in 0.5 cm^3 water was added to the silver nitrate solution. Then add 0.5 mL of NaBH_4 solution (NaBH_4 : 5×10^{-5} mol) gradually (60 mL/h) to the Ag solution. After one hour of stirring, the solution was centrifuged for 60 min at $8500 \times g$ at 5°C . The floating top has been removed. The sample was purified 3 times by adding water (10 cm^3), centrifuging at $8500 \times g$ for 60 min at 5°C , and removing the supernatant. Lastly, a solution was diluted to reach the required concentration (Kokila *et al.*, 2016).

Characterization of Ag and TiO_2 NPs:

X-ray Powder Diffraction (XRD):

XRD is one of the most used techniques to characterize NPs. XRD typically gives information on crystal structure, phase types, lattice constants, and grain size. The XRD method was used to further examine the biologically produced NPs (powder) to define their phase composition. The $\text{Cu } \alpha$ radiation ($k = 1.5418 \text{ \AA}$) was selected. The diffractogram was produced at a 2θ between 20 and 80 degrees. Using the Debye-Scherrer equation, the size of NPs was determined at 24.

$$D = (0.94\lambda) / (\beta \cos \theta)$$

Where **D**: the average crystal size; λ : X-ray wavelength ($\lambda = 1.5406 \text{ \AA}$); θ : Bragg's angle (2θ); β : full-width at half-maximum (FWHM) in radians.

Transmission Electron Microscopy (TEM):

A drop of the NPs dissolved in methanol was applied to carbon-coated grids and allowed to air dry to validate the formation and particle sizes of the synthesized materials through TEM. An accelerating voltage of 160 kV was used to capture TEM pictures utilizing the Hitachi (H-7500) TEM equipment at Mansoura University, Mansoura, Egypt.

Zeta Potential Analysis:

The dynamic light scattering (DLS) or photon correlation spectroscopy technique is widely used to determine the size distribution profile of NPs that are present in suspension or solution. The particle size distribution and zeta potential of NPs

produced physiologically were assessed by Microtrac Nanotrak wave-particle size and zeta potential analyzer.

RESULTS

Characterization of Ag:

Particle size, shape, and possible crystal structure were characterized by a transmission electron microscope (TEM) in Figure (1). Size distribution by dynamic DLS technique and surface charge characterization by zeta-potential measurements of the freshly prepared AgNPs was used and analyzed at a measurement angle of 173 degrees collecting backscatter optics. AgNPs have zeta average d.nm 152.9 (Fig. 2); and zeta potential (-20.3mV) (Fig. 3).

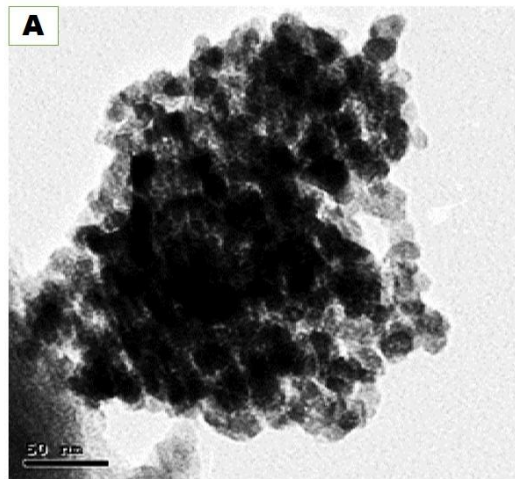


Fig.1: TEM images for Ag NPs with size 45 nm with smooth spherical shape.

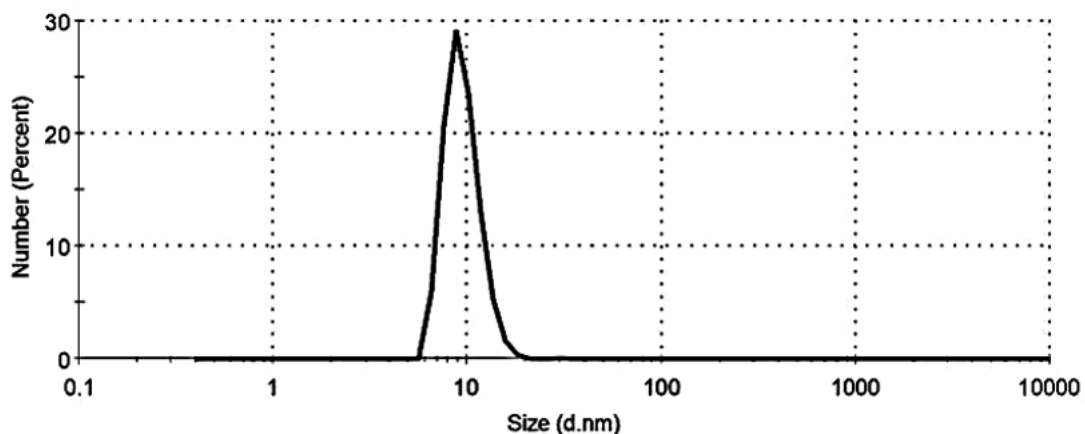


Fig.2: DLS of Ag Nanoparticles

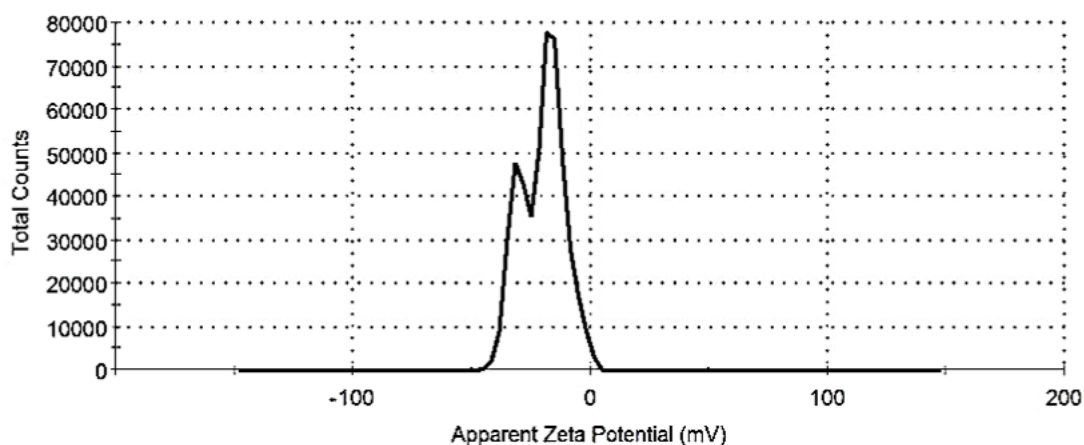


Fig.3: Zeta potential of AgNPs

Powder X-ray Diffraction of NPs:

XRD patterns of AgNPs designated that the assembly of AgNPs was face-centered cubic. In addition, all the AgNPs had alike deflection profile, and XRD peaks at 2θ of 38.50° , 44.90° , 65° , and 78° . The XRD

pattern thus clearly illustrated that the AgNPs shaped were crystalline. The chief crystalline phase was silver, and there were no obvious other phases as impurities were found in the XRD patterns in Figure (4).

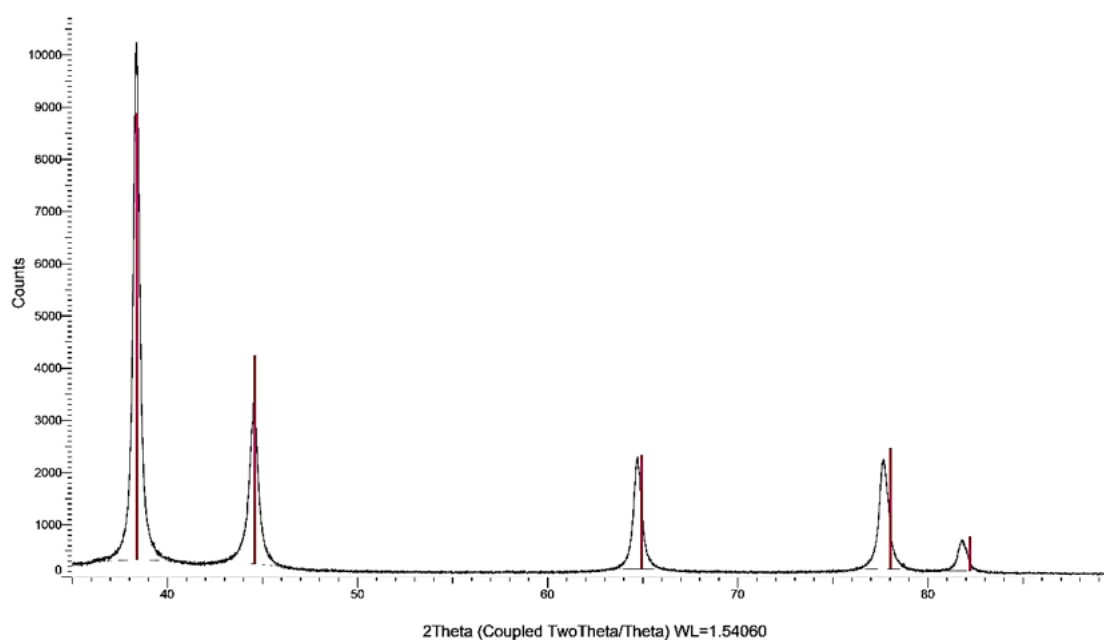


Fig. 4: XRD patterns of silver Nanoparticles.

DISCUSSION

Today, there is a lot of apprehension about the spread of antibiotic resistance and the ever-growing incidence of infectious illnesses. In numerous instances, this circumstance has caused treatments to fail. According to O'Neill (O'Neill 2014), the current trajectory might result in 10 million deaths annually from antibiotic failure by the

year 2050. Furthermore, antibiotic-resistant bacteria have reached a critical stage worldwide, mainly in emerging countries (Talebi Bezmin Abadi *et al.*, 2019) (Chokshi *et al.*, 2019). One such country is Egypt which has less severe restrictions on prescribing antibiotics (Zakaa El-din *et al.*, 2019), and the emergence of multi-resistant bacteria blowout very quickly (El-Geleel *et al.*, 2021).

Therefore, the search for novel antimicrobials to supplement or modify the current ones has become necessary. Nanoparticles are the most innovative and dazzling therapies to solve the current dilemma.

By using TEM, several investigations have evaluated the size and form of NPs (Lu *et al.*, 2013; Srinivasan *et al.*, 2019). In the current investigation, TEM analysis was used to assess the size and form of pure NPs. On the contrary, Rani *et al.*, have amalgamated 42 nm sized AgNPs using *O. sanctum* leaf extract (Rani *et al.*, 2021). Via the same plant extract, Sadanand *et al* have perceived oval and/or elliptical-shaped AgNPs with a size of 15-45 nm (Sadanand *et al.*, 2016).

In the present study, biologically synthesized solutions were characterized in the terms of particle size and zeta potential by DLS analysis. Our findings publicized that AgNPs have a zeta average of d.nm 152.9; and a zeta potential of -20.3 mV. Moreover, XRD analysis showed the crystalline nature of silver and TiO₂ nanoparticles. Inconsistent with our findings, Bahadur *et al* (Bahadur *et al.*, 2016) and Senthil Kumar and Gnanavel (Senthil Kumar and Gnanavel 2017), have also obtained similar results and the diffraction peaks showed face cubic centered for silver and titanium. Into the bargain, all the AgNPs had analogous diffraction profile, and XRD peaks at 2θ of 38.50°, 44.90°, 65°, and 78°. The prior information led to the belief that the AgNPs maintained their crystalline structure, supporting the DLS and XDR. Due to the examined medications' and manufactured NPs' physicochemical compatibility, pharmaceutical dosage forms including each drug and nanoparticles may be combined and created, providing the benefits of NPs' addition to antibiotics while addressing the major MDR problem.

Conclusion

AgNPs have a size of 45 nm when characterized through TEM. As clear, the findings show that a greater NPs concentration greatly boosts antibacterial effectiveness, while further studies are needed. AgNPs, however, can be investigated as a significant opportunity for more study

since they have a lot of prospects as an antibacterial agent, according to the findings of the current study.

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