Journal of Applied Sciences and Nanotechnology, Vol. 2, No. 3 (2022)

Journal of Applied Sciences and Nanotechnology

Journal homepage: https://jasn.uotechnology.edu.iq/

Antibacterial Studying of Silver Nanoparticles Synthesized by Chemical Reduction Method Using Different Stabilized Concentrations

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Article information

Article history:
Received: December, 09, 2021
Accepted: June, 11, 2022
Available online: September, 10, 2022

Keywords:
Silver nanoparticles,
Chemical reduction,
Stabilizers,
Antibacterial

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Abstract

Silver nanoparticles were prepared by the chemical reduction method. Silver nitrate was taken as a metal precursor and sodium borohydride as a reducing agent with polyvinyl alcohol (PVA) stabilizers of different concentrations, polyvinylpyrrolidone (PVP). X-ray diffraction (XRD), transmission electron microscopy (TEM), and atomic force microscopy (AFM) techniques have been used, these measurement results showed that the prepared material is silver nanoparticles. The average size of silver nanoparticles using the Scherrer equation with values ranging from 8.49-12.15nm. TEM images showed that the silver nanoparticles are spherical in size between 5-47nm. Nanoscale distribution of silver nanoparticles (AgNPs) prepared at different concentrations was studied by AFM. Silver nanoparticles showed high antimicrobial and antibacterial activity against Gram-positive bacteria such as Escherichia Coli and Gram-negative Staphylococcus aureus, whose bacterial activity was dependent on the concentration of PVA and PVP and the degree of intramolecular accumulation. Low concentrations of PVP lead to increase the activity, while high concentrations of PVA give better activity.

DOI: 10.53293/jasn.2022.4514.1118, Department of Applied Sciences, University of Technology

1. Introduction

Silver nanoparticles consist of sizes between (1-100) nanometres and possess physical and chemical properties such as optical, electrical, and thermal properties. Silver nanoparticles have been used in several technologies which give an advantage of their optical, conductivity, and antibacterial properties [1-3]**.** As silver has a property that makes it toxic to some types of bacteria, viruses, and fungi, as is the case with some heavy metals such as lead or mercury, but without high toxicity to humans [4, 5]. These nanoparticles may be used as antimicrobial agents in various products in clothes, shoes, paints, wound dressings, devices, and cosmetics. Plastic for its antibacterial properties, when AgNPs interact with micro-organisms (bacteria, fungi, and viruses), silver ions are released (Ag^{+}), and these ions may affect and damage the micro-organism in various ways. They attack the negatively charged cell walls of microbes to inactivate cellular enzymes and disrupt membrane permeability, As

a result, cell lysis and cell death occur [6, 7, 8]. Figure 1 shows the mechanism of penetration of the silver nanoparticles into the cell wall and how the process of disrupting the cell functions.

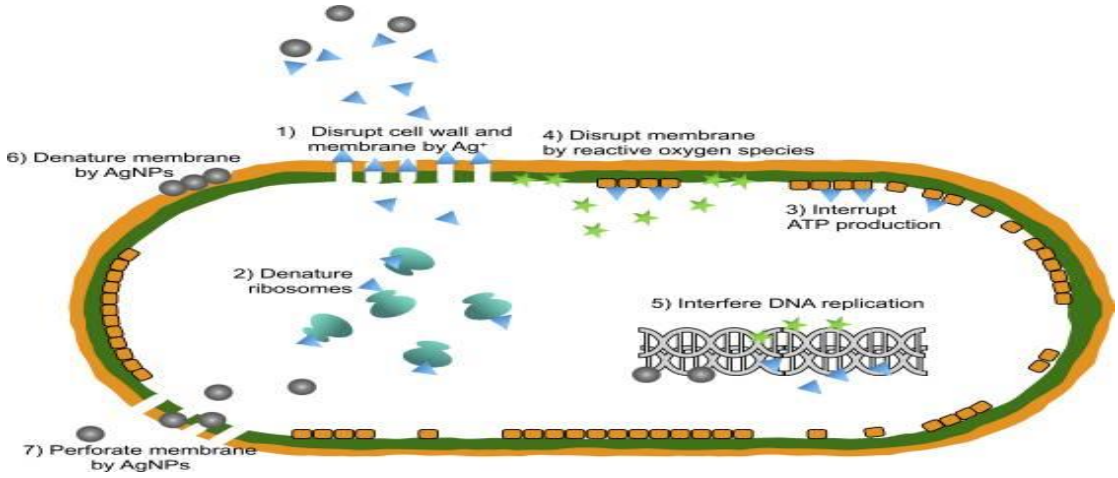


Figure 1: Shows the cell penetration process of silver nanoparticles [9].

The antibacterial actions of silver nanoparticles (AgNPs). 1) Disruption of the cell wall and cytoplasmic membrane: silver ions (Ag^+) released by silver nanoparticles adhere to or pass through the cell wall and cytoplasmic membrane. 2) Denaturation of ribosomes: silver ions denature ribosomes and inhibit protein synthesis. 3) Interruption of adenosine triphosphate (ATP) production: ATP production is terminated because silver ions deactivate respiratory enzymes on the cytoplasmic membrane. 4) Membrane disruption by reactive oxygen species: reactive oxygen species produced by the broken electron transport chain can cause membrane disruption. 5) Interference of deoxyribonucleic acid (DNA) replication: silver and reactive oxygen species bind to deoxyribonucleic acid and prevent its replication and cell multiplication. 6) Denaturation of membrane: silver nanoparticles accumulate in the pits of the cell wall and cause membrane denaturation. 7) Perforation of membrane: silver nanoparticles directly move across the cytoplasmic membrane, which can release organelles from the cell. In the case of biomedical applications of AgNPs, uniform dispersion is critical as it enables a gradual and steady release of the antibacterial agent in the form of nanoparticles or silver ions. Which depends on the type of polymer matrix in the medium. Excellent antibacterial activity of silver content can be achieved when poly (vinyl alcohol)-PVA and polyvinylpyrrolidone PVP are used as a hydrophilic mixture. PVA, PVP is widely used to manufacture biomaterials such as biocompatible, biodegradable, and water-soluble polymers, while meanwhile, inexpensive polymers. Bioactive nanocomposites with PVA also have a lot of interesting applications, among others. Others, in the medicine, cosmetics, pharmaceuticals, and packaging industries [10, 11]. Recently, Abdullah and co-workers demonstrated that nanocomposites containing AgNPs and PVA as matrix exhibit high antibacterial activity against a series of multidrug-resistant bacteria strains [12]. The PVP concentration and reaction temperature influence the particle size, morphology, and degree of polymerization of silver nanoparticles [13]. The antimicrobial activity of the size of the silver nanoparticles depends on the size and shape, one of the reasons is that the different shapes provide different areas for interaction with the microbes, thus resulting in different antibacterial efficacy [14]. Silver compounds were used to treat during World War II to effectively treat infections caused by burns and wounds, then replace the silver nitrate solution. Silver ointment «sulfadiazine», which remained until the nineties of the last century and was the first and basic medicine approved as an antibiotic and antibacterial in the treatment of burns until other compounds were added to «sulfadiazine» enhanced its effectiveness [16]. Silver nanoparticles are prepared in several ways, including physical, chemical, and biological methods. The aim of this work was to study the effect of concentrations of different fixing factors on the antibacterial activity of silver nanoparticles against bacteria, that were prepared by the chemical reduction method of (AgNps). Through the reducing agent sodium borohydride ($NaBH_4$) [17] with the fixing agents used to prevent agglomeration of silver particles. Nanoparticles of polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP).

2. Materials and Methods

2.1. Materials

Silver Nitrate ($AgNO_3$ MF: $AgNO_3$ MW: 169.88 Assay: ≥ 99.8% Glentham Life Sciences is the UK). Sodium Borohydride $NaBH_4$ MW: 37.83g / mol, Assay95% (BDH CHEMICAL LTD POOLE England). Polyvinyl Alcohol (PVA) ((MESE) product of Germany Molecular Formula: $(C_4H_6O_2.C_2H_4O)_x$ Average Molecular Weight: 67,000 Degree of Polymerization: (1,400). Polyvinyl pyrrolidone (PVP) molecular formula $(C_6H_9NO)_n$ central Drug House (CDH New Delhi India). Distilled Water. Laboratory bacteria were obtained (Escherichia coli isolated from diuretic and Staphylococcus aureus isolated from burns at Imam Ali Hospital)

2.2. Methods

Commercially purchased silver nitrate (molecular weight 169.88) was used to prepare a concentration of (0.002) M of $AgNO_3$ Silver nitrate solution where (0.039) g of Silver nitrate is weighed and placed in a beaker containing distilled water after that, the solution is placed on a magnetic stirrer to dissolve the silver nitrate. Sodium borohydride ($NaBH_4$ MW:37.83g /mol) was dissolved in a concentration of (0.1g) dissolved in 10ml of water. Polyvinyl alcohol (PVA) $(C_4H_6O_2.C_2H_4O)_X$ was prepared by dissolving different weights (1, 3, and 5)g in 100ml of distilled water placed on a magnetic stirrer that was dissolved entirely at a temperature of 70 °C At 60 rpm/min. for 15 min.

2.2.1. Silver Nanoparticles

Take 50ml of silver nitrate solution for each concentration and place it on the magnetic stirrer. When it reaches a temperature (98-100) ºC, 20 ml of $NaBH_4$ is added to it from each concentration as in Table 1 until the color changes to pale yellow indicating the formation of Silver Nanoparticles. It is removed from the device and cools down to room temperature. A 10 ml is taken and placed it on the magnetic stirrer. It is heated to a temperature of 74 ºC. Then 5ml of PVA or PVP is added to it in the form of drops for 30 seconds for each concentration, it showed different colors. The process takes 22-25 min.

Table 1: Silver nitrate and sodium borohydride concentrations with stabilizing agents (PVP, PVA).

Sequencing	$AgNO_3$(M)	$NaBH_4$(%)	stabilizing agents
1	0.002	1	1%PVA
2	0.002	1	3%PVA
3	0.002	1	5%PVA
4	0.002	1	1%PVP
5	0.002	1	3%PVP
6	0.002	1	5%PVP

3. Characterization of Synthesized Silver Nanoparticles

3.1. XRD Analysis

Silver nanoparticles were synthesized by (XRD-6000) SHIMADZU-Japan by centrifugation at 10,000 rpm for 15 min, and the pellets were re-dispersed as double sterile and centrifuged at 10,000 rpm for 10 min. Purified pellets were dried at 50 °C in an oven and analyzed by an X-ray diffraction (XRD) unit (Pan Analytical, X-Pert pro, The Netherlands). X-ray diffraction (XRD) measurement of the synthesized silver nanoparticles was performed by chemical reduction using sodium borohydride as a reducing agent. Using a Cu-Kα radiation source in the scattering range m (2θ) of 20-80 on the device operating at a voltage of 40 kV and a current of 30 mA. The presence, crystalline nature, phase diversity, and grain size of the synthesized silver nanoparticles were determined by X-ray diffraction spectroscopy The particle size of the prepared samples was determined using the Scherrer equation as follows [9, 18]:

$$\mathbf{D = (0.9\,\lambda)/(\beta cos\theta\,)} \quad (1)$$

Where D is average crystallite size and β is line broadening in radians (full width at half maximum of the peak in radians). λ is the wavelength of X-ray (1.546A°), and θ is Braggs angle (diffraction angle). K is constant (geometric factor = 0.94).

3.2. TEM Tests

A transmission electron microscope (Carl Zeiss - Germany - EM10C-100Kv) was used, where the samples were prepared in liquid form to conduct a TEM examination to know the size and shape of silver nanoparticles more accurately than (SEM) and it also gives more accurate information regarding size and shape.

3.3. Atomic Force Microscopy Tests

It was used by atomic force microscopy (TT-2 AFM - WORKSHOP USA) to determine the grain size distribution, shape, absorption and structure of silver nanoparticles (AgNPs) samples prepared for AFM assay.

3.4. Antibacterial Test

The antibacterial activity of silver nanoparticles prepared by chemical reduction was analyzed against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). Bacteria were spread into dishes containing Mueller-Hinton agar medium and incubated at 37 °C for 24 h. to grow bacteria. Then, a 6 mm in diameter drill was carried out in the center with the help of a gel hole. 10µl of silver nanoparticles were taken, and the plates were incubated in an incubator at 37°C for 24 hours. Antibacterial activity is measured based on the area of inhibition around the pit.

4. Results and Discussion

4.1. Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles was observed when sodium borohydride solution was added to $AgNO_3$ solution at a temperature (98 ℃) to change color to light yellow or dark brown depending on the concentration of sodium borohydride and molarity of silver nitrate. Figure 2 shows the prepared silver particle solution. This color change is evidence of the formation of silver particles as a result of the surface plasmon resonance phenomenon. The plasmon surface resonance property occurs in some metals such as silver due to the particle diameter reaching the nanometer scale [19] and due to the excitation of free electrons in nanoparticles [20]. Then add the stabilizing agents, polyvinyl alcohol or polyvinylpyrrolidone, without noticing the color change from light yellow to greenish-yellow, green, brown or red. Figure 3, showed the shape of the solution of silver particles after adding stabilizing agents according to concentration. Because of the increase in hydrogen bonds within the mixture, which in turn leads to an increase in the bonding between polymer molecules and their linking to oxygen sites and the formation of strong bonds within the mixture.

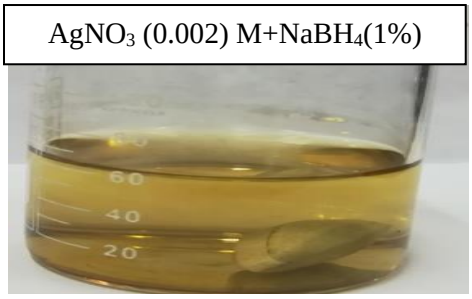
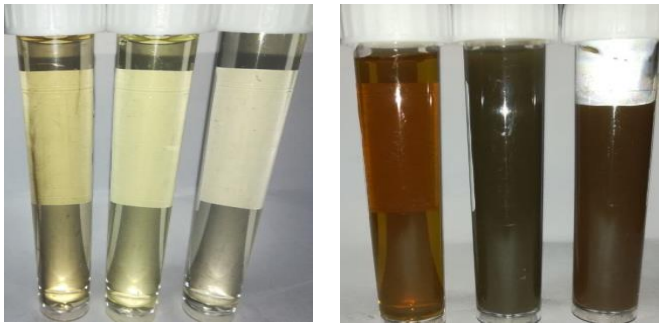


Figure 2: Silver nanoparticles before adding stabilizing agent.



1,2,3 With PVA 4,5,6 With PVP

Figure 3: Silver particles after adding the fixing agents.

4.2. XRD Analysis

X-ray diffraction (XRD) results showed that the prepared material is silver nanoparticles at the diffraction peaks (32.1°, 38.4°), respectively. The XRD spectrum also confirmed that the average size of silver nanoparticles was obtained using the Scherrer equation with values ranging between (12.15, 8.49,) and that their crystalline nature can be customized from Miller's coefficients (122), (111) [21, 22] as in Figure 4 the diagram of the Bragg peaks for silver nanoparticles is shown below:

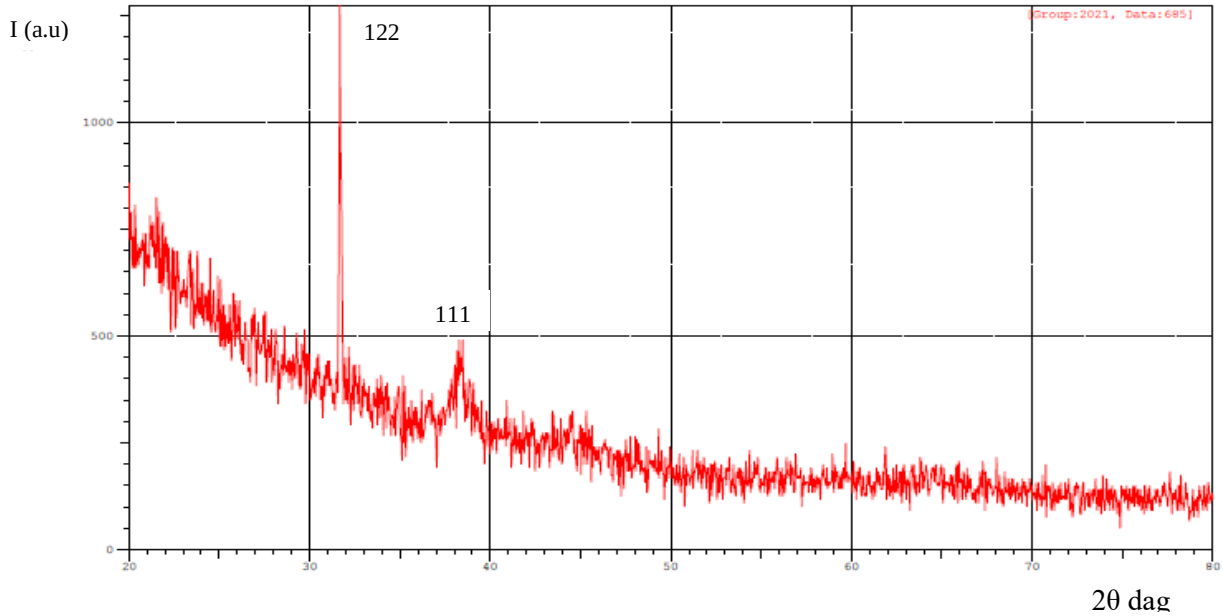
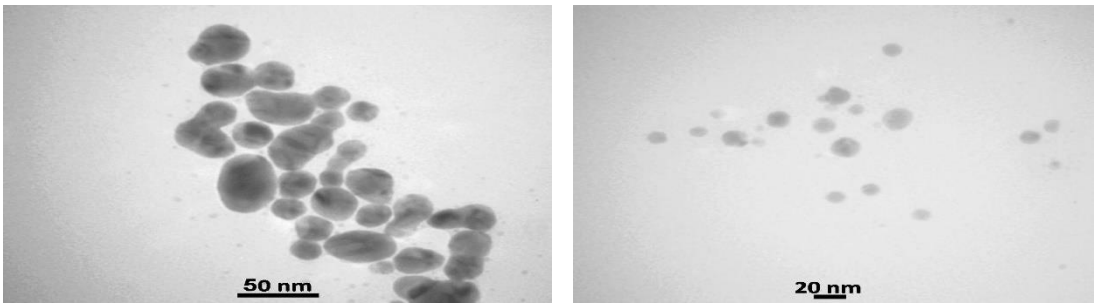


Figure 4: XRD diagram of silver nanoparticles.

4.3. Surface Morphology Analysis

The surface morphology, size and shape of silver nanoparticles were analyzed by transmission electron microscopy. Figure 5 shows TEM images of chemically reduced silver nanoparticles. TEM images showed that silver nanoparticles are spherical in shape and with a very small size ranging from (5-15) nm when adding PVA in a high concentration as in Picture A, while adding PVP with medium or low concentration gives a spherical shape and relatively large sizes ranging from (20-47) nm in comparison with the addition of PVA as in the picture B.



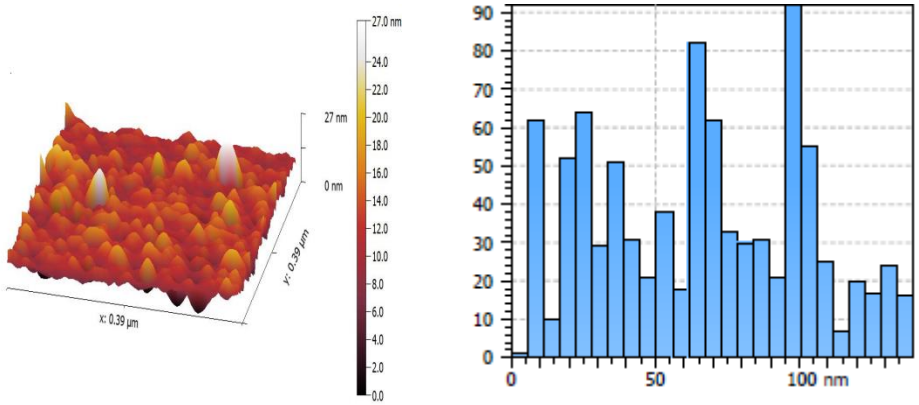
A) $AgNO_3$ 0.002 M +$NaBH_4$1%+PVA5% B) $AgNO_3$0.002 M+$NaBH_4$1%+PVP3%

Figure 5: TEM images of chemically reduced silver nanoparticles.

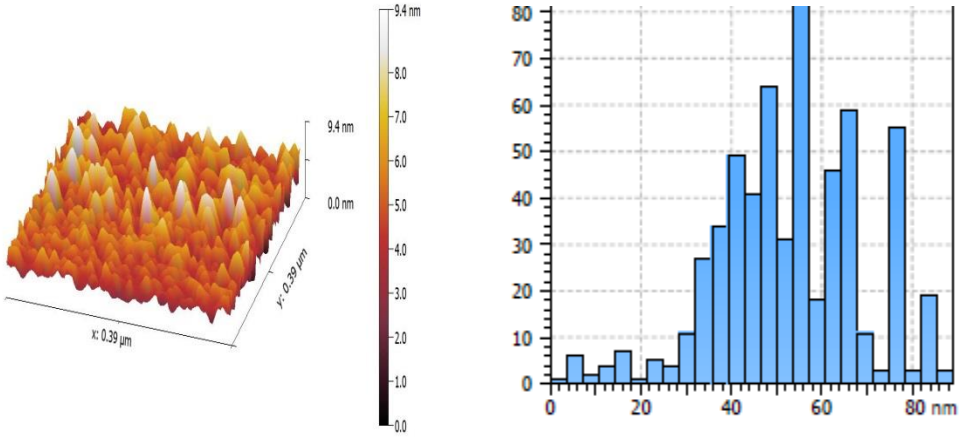
4.4. Surface Topography Structure Analysis

To determine the surface roughness of prepared silver nanoparticles (AgNPs) we use AFM assay. Figure 6 shows 3D AFM and histogram images of AgNPs prepared by chemical reduction with different conditions, which is affected by the concentration of silver nitrate, reducing agent, and used stabilizer agent. When adding a high concentration of 5% PVA a presence of different sizes of silver nanoparticles ranging between (20-95) nm as shown in Figure 6-A. However, adding 3% PVP with medium concentration gives sizes ranging from (30-57)

nm as in Figure 6-B. These results prove that the fixing factor affects the size of silver nanoparticles.



A) $AgNO_3$ 0.002 M+$NaBH_4$ 1%+PVA5%



B) $AgNO_3$ 0.002 M+$NaBH_4$ 1%+PVP3%

Figure 6: 3D AFM & histogram images of AgNps at different conditions A) $AgNO_3$ 0.002 M+$NaBH_4$ 1%+PVA 5%, left image 3D AFM Right histogram image B) $AgNO_3$ 0.002 M+$NaBH_4$ 1%+PVP 3%, left image 3D AFM Right histogram image.

4.5. Antibacterial Activity

Silver nanoparticles have wide applications in many fields, including medicine, health and technology. Silver nanoparticles have been used as an antibacterial agent due to their high antimicrobial activity, small size, spherical shape or highly effective membrane shape, and membrane due to surface to large volume ratio inhibition of bacterial growth in aqueous and solid media [23]. This study examined synthetic silver nanoparticles using the reducing agent and stabilizing agents $NaBH_4$ (PVA, PVP) as antibacterial. Silver nanoparticles were tested for antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Figure 7 shows the results of Antibacterial activities of silver nanoparticles evaluated according to the inhibition diameter. Table 2 shows the comparison between the inhibition zone diameters for (*Escherichia coli*) - (*Staphylococcus aureus*) and Table 3 shows the effect of each concentration and its activity against bacteria. The maximum inhibition zone 17 mm against the presence of PVP (*Escherichia coli - Staphylococcus aureus)* respectively, and the lowest inhibition area in (*Escherichia coli*) was 14 mm with a low PVA concentration. Gram-negative bacteria are more susceptible to silver nanoparticles. The cell wall of Gram-negative bacteria is narrower than that of Gram-positive strains. A thicker cell wall may reduce the penetration of nanoparticles into cells [24]. Alterations in the bacterial membrane could lead to the first bacterial contact with AgNP, triggering an antibacterial mechanism by facilitating the entry of AgNPs into bacterial cells. This is followed by an explosive release of silver ions into the bacterial cells, causing a bactericidal effect.

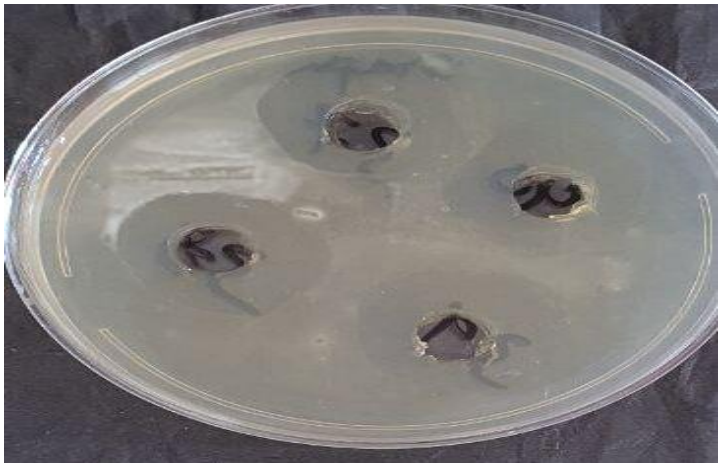


Figure 7: Activity of silver nanoparticles against bacteria.

Table 2: Comparison of inhibition diameters of silver nanoparticles on bacteria (*S.aureus*, *E.coli*).

Sample	*S.aureus* (mm)	*E.coli* (mm)
1	15	14
2	15.5	14
3	16	15
4	17	15
5	16	17
6	NO	NO

Table 3: Show the effect of each concentration and its activity against bacteria**.**

Sequencing	$AgNO_3$(M)	NaBH4(%)	stabilizers	Activity *S-aureus*	Activity *E-coli*
1	0.002	1	1% PVA	high	high
2	0.002	1	3% PVA	high	high
3	0.002	1	5% PVA	very high	high
4	0.002	1	1% PVP	very high	high
5	0.002	1	3% PVP	very high	very high
6	0.002	1	5% PVP	Low	Low

5. Conclusions

Silver nanoparticles AgNPs were synthesized using sodium borohydride ($NaBH_4$) as a reducing agent, polyvinyl alcohol (PVA), and polyvinylpropylene (PVP) stabilizing agents at different concentrations, and the inhibition capacity of each concentration was studied on bacteria and fungi. It was also found that silver nanoparticles were obtained from AgNPs. Which range in size from (5-15) nm with a spherical shape in the presence of PVA and a size ranging from (20-47) nm in the presence of PVP through TEM examination. By examining the structure of the surface by AFM, it is proven for a sample with the presence of PVA in a high concentration gives small and large sizes ranging from (20-95) nm, while it has the addition of PVP ranging in size from (30-57) nm, although PVP gives medium-sized samples that give the best result against bacteria despite the presence of samples PVA, which contains different sizes that fit the cell pores, now PVP gave the highest activity against laboratory bacteria. The antibacterial activity was measured by the diameter of the inhibition and as a result, the study proved that the silver particles are able to kill laboratory bacteria including highly resistant bacteria such as *Staphylococcus aureus* and *Escherichia coli*). A high birth rate of (0.002) M gave a high inhibition capacity against *Staphylococcus aureus* and *Escherichia coli*.

Acknowledgement

We would like to express our gratitude to the laboratories of the Department of Applied Sciences at the University of Technology, especially the Biology Laboratory for Postgraduate Studies, for their support

Conflict of Interest

The authors declare no conflict of interest.

References

[1] K. S. Shaker, S.S. Shaker "Study the Effect of Addition Silver Nanoparticle on Structure and Morphological Properties for PVDF Hollow fiber" *Engineering and Technology Journal*, vol. 35, no. 1, pp. 33-36, 2017.

[2] A. Q. D. Faisal, Z. N. Jameel, and A. A. Moosa."Preparation of Silver and Gold Nanoparticles By Using Nd - YAG Pulse Laser Ablation" *Engineering and Technology Journal*, vol.32, no.1, pp.138-144, 2014.

[3] R. A. Hikmet, N. N. Hussein." The Biological Activity of Mycosynthesized Silver Nanoparticles Against some Pathogenic Bacteria"*Journal of Applied Sciences and Nanotechnology*, vol. 2, no. 1, 2022.

[4] A. Panáček, M. Kolář, R. Večeřová, R. Prucek, J. Soukupova, V. Kryštof, et al. "Antifungal activity of silver nanoparticles against Candida spp" *Biomaterials*, vol. 30, no. 31,pp. 6333-6340 , 2009.

[5] S. Galdiero, A. Falanga, M. Vitiello, M. Cantisani, V. Marra, and M. Galdiero. "Silver Nanoparticles as Potential Antiviral agents" *Molecules*, vol.16, no.10, pp. 8894-8918, 2011.

[6] Q. L. Feng, J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim, and J. O. Kim. "A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus"*J Biomed Mater Res*, vol.52, no.4, pp. 662-8, 2000.

[7] O. Choi, K. K. Deng, N. J. Kim, L. Ross Jr, R. Y. Surampalli, and Z. Hu."The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth", *Water Res*, vol. 42, no.12, pp. 3066-3074, 2008.

[8] S. S. Hamdi. "Synthesis of Ag –TiO2 Thin Films by Spin Coating process" *Engineering and Technology Journal*, vol.34, no.13, pp.2443-2449, 2016.

[9] B. V. Sandeep, Sudhakar Pola."Synthesis, Characterization, and Evaluation of the Antibacterial Activity of Allophylus serratus Leaf and Leaf Derived Callus Extracts Mediated Silver Nanoparticles" *Journal of Nanomaterials*.Volume2017|Article ID 4213275 | , 2017.

[10] J. H. Nahida. "Study of starch, sugar blending effect on the biodegradability of (PVA) for packaging applications" *Iraqi Journal of Physics*, vol.15, no.32, pp. 43-56, 2019.

[11] A. Krzywicka, E. Megiel."Silver-Polystyrene (Ag/PS) Nanocomposites Doped with Polyvinyl Alcohol (PVA)—Fabrication and Bactericidal Activity" *Nanomaterials*, vol. 10, no. 11, pp. 2245, 2020.

[12] O. M. Abdallah, K. Z. EL-Baghdady, M. M. Khalil, M. I. El Borhamy, and G. A. Meligi, "Antibacterial, antibiofilm and cytotoxic activities of biogenic polyvinyl alcohol-silver and chitosan-silver nanocomposites"*Journal of Polymer Research*, vol. 27, no. 1, pp. 1-9, 2020.

[13] Q. Xiliang, C. Yang, L.Tiesong, He Peng, Wang Jun, Liu Ping, and Gu Xiaolong"Large-Scale Synthesis of Silver Nanoparticles by Aqueous Reduction for Low-Temperature Sintering Bonding" *Journal of Nanomaterials*, vol. 2014, no. 8, pp 8, 2014.

[14] S. Pal, Y. K. Tak, and J. M. Song. "Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium Escherichia coli" *Appl Environ. Microbiol*. vol. 73, no. 6, pp. 1712–1720, 2007.

[15] G. A. Martínez-Castañón, N. Niño-Martínez, F. Martínez-Gutierrez, et al, "Synthesis and antibacterial activity of silver nanoparticles with different sizes" *Journal of Nanoparticle Research*, vol. 10, no.8, pp. 1343–1348. 2008.

[16] B. Atiyeh, M. Costagliola, S. Hayek, S. Dibo "Effect of silver on burn wound infection control and healing Review of the literature" *Burns*, vol.33, no. 2, pp.139-48, 2007.

[17] M Mukhlis, Salah M. "Spectral Study of Silver Nanoparticles Prepared by Chemical Method" *Eng. &Tech.Journal*, vol. 32, no. 5, pp. 869-876, 2014.

[18] I. F. Hasan, K. S. Khashan, and A. A. Hadi. "Study of the Effect of Laser Energy on the Structural and Optical Properties of TiO2 NPs Prepared by PLAL Technique" *Journal of Applied Sciences and Nanotechnology*, vol. 2, no. 1, 2022.

[19] S. Bamsaoud, ‘'The Effect of Biologically Synthesized Silver Nanoparticles on the Germination and Growth of Cucurbita pepo Seedlings" *Journal of the Arab American University*, vol. 3 no. 2, pp. 34-47, 2017.

[20] K. Roy, S. Biswas, P. C. Banerjee. "Green synthesis of silver nanoparticles by using grape (Vitis vinifera) fruit extract: Characterization of the particles and study of antibacterial activity” *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 4, no. 1, pp. 1271–1278, 2013.

[21] Yin, Iris Xiaoxue, et al. "The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry" International Journal of Nano medicine, vol.15, pp. 2555—2562, 2020.

[22] M. Al-Kinany, Ghaleb A. Al-Dahash and Jasim Al-Shahban"Effect of laser Fluence Energy on Morphological, Structural and Optical Properties of Gold and Silver Thin Films Prepared by Pulse Laser Deposition Method" *Engineering and Technology Journal*, vol.33, no.9, pp. 1561-1570, 2015.

[23] M. G. Guzmán, J. Dille, and S. Godet. "Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity" *World Academy of Science, Engineering and Technology*. 2008.

[24] G. D. Avila-Quezada, G. P. Espino-Solis."Silver Nanoparticles Offer Effective Control of Pathogenic Bacteria in a Wide Range of Food Products" *Pathogenic Bacteria*, Edited by Sahra Kırmusaoğlu and Sonia Bhonchal Bhardwaj, EBOOK (PDF) ISBN978-1-83881-934-7, 2019.