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Assessment of Antibacterial Activity in Vitro: Eco-Friendly Synthesis and Characterization of Silver Nanoparticles

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ABSTRACT

Silver nanoparticles (AgNPs) are one of the most essential and interesting nano materials between different metallic nanoparticles that are implicated in biomedical utilization. The expansion number of resistant bacteria create an inquiry for new antibiotic methods. Metallic nanoparticles have derived as a new platform against different microorganisms. The nanoparticles can by oxidative stress damaging the membrane of bacteria and DNA. Synthesis novel sliver nanoparticles using different reducing and stabilizing agents and Study antibacterial activity of the synthesized silver nanoparticles. Silver nanoparticles (AgNPs) were synthesis by utilizing green, simple and easy approach chemical reduction method using glucose as reducing and gelatin as stabilizing agent. The optimum conditions of AgNPs synthesizing were obtained by varying the concentration of $AgNO_3$, concentration ratio of glucose /$AgNO_3$ and temperature. The synthesized AgNPs were determined by UV—vis spectrum. Atomic Force Microscopy (AFM), Scanning electron microscopy (SEM), and Fourier transmission infrared spectroscopy (FTIR) analysis. Chemistry method for producing. The obtained AgNPs with particle size 75.7nm. Silver nanoparticles (AgNPs) showed excellent antibacterial against Gram -negative bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and Gram-positive bacteria, *Staphylococcus aureus*, Bacillus *cereus*.

1. Introduction

Nanotechnology involves imaging, measuring, modeling and manipulating matter at the scale between approximately 1 and 100 nanometers, where obtaining a new material with this nanometer-scale and these materials have many applications in science, engineering and technology at this length scale (Bayda, et al., 2019; Baig, et al., 2021). Silver nanoparticles (AgNPs) exhibit diverse biological activities and possess various chemical and physical properties within the range of nanomaterials (Gamboa, et al., 2019). Several techniques have been employed to produce AgNPs, encompassing biological, chemical, and physical methods (Iravani, et al., 2014). Chemical redox, utilizing a wide array of organic and inorganic reducing agents, is among the most widely recognized chemical approaches for AgNP synthesis. This method is favored due to its simplicity, cost-effectiveness, and capability to generate large quantities of samples (Vishwanath & Negi, 2021).

Scientists are currently studying the green chemical synthesis of metallic nanoparticles with the aim of enhancing environmental preservation and safeguarding the well-being of living organisms in pharmaceutical and

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biomedical applications. In order to promote the principles of green chemistry in nanoparticle production, there are three primary factors that should be taken into account: the selection of an appropriate solvent, the utilization of environmentally friendly reducing agents, and the adoption of non-toxic materials for stabilizing the nanoparticles (Samuel, et al., 2022).

Glucose is used as green reducing agent to prepare AgNPs (Platania, et al., 2021). The homogeneous size distribution and exceptional antimicrobial properties of glucose nanosilver colloids make them biologically compatible and highly promising for utilization in medical and pharmaceutical applications. These colloids have the potential to be effectively employed in various areas within these fields. There are many advantages of using glucose as reducing agent: it is an inexpensive ingredient widely used in many biominerals, no residual solvent is typically present in AgNPs colloidal solutions compared to the traditional hydrazine hydrate (Platania, et al., 2021).

Gelatin, consisting of peptides and proteins, finds extensive use in the pharmaceutical, food, and cosmetic industries. Its high molecular weight enables efficient interaction with metals and effective stabilization of metal particles. The primary function of gelatin is to serve as a stabilizer, making it commonly employed in various applications, including food processing, pharmaceuticals, photography, and electrochemistry (Mikhailov, 2023; Al-Nimry, et al., 2021; Khan, et al., 2020; Ward & Courts, 1977; Mees & James, 1966).

The gelatin-coated AgNPs demonstrate remarkable long-term stability, preventing aggregation and retaining stability across a broad pH range. Moreover, these modified and stabilized AgNPs exhibit exceptional and enduring antibacterial activity, even when stored under normal environmental conditions (Khanh, et al., 2019). This study aims to synthesis of novel sliver nanoparticles using different reducing and stabilizing agents, characterization of nanoparticles using different techniques and study antibacterial activity of the synthesized silver nanoparticles.

2. Materials and Methods

Materials

Silver nitrate ($AgNO_3$) was obtained from Sigma-Aldrich Company. Glucose and Gelatin were obtained from HiMedia Company. The culture mediums: Muller Hinton agar was purchased from HiMedia Company. All reagents were used as received. Deionized water was used to prepare all of the solutions.

Syntheses of AgNPs

AgNPs were prepared by chemical reduction methods by using glucose as a reducing agent and gelatin as a capping agent, by adding (5 ml. 1M) $AgNO_3$ solution to gelatin solution (2g/190ml water) with continuous stirring to obtain $AgNO_3$/gelatin solution. Then, adding (20ml. 2M) glucose solution and left for 3h, at temperature 60°C (Darroudi, et al., 2011).

Parameters Studied in preparation of AgNPS

Three parameters were studied in the experiments, they are (A) different concentrations of $AgNO_3$ (0.00l, 0. 01, 0.1, 1 M), (B) different concentration ratio of glucose: AgNO3 (1:1, 2:1 and 3:1 M/M) and (C) different temperature: 40. 50. 60, 70 and 80 °C.

Characterization of AgNPs

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium. UV-Vis spectral analysis was done by double beam spectrophotometer (Shimadzu). Atomic Force Microscope (AFM) analysis was done using scanning prop microscopy NT-MTD (Russia) for mapping the atomic- scale topography of AgNPs surface. Fourier transform infrared (FT-IR) spectrometer (Bruker Tensor 27 FT-IR (Germany)) was used to obtain the infrared spectrum of the AgNPs. Finally, the Scanning electron microscope (SEM) (FEI SCAN type) was used to test the morphology of the prepared AgNPs

Antibacterial activity

The antibacterial activity of AgNPs were tasted by using well diffusion method against the following bacteria strains which previously isolated: *Bacillus cereus* (isolated from air), *Escherichia coli* (isolated from water), *Pseudomonas aeruginosa* (isolated from soils) and *Staphylococcus aureus* (isolated from burns) obtained from Ministry of science and Technology and *Klebsiella pneumonia* (isolated from Pneumonic) obtained from Genetic Engineering end Biotechnology for postgraduate studies/university of Baghdad.

Muller Hinton agar media, which prepared according to Manufacture Company, poured into petri-dish. Then, in each of the Petri dishes 4-5 pits were bored sing sterile cork borer (6mm diameter). The bacteria were swabbed on the agar using the sterilized swab. About 100µL of AgNPs was added in each of pits and another was filled with control ($AgNO_3$) the plates were incubated at 37^0C for 24hr. After incubation the inhibition diameter was measured. The mean and standard deviation reported for each type of AgNPs and each microbial strain is as tested in triplicate (Ali, et al., 2021).

3. Results and Discussions

Characterization of AgNPs

Glucose can reduce silver ions to AgNPs and through this process oxidizes itself to gluconic acid (the redox reaction involves the aldehyde group). Each parameter studied in the synthesis of AgNPs were characterized with UV—Vis. Spectroscopy and AFM

Effect of $AgNO_3$ Concentrations on Formation of AgNPs

At different $AgNO_3$ concentrations (0.001, 0.01, 0.1 and 1 M), the color of the AgNPs depends on the $AgNO_3$ concentration, as shown in Figure 1. The color was changed from light yellow at the lowest concentration and dark brown at the highest concentration, which indicates an increase in AgNPs concentration.

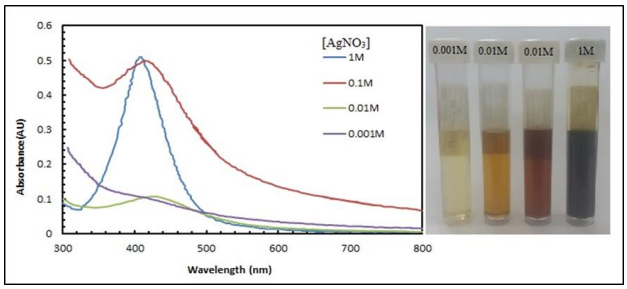

Fig. 1. UV–Vis. spectroscopy and photograph of AgNPs solutions at different [$AgNO_3$], at [Glucose]/ [$AgNO_3$] = 3M, Temperature =60°C, Stirring time=3h

The effect of $AgNO_3$ concentration on the formation of AgNPs was monitored by using UV–Vis. spectroscopy. The results of UV–Vis. spectroscopy in Figure (1) showed that at 0.001M $AgNO_3$, there is no λ_{SPR}, which indicate there is no AgNPs produce. The observed λSPR band on the curve appears broad at 415 nm and 423 nm for 0.01M and 0.1M AgNO3 concentrations, respectively, indicating the presence of AgNPs. The width of the peak suggests a distribution of particles with varying sizes. However, at 1M AgNO3 concentration, a distinct and narrow λSPR band is obtained at 427nm, indicating the presence of smaller-sized particles (Vanaja, et al., 2014).The red shift in λ_{SPR} by increasing $AgNO_3$ concentration (shifted to longer wavelength) indicates an increase in size of AgNPs (Peng, et al., 2010). On the other side, the amount of absorption increased by increasing $AgNO_3$ concentration, this means higher nanoparticles production at higher concentrations. From four explained concentrations, 1M $AgNO_3$ has the best performance in amount of production and in narrowness of range of nanoparticles' size.

Effect of Concentration Ratio of Glucose on $AgNO_3$

When increasing concentration ratio of Glucose/ $AgNO_3$, the colour of solutions for all ratios were dark brown, as shown in Figure 2. The results of UV–Vis. spectroscopy in Figure (2) showed that the intensity of the peak increases gradually with increasing in glucose/ $AgNO_3$ concentration ratio, which means at high glucose concentration the Ag^+ ions reduction increased and more AgNPs formed. At ratio (1, 2, 3), λ_{SPR} band obtained at (429,428 and 428nm), respectively, the change in λ_{SPR} is very slightly.

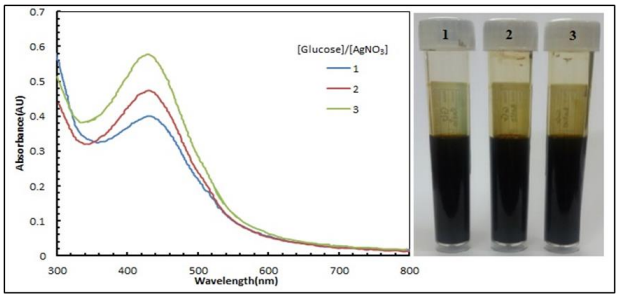

Fig. 2. UV–Vis. spectroscopy and photograph of AgNPs solutions at different [glucose]/ [$AgNO_3$], [$AgNO_3$] =1M, Time=3h, Temperature=60°C

To know of the effect of increasing concentration ratio of glucose/$AgNO_3$ on particle size, samples were characterized by AFM. Figures (3, 4 and 5) show AFM images and corresponding size distributions of AgNPs that prepared using glucose: $AgNO_3$ concentration ratio of 1, 2 and 3, with average size of 153, 129 and 123 nm, respectively.

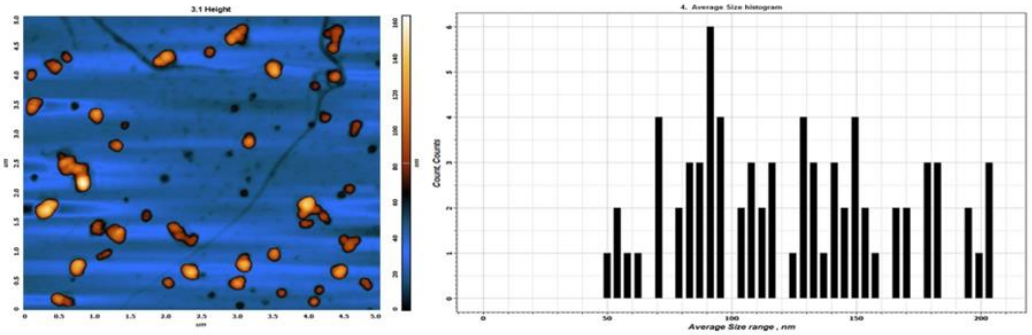

Fig. 3. AFM of AgNPs at [glucose]/[$AgNO_3$]= 1:1 (Avg. Diameter:153 nm)

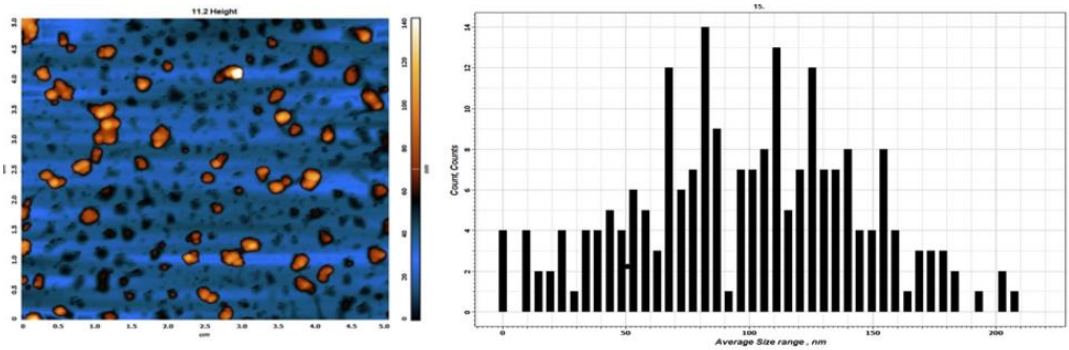

Fig. 4. AFM of AgNPs at [glucose]/ [$AgNO_3$] =2:1 (Avg. Diameter 129nm)

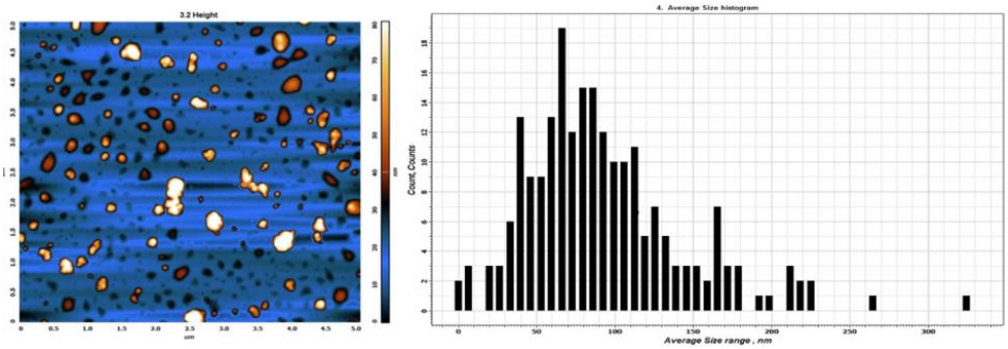

Fig. 5. AFM of AgNPs at [glucose]/ [$AgNO_3$] = 3:1 (Avg. Diameter: 123 nm)

AFM results showed that at 1M $AgNO_3$, the best size distributions (narrow distributions) of AgNPs with smaller average diameter size at this distribution when Glucose/ $AgNO_3$ concentration ratio was 3, average diameter size 123nm.

Effect of Temperature on AgNPs

To study the effect of temperature on AgNPs formation and determining the best temperature of reaction at 1M $AgNO_3$ with the ratio 3 of glucose/$AgNO_3$ concentration, the AgNPs solutions were prepared at different temperatures (40, 50, 60, 70 and 80°C). The colour of solutions, as shown in Figure 6, turned from light brown at the lowest temperature to dark brown at the highest temperature; this indicates an increase in AgNPs concentration.

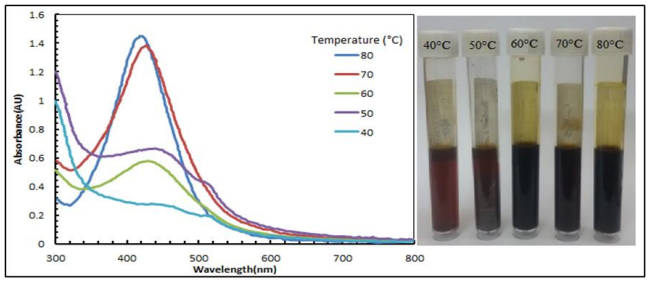

Fig. 6. UV–Vis. spectroscopy and photograph of AgNPs solutions at different temperature, at [$AgNO_3$] =1M, [Glucose]/ [$AgNO_3$] = 3M, Time=3h

The results of UV–Vis. spectroscopy in Figure (6) showed that there is no λ_{SPR} observed at 40°C. Therefore, at temperature 40°C, AgNPs are not produced. With rising temperature to (50 and 60°C), λ_{SPR} obtained at (434 and 428nm), respectively, with blue shift, indicated decreasing in AgNPs particle size and the band of peak curve is wide, means particles distribution at different sizes. At high temperatures (70 and 80 °C) the λSPR band obtained at (424 and 418nm), respectively, and it was narrower which indicating narrowness of dispersion distribution of nanoparticles. This means that the range of particles size is small. In addition, the intensity of the peak decreases by increasing temperature.

To study the effect of increasing temperature on particle size, samples which prepared at 70 and 80 °C characterized by AFM. The results were showed in Figures 7 and8.

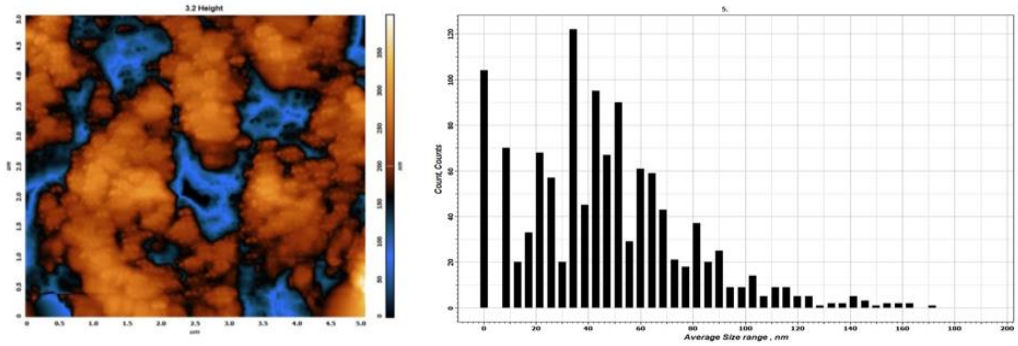

Fig. 7. AFM of AgNPs at temperature 70°C (Avg. size: 75.7 nm)

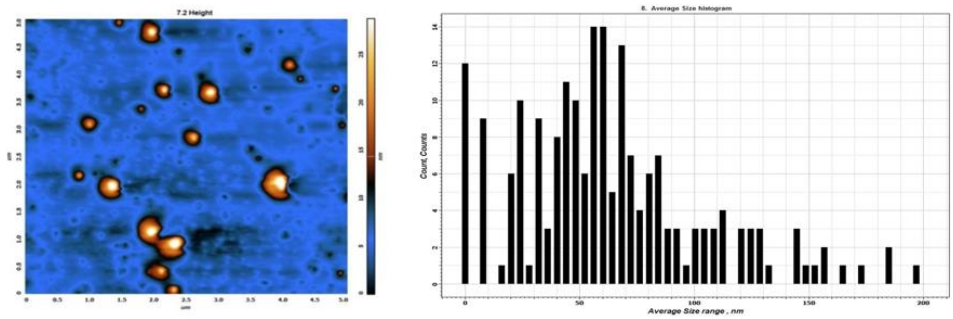

Fig. 8. AFM of AgNPs at temperature 80 °C (Avg. size: 92.7 nm)

AFM results showed that the best size distributions of AgNPs (narrow distributions range) with smaller average diameter size of this distribution were at temperature 70°C, average diameter size was 75.7nm. By raising the reaction temperature to 70°C, the reduction of Ag+ ions was enhanced. This temperature increase had a favorable effect, resulting in a slight reduction in the average diameter of the nanoparticles and improved stability of the suspension. Higher temperatures facilitated more intense chemical reduction, attributed to the enhanced activity of glucose and greater mobility of silver ions. Consequently, a larger quantity of smaller nanoparticles was formed as a result of these conditions.

However, at high temperatures, the synthesis rate becomes excessively rapid, making it challenging to control the particle size effectively. When the reducing agent is introduced into the precursor solution at 80°C, the growth, agglomeration, and nucleation of AgNPs all accelerate nearly simultaneously. Therefore, it is advisable to opt for a moderate temperature, such as 70°C, to achieve the synthesis of AgNPs with proper control over their size. This temperature provides a more suitable balance for achieving the desired particle size while maintaining control over the synthesis process. So, in this study the best factors at [$AgNO_3$] =1M, concentration ratio of Glucose/ $AgNO_3$ =3 and temperature 70°C which obtained average diameter size (75,7nm) of AgNPs. The FTIR and SEM characterization of AgNPs solution at these parameters show with details below.

FTIR spectra

The gelatin was characterized using FITR spectroscopy. Various Vibration bands at different wavenumbers were shown (Figure 9). These included a peak at 3272 cm^{-1} corresponding to the stretching of N–H bonds in the natural protein, which was associated with hydrogen bonding involving a carbonyl group in a peptide chain. Another band appeared at 3078 cm^{-1}, indicating the stretching of alkyl C–H bonds. Additionally, a peak at 2942 cm^{-1} indicated the asymmetrical stretching of CH_2 bonds. The spectrum also displayed a band at 1634 cm^{-1}, which represented the stretching of C=O bonds and hydrogen bonding involving COO−. Furthermore, there was a peak at 1539 cm^{-1}, indicating the bending of N–H bonds coupled with the stretching of C-N bonds. Other observed bands included at 1455 cm^{-1} corresponding to CH_2 bonds, 1235 cm^{-1} for N-H bending, and 1080 cm^{-1} for C–O stretching.

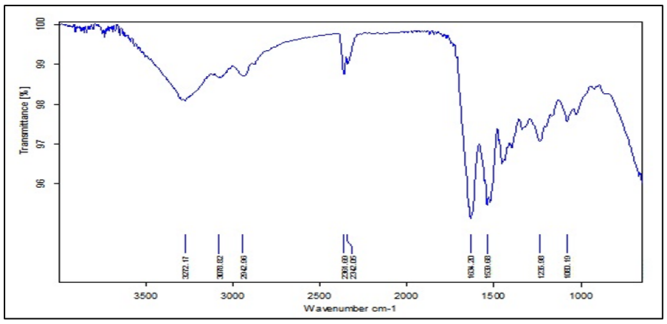

Fig. 9. FTIR spectrum of gelatin

In the spectrum of the AgNPs, shown in Figure 10, the absorption bands corresponding to the amide groups of gelatin, shifted to lower wave number values at 1539 cm^{-1}-1456 cm^{-1} to at1235 cm^{-1}-1201 cm^{-1}. These changes on the position bands may be due to the interaction gelatin with AgNPs.

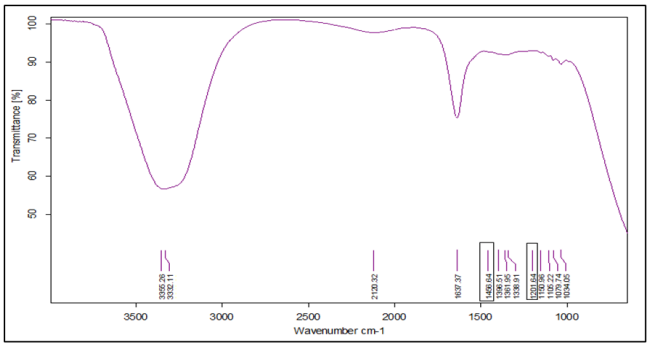

Fig. 10. FTIR spectrum of AgNPs synthesis by Gelatin and Glucose.

Figure 11 shows SEM analysis of AgNPs. It was observed large particles due to interaction of steric interactions between the gelatin (functional group, amine) capping molecules bound to the AgNPs. The presence of a capping agent suggests that the AgNPs were stabilized and not in direct contact, even within the aggregates. This stabilization mechanism prevents the aggregation of the nanoparticles. The observation of larger silver particles in the SEM measurements could be attributed to the aggregation of smaller particles, leading to their increased size.

SEM Micrograph

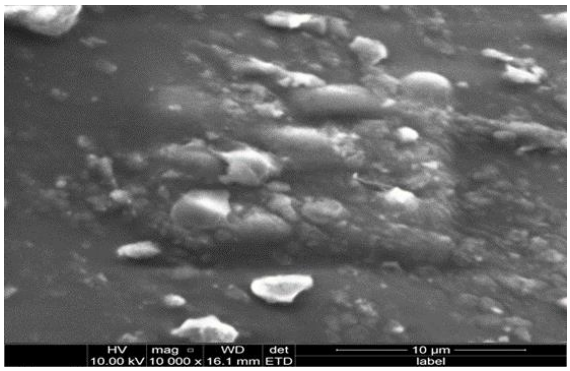

Fig. 11. SEM image of AgNPs synthesis by using Gelatin and Glucose

Antibacterial activity

The effect of AgNPs, which was prepared by using glucose and gelatin as reducing and stabilizing agents, respectively, at different ratio of [glucose]/[$AgNO_3$] (1:1, 2:1, and 3:1), was performed on bacteria strain. The results were showed in Figure 12.

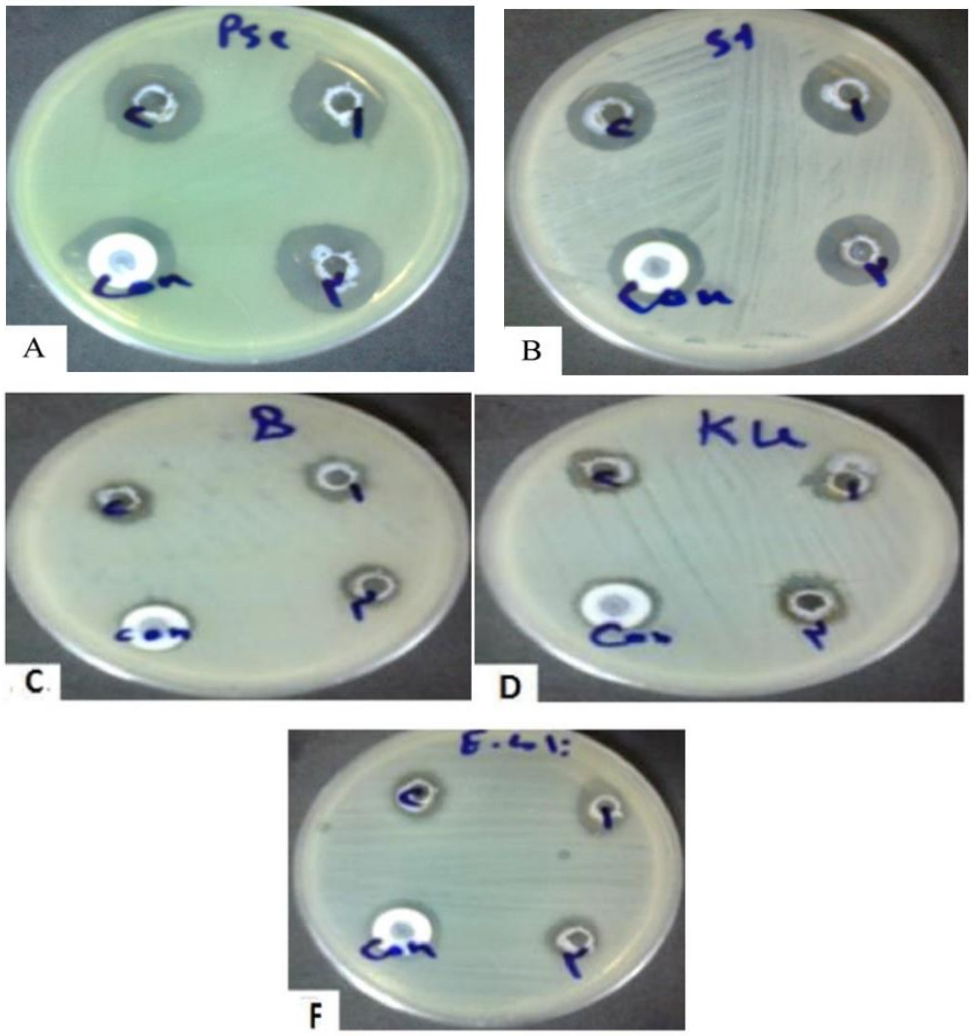

Fig. 12. Inhibition zone at different concentration of different [Glucose]/ |$AgNO_3$] (1, 2, and 3), 60 min. , stir time 3hr, temp. 60^0C, (A) *P. aeruginosa*, (B) *S. aureus*, (C) *B.cereus*, (D) *K. Pneumonia* and (F) *E.coli*

The inhibition zone that showed in (Tabe1) showed that AgNPs was more effective on *P. aeruginosa* than other bacteria.

Table 1
Inhibition zone at different concentrations ration of [Glucose]/ [$AgNO_3$]

	Inhibition zone (mm)				
[Glucose]/ [$AgNO_3$]	***B. cereus***	***K. pneumoniae***	***P.aeruginosa***	***E.coli***	***S.aureus***
1	11	11	19	11	19
2	11	11	19	11	19
3	11	9	22	11	17
Control*	14	15	20	15	19

*(-) control used $AgNO_3$ at concentration 1M.

These results were in agreement with (Jacob Inbaneson, et al., 2011), who prepared AgNPs by chemical reduction using tri-sodium citrate as reducing and capping agent. They studied the AgNPs effect against *E. coli, P. aeruginosa, K. pneumoniae* and *S. aureus*. Disc diffusion assay was performed to determine the antibacterial activity and found that the *P. aeruginosa* showed maximum sensitivity with inhibition zone (10 mm).

4. Conclusions

In antibacterial assay, AgNPs show inhibitor effect against both of Gram-positive and Gram-negative bacteria at different condition of prepared. AgNPs that prepared using glucose as reducing agent and gelatin as stabilizing agent, given high antibacterial activity at different [glucose]/[$AgNO_3$].

Competing Interests

The authors had no competing interests.

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