



Albumin/Ag Nanoparticles Synthesized by Using UV-Irradiation and Estimation of Their Antibacterial Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nano-scale (less than 100 nm) materials have received wide attention because of their rare properties that differ significantly from a bulk sample of the same material. In this research, a physical irradiation method by using UV lamp was established for preparation nanoparticles colloids of silver nanoparticles (Ag-NPs) with an eco-friendly stabilizer (Albumin). The preparation process was carried out using silver nitrate (AgNO₃) in aqueous albumin at room temperature. The effects of UV-irradiation time and the concentrations of Ag⁺ and albumin on the particle size have been investigated. Moreover, the antibacterial effect of the Ag/Albumin nanoparticles has been tested. The results showed that, the UV-irradiation can influence the Ag-NPs size, which the of Nps decreases with the UV-irradiation increases. The morphological study demonstrated well-dispersed spherical Ag-NPs with an average diameter of about 29 nm at the optimum conditions. The

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antibacterial results exhibited a significant inhibitory effect for the Ag-NPs that synthesized in this work. The current fabrication method of the silver nanoparticles colloid could be extended to other metals such as Cu- Nps and may possibly find various additional applications.

Keywords: Silver nanoparticles; green synthesis; albumin; UV-irradiation; antibacterial.

1. INTRODUCTION

Every day has been creating, what is a new in the numerous scientific fields and there is no suspicion that nanotechnology has become the most vital areas in chemistry, physics, biology, and others. Nanoscience is the science that study and characterization of materials in the nanoscale which, at least have one dimension between 1 and 100 nm in size. Nanoparticles are known as particles at the nanoscale, they suggestion new and improved properties depends on its size compared to the bigger particles of the similar substance. In current years, the preparation of metal nanoparticles has garnered more attention due to their exclusive properties compared to macro-sized metal phases. Silver nanoparticles (Ag-NPs) have a different scientific, industrial or consumer applications owing to the exceptional electrical, optical, thermal, catalysts and biological properties [1-9].

The Ag-NPs are accomplished killing 650 microbial germs without hurting the human body, therefore, Ag-NPs are used in association with medical instruments and apparatus [10]. However, several new synthetic methods have been explored for their preparation (i.e., chemical, physical, biochemical methods and photochemical) [11,12]. Most of the chemical methods that have been used for the synthesis of nanoparticles are too costly and include the uses of toxic and hazardous chemicals which are lead to various biological risks. Furthermore, the environment is suffering great damage because of a large amount of unwanted chemical and hazardous materials.

Numerous techniques have been practical to prepare metallic nanoparticles, instance, utilizing of light at different wavelengths in solutions of metallic producer in the presence of chemical materials as the stabilizers or size controller [13]. Recently, the photochemical reduction method of creating nanoparticles has become an essential technique in nanotechnology, it is can be produced Ag-NPs at room temperature also, it is more suitable and environmentally friendly than others by using green stabilizer [14,15]. Previous

studies examining the UV-irradiation method for preparing Ag-NPs have used a range of stabilizers such as polyvinyl pyrrolidone (PVP) [16], polyurethane [17], polymethacrylic acid [18] and gelatin [19].

Albumins are commonly found in blood plasma, substances containing albumins, such as egg white, also in the human blood plasma, serum albumin are the main protein [20,21].

In the literature, the use of albumin for the synthesis of nanoparticles by irradiation with ultraviolet light and the consideration of albumin as a stabilizer for nanoparticles some literature reported. Instant, Junling wang, et al. 2019 reported, Bovine Serum Albumin (BSA) is used as the stabilizing reagent for synthesis of silver nanoparticles (Ag-BSA NPs) in aqueous solution, instead of the typical thiol capping agents and unfriendly organic polymers which the BSA capped silver nanoparticles are extremely stabilized and concentrated in aqueous solution without any aggregation [22]. Also, In Meena pandey pant, et al. 2014 study the UV radiation sensitivity of Bovine Serum Albumin (BSA) bound silver nanoparticles (62 nm diameter) to various power density between 468 mJ/cm² to 1872mJ/cm² under physiological conditions [23]. However, Renquan lu et al. 2012 present, the proteins of egg white, which have different functional groups, played important roles in reducing Ag⁺ and maintaining product attributes such as stability and dispersity [24]. Moreover, Kalaiyarasan Thiyagarajan et al. 2018 in the present study, a simple, sustainable, cost-effective and green method has been developed to prepare highly stable aqueous colloidal silver nanoparticles (AgNPs-EW) using the ovalbumin, ovotransferrin and ovomucoid of egg-white as reducing and capping agents accomplished under the irradiation of direct sunlight. In addition and studied the antibacterial activity against *Salmonella typhimurium* and *Escherichia coli*, the results showed that the AgNPs-EW had the highest antibacterial activity [25]. Hence, in this research explore a new photochemical synthesis of Ag-NPs using UV-irradiation with albumin, which can be accomplished at room temperature. In the current research, albumin was introduced

as a green stabilizer to prevent particle agglomeration in the fabrication of nanoparticles. Four clinical isolates of bacteria [two gram-positive (*Staphylococcus aureus* and *Streptococcus sp.*) and two gram-negative (*Escherichia coli* and *Klebsiella sp.*)] were used to evaluate the antibacterial activity of Ag-NPs.

2. EXPERIMENTAL

2.1 Materials

Albumin egg powder and AgNO₃ were supplied by Sigma–Aldrich as powder material and used without further purification.

2.2 Methods

2.2.1 Fabrication of silver/albumin nanoparticles AgNPs

To optimize the reaction condition several parameters have been optimized which are a time of the UV-irradiation, AgNO₃ concentration and albumin concentration. Firstly, to optimize the time of UV-irradiation, a 100 ml of AgNO₃ solution (0.1 M) was added to 400 ml of albumin solution (1% w/v). The solution was stirred for an hour to obtain AgNO₃/Albumin. AgNO₃/Albumin solution was divided into eight samples. The samples were exposed to high-intensity UV-irradiation at room temperature, by a low pressure mercury lamp: 93110, E27 of spectral lamp company, $\lambda=185$ nm and P = 6W; under the same condition, for different period of time [26], at 0, 10, 20, 30, 40, 50, 60 and 70 min. Secondly, to optimize the AgNO₃ concentration, five samples were prepared by adding 10 ml of AgNO₃ at different concentrations 0.05, 0.1, 0.15, 0.20 and 0.25 M and fixed albumin concentration of 1% (w/v). The samples were stirred for an hour to obtain AgNO₃/Albumin. Then the samples were exposed to high-intensity UV-irradiation with UV-lamp for 60 min. Finally, to optimize albumin concentration, five samples were prepared by adding 10 ml of 0.2M of AgNO₃ to 40 ml of albumin at different concentrations 0.5, 1, 1.5, 2 and 2.5 % (w/v) respectively. The samples were stirred for an hour to obtain AgNO₃/Albumin and then they were exposed to high-intensity UV- irradiation with UV-lamp for 60 min.

2.2.2 Evaluation of antibacterial activity

Antibacterial activities of Ag-NPs were evaluated using the well diffusion method on Mueller-Hinton

agar [27]. Media was poured on two replicates Petri plates of each (*Escherichia coli*, *Klebsiella sp.*, *Staphylococcus aureus* and *Streptococcus sp.* labelled as B1, B2, B3, and B4, respectively). After the media was solidified the four wells (7 mm diameter) were made in each plate and 100 μ l of the different concentrations of Ag-NPs (0.031, 0.063 and 0.125 M) and 100 μ l of sterilized distilled water (negative control) were added in these wells. Also, the bacteria were added to media at 37°C. After 24 hours of incubation, each plate was examined and measured in the diameters of the zones of complete inhibition including the diameter of the wells.

2.3 Characterization

2.3.1 UV/Vis spectrophotometer

UV-Vis is considered as one of the most important spectroscopic methods used for metal nanoparticles characterization. Because of unique surface plasmon resonance (SPR) shown by specific metal and metal oxide nanoparticles (Au, Ag, Cu, and Pt), the use of the technique is essential for early detection of nanoparticles presence. In this work, a UV-visible spectrophotometer [UV 1650 PC-Shimadzu B (Shimadzu Osaka, Japan)] was used in detection of SPR bands of nanoparticles obtained from the synthesis. About 70 μ L of the sample was poured into cuvette. The spectra were run in the range of 200 to 800 nm.

2.3.2 Transmission Electron Microscopy (TEM)

The transmission electron microscope (TEM) was utilized in determining the actual sizes and shapes of the synthesized nanoparticles. TEM observations were carried out using an H-7100 electron microscope (Hitachi, Tokyo, Japan), and the particle size distributions were determined using the UTHSCSA Image Tool Version 3.00 program. The equipment is a typical microscope comprised of electromagnetic lenses, which enables it to focus on a sample and take images at nanometric size. Sample preparation was done by dilution with deionised water. The samples were then sonicated, dropped on a copper grid and oven dried 20 mins.

2.3.3 Scanning Electron Microscope (SEM)

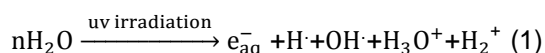
Scanning microscopy (SEM) measurements were made in a Jeol JSM-7600F Scanning Microscope JEOL, (Eching b. München,

Germany) instrument. The instrument is mostly used for surface morphology determination of materials. Samples were powdered and placed on a copper grid using carbon adhesive. The samples were then dried and sputter-coated with gold film using a sputter coater Baltec SCD005 Sputter coater (Bal-tec. Canonsburg, Pennsylvania, USA).

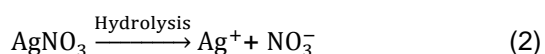
3. RESULTS AND DISCUSSION

AgNO₃/Albumin is a colourless suspension, after applying UV- irradiation the colour of the prepared samples slowly changed from colourless to light brown, brown and lastly dark brown as shown in Fig. 1, that is indicative the formation of Ag-NPs in albumin suspension [28]. 0 min denotes the AgNO₃/Albumin suspension without any irradiation and 10, 20, 30, 40, 50, 60 and 70 min of UV- irradiation. The change in the colour depends on the increase of irradiation time.

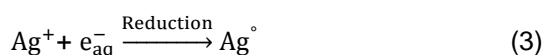
The mechanism for the synthesis of Ag-NPs using UV irradiation is detailed by the Equations (1-3). After exposure of AgNO₃/Albumin aqueous suspensions to UV-irradiation, hydrated electrons, primary radicals and molecules are formed [28] as in Equation 1.



AgNO₃ disintegrates to Ag⁺ and NO₃⁻ ions when dissolved in the aqueous solution, as shown in Equation 2.



The e_{aq}⁻, is a reducing agent. Therefore, in the following step, it reduces silver ions down to the zero-valent state as in Equation 3.



The interaction between Albumin and AgNP have been reported in previous study such as changes in alpha helical structure during adsorption of protein on NP surface to a certain extent [29].

3.1 UV-visible Spectroscopy Analysis

The creation of Ag-NPs was recognized by the surface plasmon resonance (SPR) of AgNO₃/Albumin and Ag in albumin between 300–700 nm. The SPR bands are influenced by the, size, shape and morphology of the prepared

nanoparticles [30]. Fig. 2 shows the preparation of Ag-NPs in albumin using different UV-irradiation times. The characteristic silver SPR band was identified around 460-480 nm in Fig. 2, which indicated the formation of Ag-NPs [31]. As shown in Fig. 2 before irradiation the solution by UV irradiation, there was no absorption band that means, there are no Ag-NPs in the solution. However, after irradiating the solution at different times of UV-irradiation 10, 20, 30, 40, 50, 60 and 70 min an absorption bands is observed. The intensity of the SPR band also increased as the time increased which indicates that, the concentration of Ag-NPs increased as well [32] that because of the fragmentation of Ag-NPs by photo-induction led to an increase in the whole number of particles in the solution [33]. However, Fig. 2 has shown the irradiation time at 60 and 70 min gives almost the same results in the wavelength and the absorbance which means after 60 min UV-irradiation there is no more reduction of silver ion. According to the SPR band in Fig. 3, when the concentration of AgNO₃ was increased, the SPR detected around (450-470 nm) that improve the formation of Ag-NPs. However, when increased the concentration of AgNO₃ to (0.1, 0.15 and 0.2M), the absorbance presenting to lower wavelength (a blue shift), as shown the results in Fig. 3 when the initial Ag⁺ concentrations are increased, Ag-NPs with higher yields and smaller sizes are produced [17]. Furthermore, when the concentration of AgNO₃ was increased to 0.25 M, the intensity of the absorption band was decreased, this refer to agglomeration was occurring in the solution [34]. This could be because after reaching a certain NPs size, the stabilizer was unable to hold the size of nanoparticle effectively, which resulted in its very large size [28].

UV-visible spectroscopy data determined the formation of Ag-NPs by observing the (SPR) bands. Fig. 4 shows the preparation of Ag-NPs in albumin by using different concentrations of albumin. Normally, the absorption spectrum of NP_s depends on the shape, size, and size distribution of the nanoparticles [35]. However, SPR band characteristic of Ag-NPs was identified around 450 to 470 nm, which indicates the formation of Ag-NPs [36]. As shown in Fig. 4, when the concentrations of albumin were increased 0.5, 1, 1.5, 2, and 2.5%, the intensity of the SPR peak also regularly increased. The increase of the absorbance was indicative that the concentration of Ag-NPs increased [37]. Furthermore, Fig. 4 shows that with an increase the concentrations of albumin, the absorbance

also increased and shifted to lower wavelength to blue-shift, which referred to a decrease in the particle size [38,39]. Also, it was observed that the 2 and 2.5% of albumin solution had a larger absorbance compared to other samples. The increase of the absorbance indicated that the concentration of Ag-NPs increased [40].

3.2 Morphology Results

Fig. 5 shows a typical TEM image of the prepared spherical Ag-NPs and indicates the dominant size of Ag-NPs ranges from about 29 nm with high distribution. Furthermore, The SEM

result in Fig. 6 shows the surface morphology of the well-dispersed Ag-NPs formed in albumin with a spherical shape which agreement with TEM result.

3.3 Antibacterial Activity

The different concentrations of Ag-NPs exhibited significant inhibitory ($P < 0.05$) the antibacterial activity against four clinical isolates of bacteria Fig. 7 and Fig. 8. Ag-NPs showed maximum antibacterial activity against *Staphylococcus aureus* (10 ± 1.4) and *Streptococcus* sp. (10.5 ± 0.7) and lowest activity against *Escherichia coli*

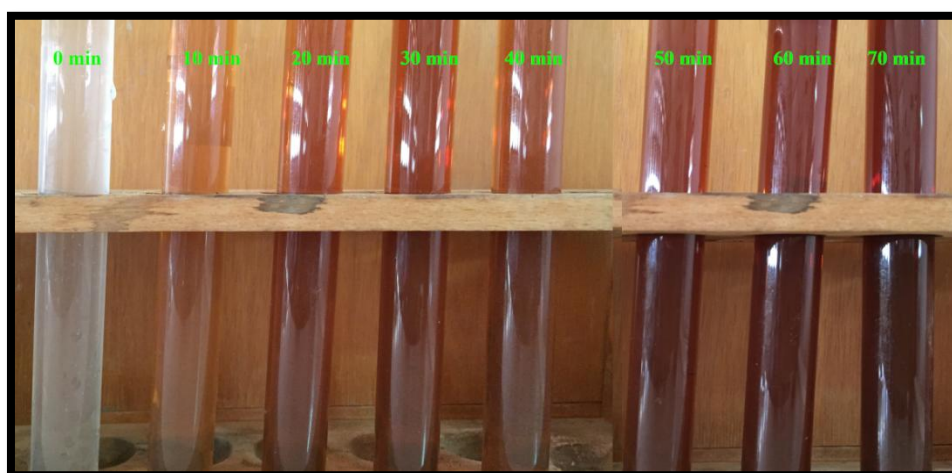


Fig. 1. Picture of AgNO₃/Albumin (0 min) and Ag/Albumin (10- 70 min) suspensions at different UV irradiation times

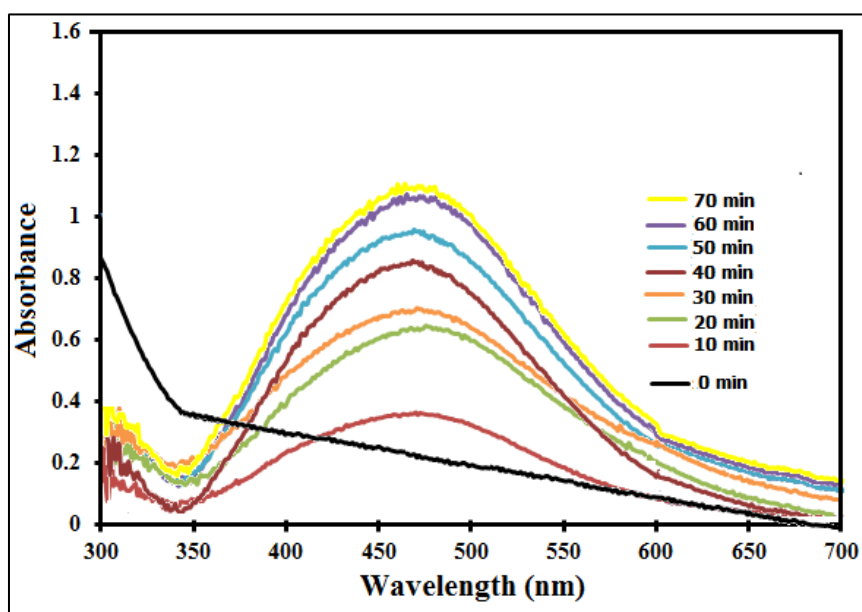


Fig. 2. UV-visible absorption spectra of Ag-NPs in Albumin at different UV- irradiation times 10, 20, 30, 40, 50, 60 and 70 min

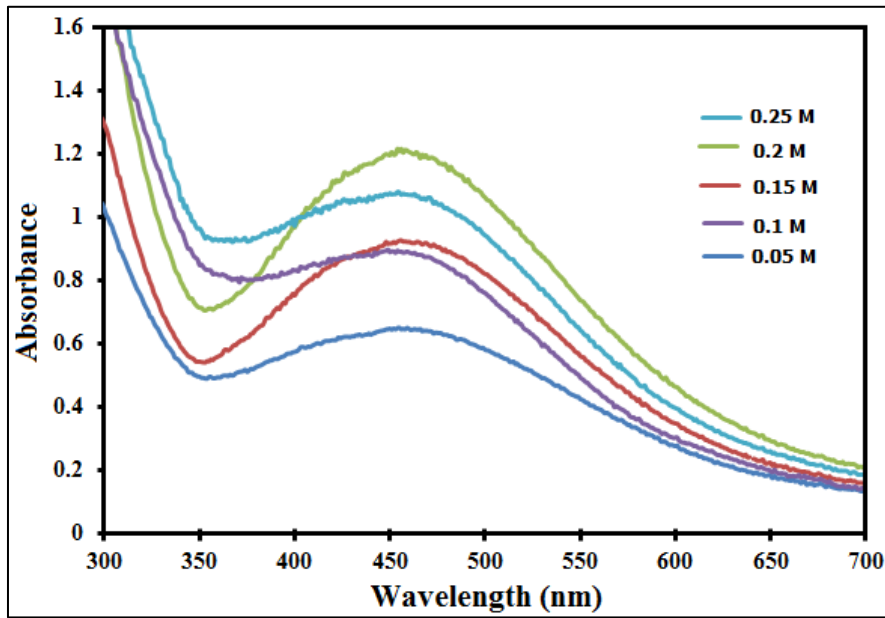


Fig. 3. UV-visible absorption spectra of Ag-NPs in albumin suspensions, at different concentration of AgNO₃ 0.05, 0.1, 0.15, 0.2 and 0.25 M at 60 min of UV-irradiation

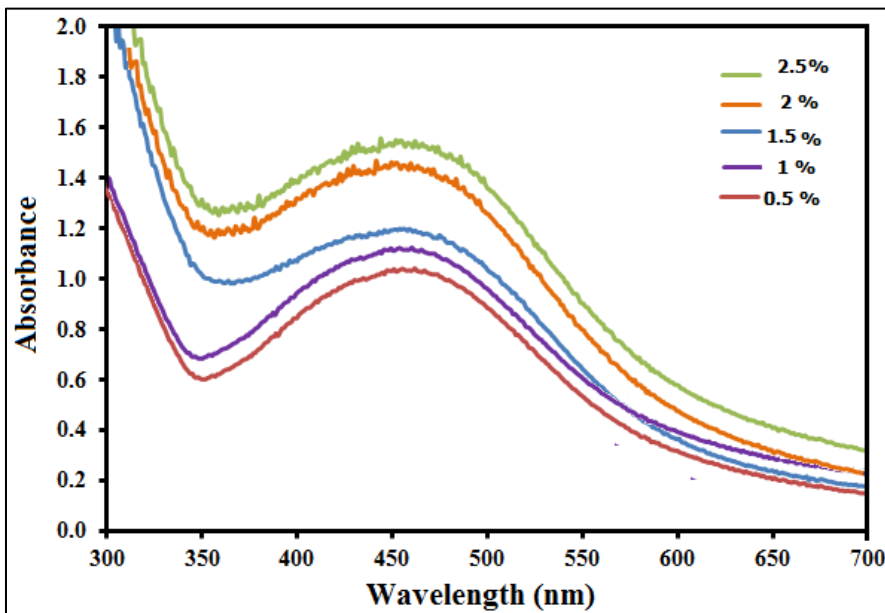


Fig. 4. UV-visible absorption spectra of Ag-NPs in albumin at different concentration of albumin 0.5, 1, 1.5, 2, and 2.5 % at 60 min of UV- irradiation

and *Klebsiella* sp. (8 ± 0). The results are expressed as mean values \pm standard deviation (SD). LSD multiple range tests were used to compare the mean of the treatments at $P < 0.05$. The Significance of the difference between mean values was determined by two way analysis of variance. The results show antibacterial resistance of Ag-NPs against these

two different types of bacteria. However, when the concentration of Ag-NPs was increased the inhibition zone increased, according to increase the number of Ag-NPs with small size. The small sized particles with greater surface area provide more contact with the cell membrane, thus easily penetrated into the cell wall and damaged the bacterial membrane [41].

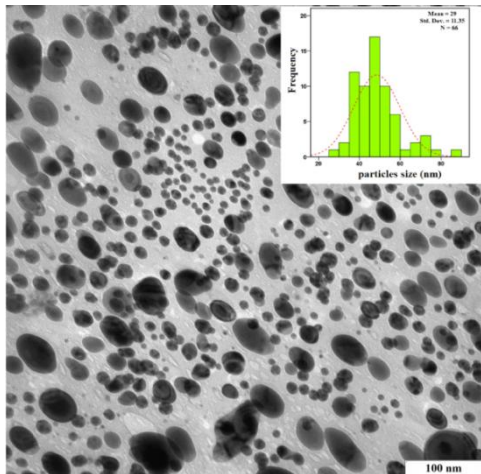


Fig. 5. TEM image of Ag-NPs synthesized under UV- irradiation at 60 min (AgNO₃=0.2 M, albumin =0.25%)

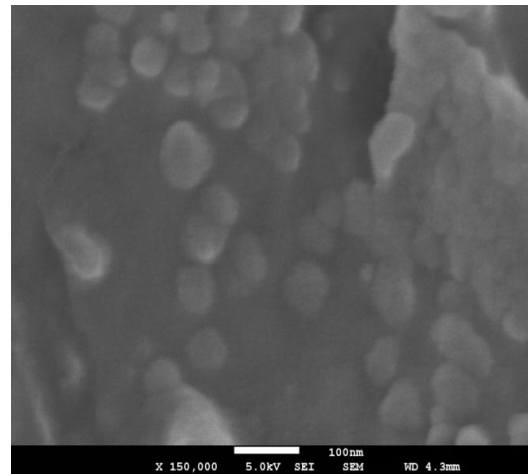


Fig. 6. SEM image of Ag-NPs synthesized under UV- irradiation at 60 min (AgNO₃=0.2 M, albumin =0.25%)

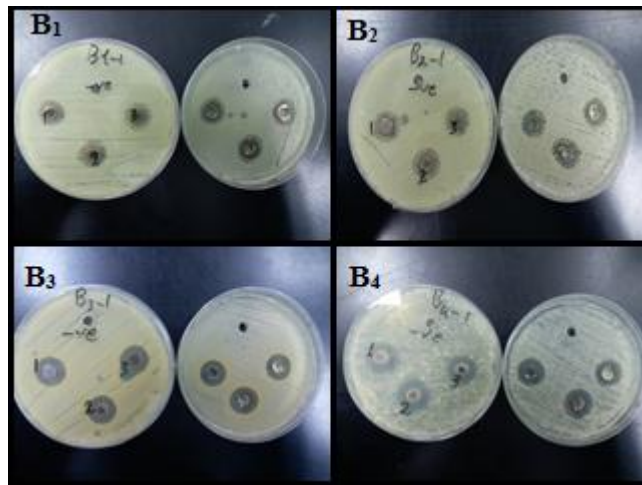


Fig. 7. Inhibition zone of the different concentrations of of Ag-NPs (1= 0.125, 2= 0.063 and 3= 0.031M) and sterilized distilled water (-ve= negative control) on *Escherichia coli* (B1), *Klebsiella* sp. (B2), *Staphylococcus aureus* (B3) and *Streptococcus* sp. (B4)

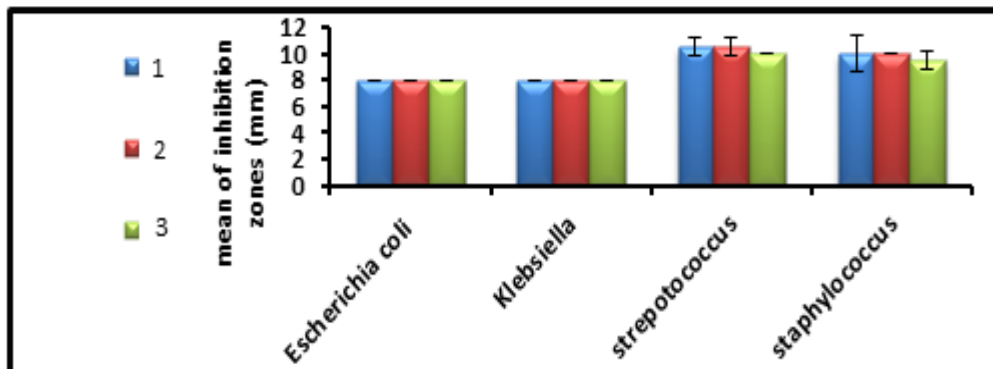


Fig. 8. Mean of inhibition zone \pm standard deviation of the different concentrations of Ag NPs (1= 0.125, 2= 0.063 and 3= 0.031M) on *Escherichia coli* (B1), *Klebsiella* sp. (B2), *Staphylococcus aureus* (B3) and *Streptococcus* sp. (B4)

4. CONCLUSION

UV- irradiation as a green and a physical reducing method for the synthesis of Ag-NPs in albumin at different irradiation times successfully used. The Ag-NPs are effectively shaped as demonstrated by the greatest surface plasmon resonance crest at 450–480 nm as shown by UV-Vis spectroscopy by using different time of UV- irradiation. Moreover, using different concentrations of AgNO₃ under UV-irradiation was reported. The increase in the concentration of AgNO₃ leads to increase the concentration of Ag-NPs, this was proven by the UV-visible spectroscopic analysis. Different concentrations of albumin used in synthesised Ag-NPs in albumin under UV-irradiation. The results showed that, the concentration of Ag-NPs increases with increasing the concentration of albumin up to 2.5 wt%. The morphology study improved the formation of Ag-NPs with size 29 nm and spherical shape. Antibacterial result showed that, the Ag-NPs that synthesised by UV-irradiation are useful as an antibiotic.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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