

Antibacterial Effect of 405 nm Diode Laser and the Shape of Silver Nanoparticles on *Escherichia coli*

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Abstract

Background: Silver nanoparticles (Ag-NPs) have gained significant attention for their unique properties and potential applications, particularly in the field of antibacterial activity. The synthesis of Ag-NPs through the laser ablation method in liquid allows for the production of different shapes, influencing their optical and biological properties. **Objectives:** The present study was aimed amount-dependent effects of different Ag-NP shapes on *Escherichia coli*. The evaluation of the impact of irradiation on the antibacterial activity of Ag-NPs was conducted by using a 405 nm laser. **Materials and Methods:** Laser ablation method in liquid was used to prepare Ag-NPs with varying shapes. Optical property analysis: UV-vis spectroscopy was performed to examine the optical properties of the synthesized Ag-NPs. Dynamic light scattering and transmission electron microscopy were employed to determine the size and morphology of the Ag-NPs. The impact of Ag-NPs on *E. coli* was studied, considering both concentration and shape dependencies. The antibacterial activity of irradiated and nonirradiated Ag-NPs was compared. **Results:** The results of the study revealed the following key findings. Shape dependency: the antibacterial activity of Ag-NPs was found to be shape-dependent, with rod-shaped Ag-NPs exhibiting complete inhibition of *E. coli* at a lower concentration (0.1 mL) compared to spherical-shaped Ag-NPs (1 mL). Irradiation effect: irradiated Ag-NPs using a 405 nm laser at 20 mW demonstrated higher antibacterial activity than their nonirradiated counterparts. **Conclusions:** This study explores the antibacterial potential of Ag-NPs and silver nanorods (Ag-NRs) against *E. coli* bacteria. Notably, the antibacterial efficacy is significantly heightened through the irradiation of Ag-NPs by a laser beam with a wavelength of 405 nm. Comparative analysis reveals that Ag-NPs exhibit superior antibacterial activity when compared to Ag-NRs against *E. coli*.

Keywords: Antibacterial activity, *Escherichia coli*, silver nanorods, spherical silver nanoparticles

INTRODUCTION

Investigating antimicrobial mechanisms in silver-based nanocomposites contributes significantly to nanobiotechnology.^[1] Nanocomposite materials have received significant attention due to their unique optical, electrical, magnetic, biological, and mechanical properties.^[2] The bactericidal activity of nanocomposite materials depends on their size, concentration, morphology, and surface properties.^[3] In addition, it was stated that the bactericidal effect of silver nanoparticles (Ag-NPs) decreases with increasing particle size and is affected by particle morphology.^[4] Ag-NPs exhibit effective antibacterial properties due to their small size, which causes damage to the DNA structure of microorganisms.^[5] Ag-NPs can disrupt bacterial growth by

producing reactive oxygen species (ROS) that can attack the microbial antioxidant defence system, leading to cell damage.^[6] Ag-NPs can adhere to membrane proteins, causing an increase in membrane stiffness that leads to membrane disruption. This leads to impair membrane permeability, respiratory chain, cell division, and ion transport.^[7] Surface plasmon resonance (SPR) manifests a resonance effect due to the interaction of conduction electrons of metal nanoparticles with incident photons. Owing to its intrinsic sensitivity to surface properties,

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SPR has attracted tremendous attention in biomedicine and nanosensor applications.^[8,9] Laser radiation in the SPR mode may change the electronic state of Ag-NPs, which increases the antibacterial activity of the system as a whole.^[10] Antimicrobial Photodynamic therapy uses photosensitizers to generate ROS that damage cell membranes and DNA, leading to bacterial cell death.^[11,12] Photodynamic therapy requires photosensitizer, light, and oxygen.^[13] The size and shape of the Ag-NPs influence the production of Ag⁺ ions. Accordingly, the Ag-NPs with small sizes and a sphere-shaped or quasi-spherical arrangement are more susceptible to releasing Ag⁺ ions due to their greater surface area.^[14] Numerous studies have experimentally demonstrated that the antipathogenic activity of Ag-NPs is superior to silver ions.^[15] Electrostatic interaction between bacteria and Ag-NPs can influence charged nanoparticle penetration through biofilm.^[16] Some experiments have found that Ag-NPs can damage mitochondrial functions through ROS. Specifically, Ag-NPs can be easily oxidized by O₂ and other molecules.^[17] In this research, the effect of the shape of Ag-NPs on the biological activity of *Escherichia coli* bacteria in different quantities with and without the effect of laser has been studied [Figure 1].

MATERIALS AND METHODS

Bacteria

E. coli is a Gram-negative, nonspore, rod-shaped, and facultative anaerobe. To ensure bacteria validity, we must keep them at a temperature of 0°–5°, and they will live for a week in the environment outside the incubator. These Gram-negative bacteria were used to evaluate the antibacterial effect of Ag-NPs.

Synthesis of spherical Ag-NPs

To investigate the effects of Ag-NPs, we fabricated these structures using laser ablation in liquids method.

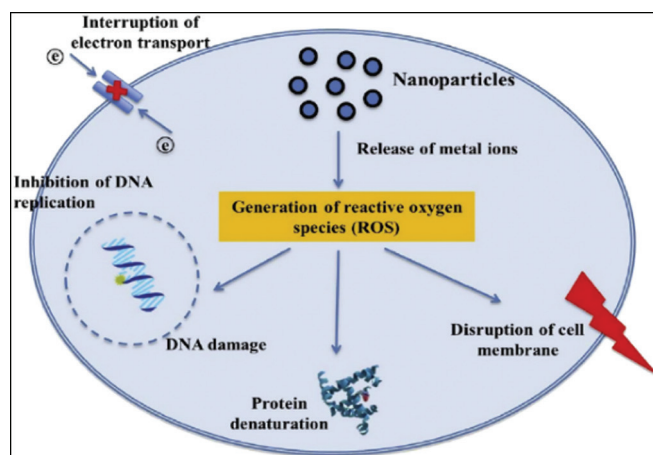


Figure 1: The effect of nanoparticles on cell components.^[1]

Specifically, a pulsed Nd:YAG laser at 1064nm with an energy of 80 mJ per pulse and a pulse width of 4 ns used to ablate silver target. The Q-switch Nd:YAG laser was directed toward a pure silver plate to produce the nanoparticles, operating at a 10 Hz repetition rate, and ablation was carried out for different ablation times, 2, 3, and 4 min, to obtain three different concentrations, 12.5%, 25%, and 50%, respectively. The laser beam was focused on the silver target after being reflected from a mirror and passing through a convex lens with a focal length of 100mm. The target was immersed in a 15mL solution of double distilled water and stirred during the ablation process. The ablation process was carried out at room temperature.

Synthesis of silver nanorods

The nanorods were synthesized by laser ablation of Ag target with 1064 nm laser irradiation and 100 mJ in double distilled water under the application of electric field. More specifically, two electrodes were immersed in the liquid that apply electric field with a voltage 10 V to produce rod-shaped Ag-NPs.

Laser

A semiconductor laser with a continuous wavelength of 405 nm at 20 mW output power is used to irradiate bacteria samples.

Addition of spherical-shaped Ag-NPs

The antibacterial activity of Ag-NPs and irradiated Ag-NPs was evaluated against *E. coli* bacteria. The antibacterial activity of the prepared Ag-NPs was evaluated using the bacterial counting method. The nanoparticles were added to 10 plates containing 1 mL of *E. coli* suspension; five plates were exposed to 405nm laser irradiation. The volume of added Ag-NPs was 0.05, 0.1, 0.5, and 1 mL. A defined volume was added to each plate, with one plate serving as the control. All of plates were kept at 37°C for 24h.

Addition of rod-shaped Ag-NPs

Rod-shaped prepared Ag-NPs were added to six plates of *E. coli* bacterial and three of them were irradiated by 405nm wavelength of laser. The volume of added Ag-NPs was 0.1 and 1 mL. A defined volume of Ag-NPs was added to each plate containing 1 mL of *E. coli* suspension, with one plate serving as a control. All of plates were kept at 37°C for 24h.

Counting method

An experiment was conducted to assess the antibacterial efficacy of Ag-NPs against *E. coli* through a growth inhibition test. The number of live bacteria and their colony-forming units (CFU/mL) were counted. In the first stage of serial dilution preparation, a liquid sample was

diluted by a factor of 0.1 (1 mL of the first tube was added to 9 mL of solution in the second tube). A total of 0.1 mL was taken from each prepared tube (serial dilution) and transferred to a Mueller Hinton Agar solid culture center. The entire sample was spread onto the culture medium using a sterile swab. The cultures were then incubated at 37°C for 24 h, and the number of colonies in the samples was counted. The number of colonies in each plate was counted, and by multiplying it with the dilution factor, the total number of colonies was obtained.

RESULTS AND DISCUSSION

UV-vis spectroscopy

Figure 2A shows the UV-vis spectra of Ag-NPs colloidal solutions produced in distilled water using different selected ablation times, utilizing CECIL ENGLAND (7200CE). It is obvious from Figure 2A that the absorption peak was increased by increasing the ablation time. This is attributed to the increasing the concentration of prepared Ag-NPs. Figure 2B shows that the spectrum of nanorods is broader than that of nanoparticles due to their different shapes and sizes, which affect their plasmonic properties. Ag-NPs have a spherical shape and smaller size, which leads to a narrow plasmon resonance peak in the spectrum. This is because the electrons in the nanoparticles are confined to a smaller volume. In contrast, silver nanorods (Ag-NRs) have a more elongated shape and larger size, which leads to a broader plasmon resonance peak in the spectrum. This is because the electrons in the nanoscale have a larger swing size. It is obvious from Table 1 that at a time of (2 min), the maximum wavelength of absorption is at 403 nm, and with increasing irradiation time, we notice that there is a shift towards longer wavelengths (redshift). This means the size of nanoparticles increases with increasing irradiation time, and this is evident from the table shows that absorbance increases with increasing irradiation time, and this can be explained by the increase in the number of particles of the removed material with time.

Transmission electron microscopy

Figure 3A depicts transmission electron microscopy (TEM) image of Ag-NPs prepared at 80 mJ laser energy for ablation time of 4 min. The figure shows the spherical shape of the nanosilver material, which has apparent granular size of different dimensions, small and spherical. The nanoparticles showed no coalescence and maintained a well-defined spherical shape with a diameter of 19 nm. Figure 3B shows the size and shape of the nanorods obtained by applying a voltage of about 10 V to the sample, using a laser energy of 100 mJ. The figure indicates the clear coalescence of the nano atoms, as the image appeared in the form of a rod and very small dimensions, showing the state of plasmon resonance in a form. It is better, as the nanometer dimension is estimated according to the apparent measurement below (15 nm).

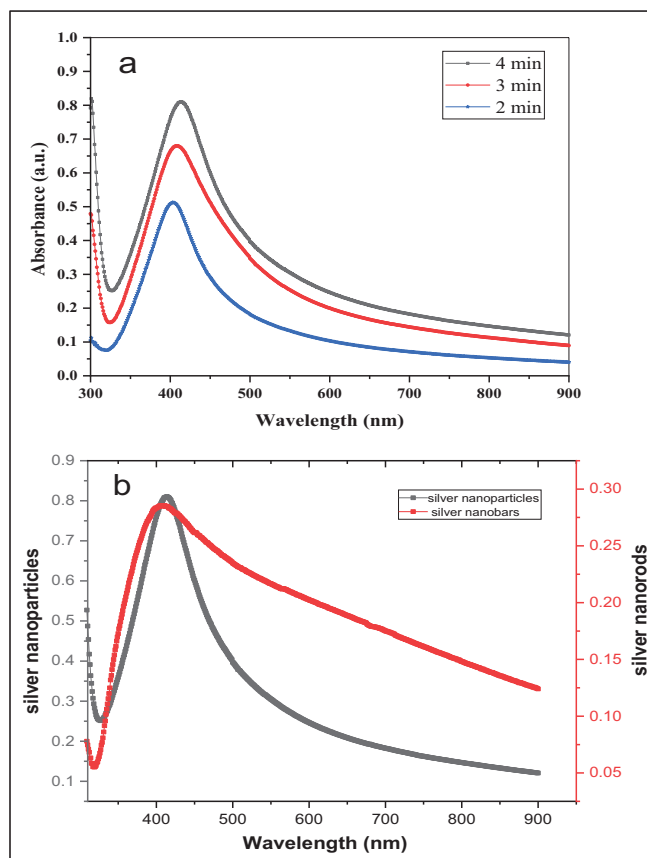


Figure 2: (A) Absorption spectrum of spherical Ag-NPs with different irradiation times. (B) Comparison of the absorption spectrum of Ag-NPs and Ag-NRs

Table 1: The wavelength, absorbance, and FWHM for each concentration of nanoparticles

Wavelength (nm)	Absorbance (a.u)	FWHM (nm)
413.13 (4 min)	0.811	91.51
407.93 (3 min)	0.676	84.16
403.23 (2 min)	0.511	31.74

FWHM = full width at half maximum

Dynamic light scattering

Dynamic light scattering (DLS) technology was used to evaluate particle size and distribution. While DLS primarily measures intensity distribution, the corresponding volume distribution can be calculated from the intensity data. The relative intensity of scattered light as a function of particle size. Figure 4 depicts these Ag-NRs size distribution, which is around from 500 to 1000 nm rod distributions. A polydispersity index (PDI) near zero indicates a monodisperse (narrow size distribution), while a larger PDI denotes a polydisperse (wide size distribution). It is obvious from Figure 4 that the PDI, which indicates the produced nanorod Ag-NPs, was polydisperse value 242.18–521.33 nm.

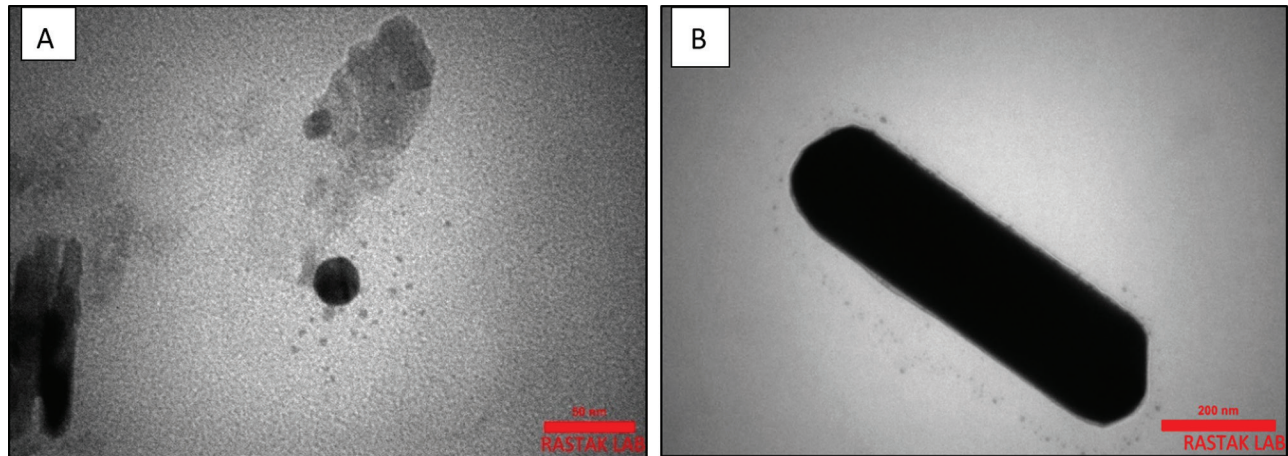


Figure 3: The TEM image: (A) Ag-NPs and (B) Ag-NRs

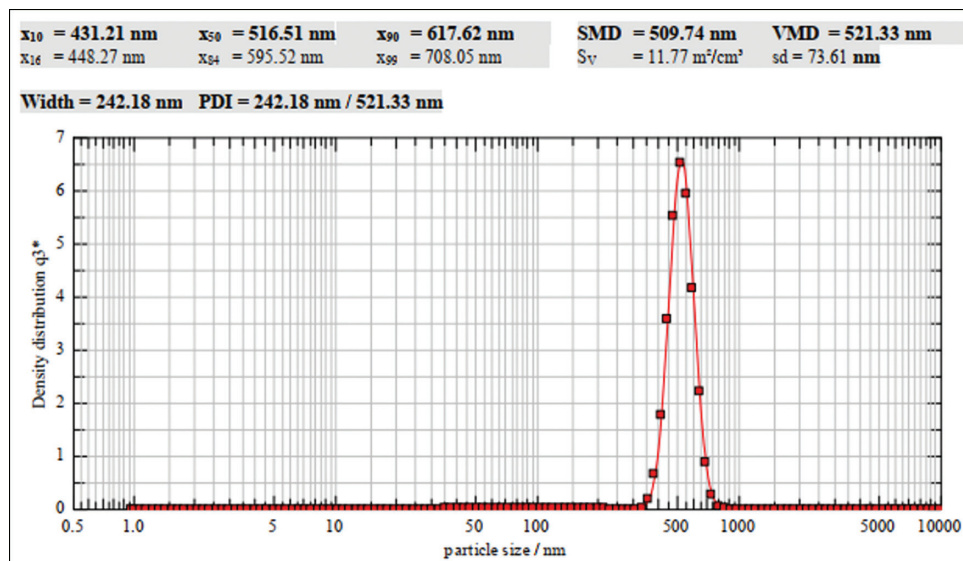


Figure 4: DLS spectrum of Ag-NRs solution

Effect of Ag-NPs without irradiation and under irradiation

It is clearly from Figure 5 that increasing the amount of nonirradiated spherical Ag-NPs to decrease of bacterial viability where the number of bacteria was decreased from 2.74×10^8 to 1.8×10^8 CFU/mL at the larger amount (1 mL) as compared with control group. Furthermore, in all amounts of nanoparticles, there was a decrease in the number of bacterial cells. The highest decrease in the number of bacterial cells was when treated with 1 mL of irradiated Ag-NPs and the number of viability bacteria was 1.7×10^8 CFU/mL. The lowest decrease in the number of bacterial cells was when they were exposed to Ag-NPs at 0.05 mL and laser light. The results indicated that the photoactivated Ag-NPs' antibacterial activity increased with the amount of Ag-NPs. The findings of this study can be compared to other studies reporting photovoltaic applications on metallic NPs. Sadeghi *et al.*^[18] investigated the antibacterial activity of nanosilver

forms against the microorganisms *Staphylococcus aureus* and *E. coli*. It was discovered that the surface areas of nanosilver shapes play a crucial role in regulating antibacterial activity. Ag-NPs exhibited the highest antibacterial activity and pathogen-specific surface area. Guzman *et al.*^[19] attributed the antibacterial activity of Ag-NPs to their electrostatic interaction with negatively charged bacterial cells. DNA loses its ability to replicate upon exposure to Ag⁺, cellular proteins become inactive, and Ag⁺ binds to the functional groups of proteins, resulting in protein denaturation. Al-Sharqi *et al.*^[20] examined the antibacterial activity of Ag-NPs in four different concentrations. They found that laser-activated Ag-NPs reduced the live population of *E. coli* to 14%, while the survival rate of *S. aureus* decreased to 28% after 10 min of exposure. The effects depended on Ag-NP concentration and duration of exposure to laser light.

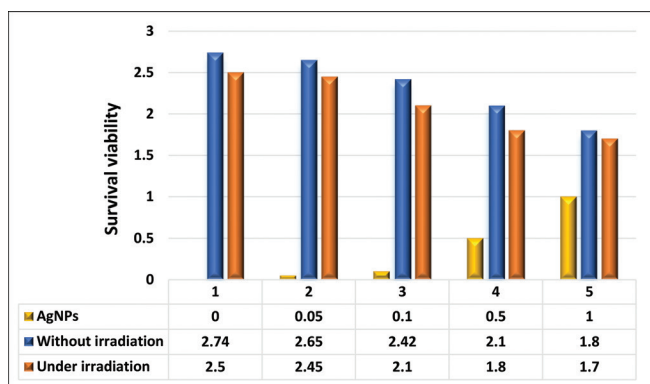


Figure 5: The effect of Ag-NPs on the survival of *E. coli* at various concentrations with and without irradiation

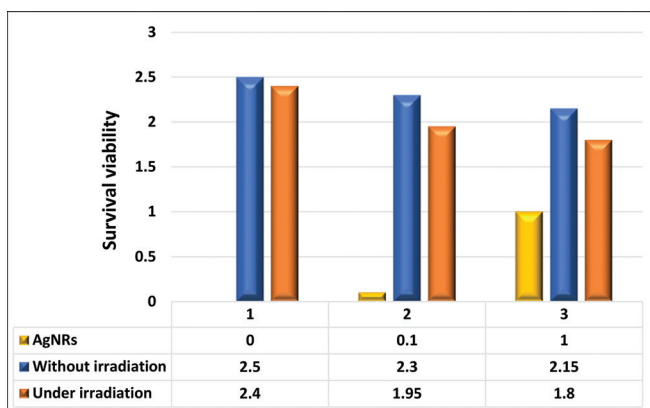


Figure 6: Effect of Ag-NRs on the survival of *E. coli* for different amount of Ag-NPs with and without irradiation

Effect of Ag-NRs without irradiation and under irradiation

The chart in Figure 6 shows the results of the effect of Ag-NRs alone and the effect of Ag-NRs irradiated with a 405 nm laser for 4 min of irradiation. We noticed that, when adding Ag-NRs alone, the number of live bacteria decreased due to the increase in Ag-NRs, when 1 mL of nanorods, the number of bacteria was 1.95×10^8 CFU/mL. As for the combined treatment with Ag-NRs and laser, we noticed a significant decrease in bacteria compared to the treatment with Ag-NRs alone. The highest reduction was at 1 mL of the Ag-NRs and upon laser irradiation, where the number of viable bacteria became 1.8×10^8 CFU/mL, compared to the control. El-Gendy *et al.*,^[21] by exposing a femtosecond laser to blue light, demonstrated the possibility of enhancing the bactericidal effect of modest concentrations of Ag-NPs. Using 50 mW laser radiation and 400 nm femtoseconds, the growth dynamics of *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and methicillin resistant *S. aureus* were measured. The combined therapy was more antimicrobial than either Ag-NPs or photoirradiation alone. Methicillin-resistant *Staphylococcus aureus* was more resistant to the bactericidal effects of Ag-NPs than *P. aeruginosa* and *L. monocytogenes*.^[22] Ag-NPs' form has been found to impact how effective they are

against microbes significantly. Since they exhibited more significant antibacterial activity than spherical Ag-NPs, rod-shaped Ag-NPs could be used at lower concentrations. The presence of facets with a high atom density, specifically (111) facets on rod-shaped Ag-NPs, favored their bactericidal activity. In contrast, the presence of (100) facets on spherical Ag-NPs resulted in comparatively lower bactericidal activity.

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Ethical approval

Not applicable.

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Conflicts of interest

There are no conflicts of interest.

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