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Studying the Effect of Gold Nanoparticles Prepared by Pulse Laser Ablation in a Liquid on Bacterial Activity

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Abstract

Gold nanoparticles (AuNPs) were synthesized using the pulsed laser ablation technique in liquid. An (Nd: YAG) laser operating at a fixed energy of 600 mJ and a frequency of 4.3 Hz was employed. The absorption spectrum of the synthesized particles was measured using an ultraviolet-visible spectrometer (uv-vis), with peak values observed in the range of (523–530) nm. Scanning electron microscope (SEM) analysis results revealed that the particles exhibited a spherical shape and had an average size ranging from (38 - 92) nm. X-ray diffraction confirmed the crystalline structure of the material, which was determined to be cubic. Transmission electron microscopy (TEM) images demonstrated that the particle diameter was (31 and 21) nm for the respective frequencies, which corresponded to 1200 pulses. Furthermore, the biological activity of the AuNPs was assessed against positive and negative bacteria.

Keywords- pulsed laser ablation technique, AuNPs, (UV-Vis) spectroscopy, SEM, TEM.

I. INTRODUCTION

The term "nanoparticles" (NPs) refers to particles in the condensed phase with sizes ranging from (1 to 100) nm [1]. NPs possess a remarkably small size and a significant surface area relative to their size [2], nanotechnology was first introduced in 1974 [3]. Three primary methods can be used for synthesizing NPs: physical, chemical, and biological approaches [4]. Among the physical techniques used, pulsed laser ablation is recognized for its ability to produce high-purity particles. This approach is considered cost-effective, environmentally friendly, and straightforward, because it requires specific experimental conditions. By utilizing water and a target material, highly crystalline nanostructures can be readily produced in a single step, thereby ensuring excellent purity [5].

The key advantages of pulsed laser ablation technology include the ease of controlling experimental settings, the ability to create NPs under various atmospheric conditions, and its versatility with different materials [6]. Collecting magnetic NPs produced through pulsed laser ablation in liquid (PLAL) is a straightforward process. Several studies in this field have demonstrated that magnetic NPs can be easily separated using magnets [7]. However, due to the release of larger particles during laser ablation, NPs tend to aggregate in solutions, leading to broader size distributions.Particle size reduction can be achieved by employing a range of surfactants and polymers to effectively control the expanded size distributions [8]. In this technique, the creation of nanoparticles is accomplished through the straightforward ablation of a solid target positioned in a liquid media. Such simplicity also makes it possible to create materials [9].

Gold NPs (AuNPs) exhibit distinct optical characteristics in the visible spectrum because of the surface plasmon oscillation of free electrons induced by light [10]. This unique property increases the surrounding medium's temperature upon light absorption, and it has various properties and applications [11]. The colors exhibited by these NPs at the nano scale depend on their size, shape, and the medium in which they are situated. AuNPs are widely used in biotechnology and biomedicine because of their inert nature, durability, high contrast, non-toxicity, and compatibility. They have been identified as the least toxic and safest agents for drug delivery purposes [12,13]. Simply altering the laser's wavelength, spot size, fluence, pulse duration, repetition rate, and liquid medium might improve the production of gold nanoparticles [14–16]. The main objective of this work is to find out the response of bacteria to the impact of gold nanoparticles on them.

II. METHODS

The manufacturing of AuNPs involved the use of an Nd: YAG laser with specific parameters. The laser had a wavelength of 1064 nm, a frequency of (3 and 4) Hz, and an energy of 600 mJ. PLAL was employed to create AuNPs from gold material with a high purity level of 99.99%. The gold material was submerged in a 100% ethanol alcohol solution during the process. The different pulse types used were (800, 1000, and 1200) pulses. To carry out the process, the sample was placed at the bottom of a jar filled with alcohol, and a quartz lens was used to focus the laser beam on the surface of the sample. This instance ensured the required energy density for ablation. A total of 3 ml of liquid was used for the process. The Nd: YAG laser, known for its reliability and ease of operation, is widely used as a solid-state laser. Notably, the working temperature of the laser device should not exceed 37 ℃. Therefore, careful monitoring and management of the laser's temperature are essential during the process.

This ensures the protection of the device from damage and defects in the YAG crystal active medium. Additionally, the pulse counter needs to be reset to prepare the laser for subsequent operation. In the production of the prepared membrane material, the drop casting technique was employed due to its significance and its simplicity in generating thin films. The AuNP solution is carefully deposited onto horizontally positioned glass substrates using a pipette, following thorough cleaning stages to ensure their cleanliness. Notably, the presence of contaminants significantly affects the accuracy of the obtained readings.

III. RESULTS AND DISCUSSION

A. Ultraviolet-visible (Uv-Vis) spectrometer

Figure 1 displays the absorption spectra of AuNPs prepared using different pulse counts, frequencies when (3,4)Hz, and constant energies. The presence of visible absorption peaks confirms the formation of nano-sized metals. Specifically, at pulse counts of 800, 1000, and 1200, and frequencies of 3 Hz and 4 Hz, the surface Plasmon resonance becomes evident, occurring within the range of (523–530) nm. These peaks arise due to the gradual increase in NP production and associated changes in absorbance values as a result of the increasing number of pulses, as stated in [17].

An increase in the number of pulses and frequency leads to a blue shift, resulting in the emergence of these peaks that support the production of NPs in the solution. The observed peak that corresponds to surface plasmon resonance (SPR) can be attributed to Mie scattering. Mie scattering occurs when spherical NPs with radii matching the wavelength of the incident light interact with an electromagnetic wave [18].

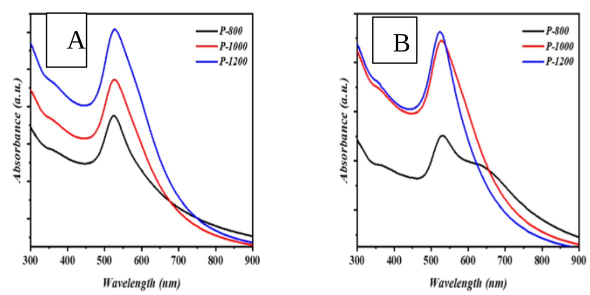


Figure 1. Absorption spectrum of gold nanoparticles at A/frequency (3 Hz) B/ frequency (4 Hz)

B. X-ray diffraction

Figure 2 illustrates the X-ray diffraction of of gold nanoparticles at frequency (3,4)Hz, revealing a cubic shape. The dominant crystallographic plane observed was (111), accompanied by multiple peaks corresponding to AuNPs. The analysis confirmed that the particles possess a polycrystalline structure and exhibit a face-centered cubic form. The experimental results for gold, presented in Table 1 showed structural properties of AuNPs, are in agreement with the research findings [19]. The international card (JCPDS-05-0669) was used to match and compare results.

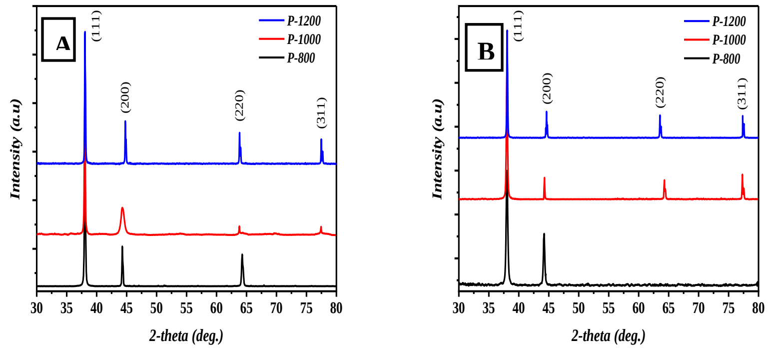
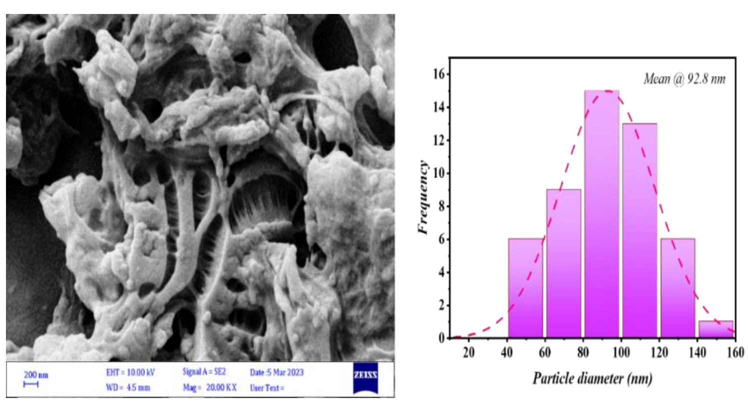


Figure 2. X-ray diffraction of gold nanoparticles at
A/frequency (3 Hz)
B/ frequency (4 Hz)

Frequencies (Hz)	Pulse	Geometric Crystal System	2θ (deg)	hkl	FWHM (deg)	d_{hkl} (nm)	D (nm)	Card No.
3	800	Cubic	38.0186	111	0.158	2.3649	56	05-0669
	1000	Cubic	38.0194	111	0.2141	2.36485	41	05-0669
	1200	Cubic	38.0692	111	0.2701	2.36188	32	05-0669
4	800	Cubic	38.0314	111	0.311	2.36414	28	05-0669
	1000	Cubic	38.0161	111	0.313	2.3636	27	05-0669
	1200	Cubic	38.0283	111	0.3778	2.36432	23	05-0669

C. Field emission scanning electron microscopy AuNPs and transmission electron microscopy

Figure 3 showcases the findings obtained from field emission scanning electron microscopy (FE-SEM), including images depicting the distribution of particle diameters and the average diameter of AuNPs. These NPs were deposited using pulse rates of 800, 1000, and 1200, and frequencies of 3 Hz and 4 Hz. The diameter range of the generated AuNPs is displayed, ranging from (38 to 92) nm. Table 2 showed that average nanoparticle size values presents the results obtained for AuNPs. The observed AuNPs exhibit irregularly shaped semispheres, and their forms vary as the number of pulses changes. To determine the size of the NPs, transmission electron microscopy (TEM) can be employed, as depicted in Figure 4 showed image of AuNPs at frequency (3 and 4) Hz when 1200 pulse . The results for AuNPs are summarized in Table 3 showed the average nanoparticle size values.



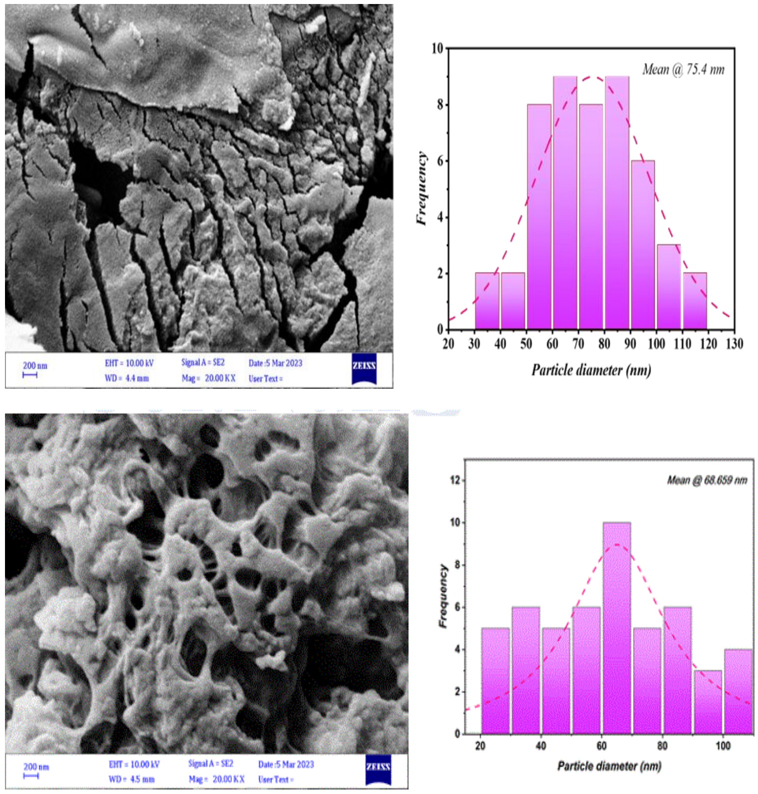
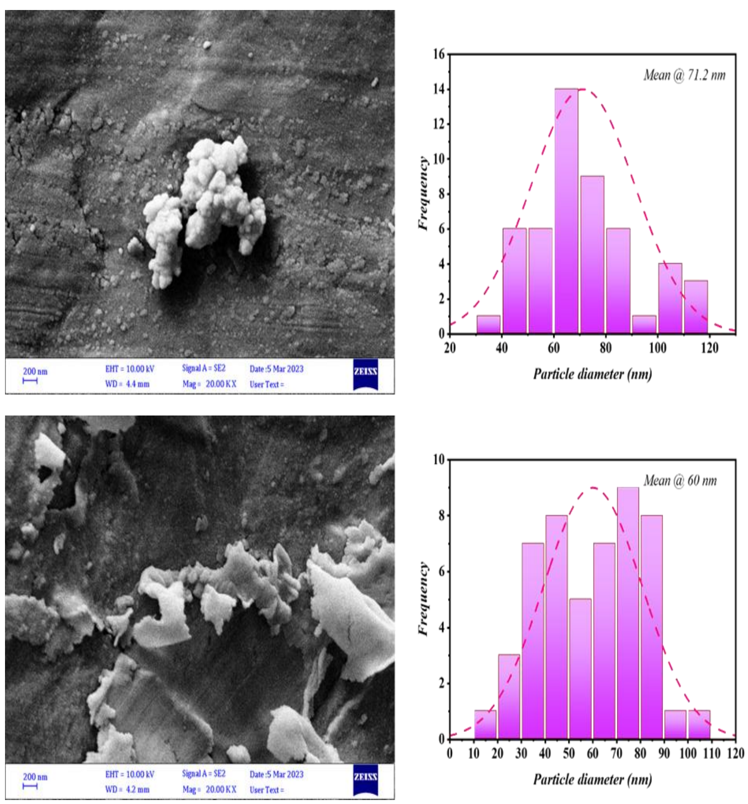


Figure 3-A. FE-SEM images and distribution of AuNPs at frequency (3 Hz)



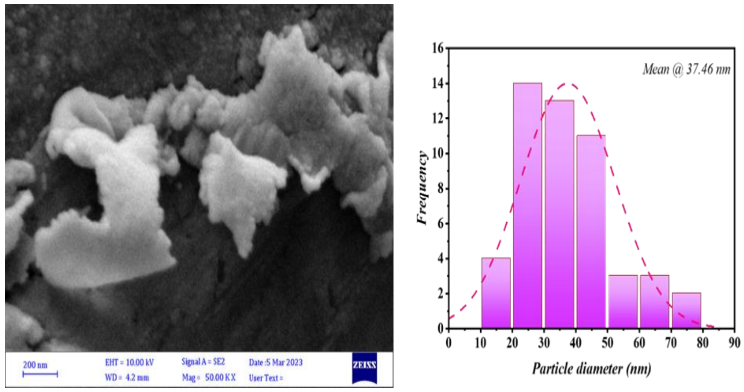


Figure 3-A. FE-SEM images and distribution of AuNPs at frequency (3 Hz)

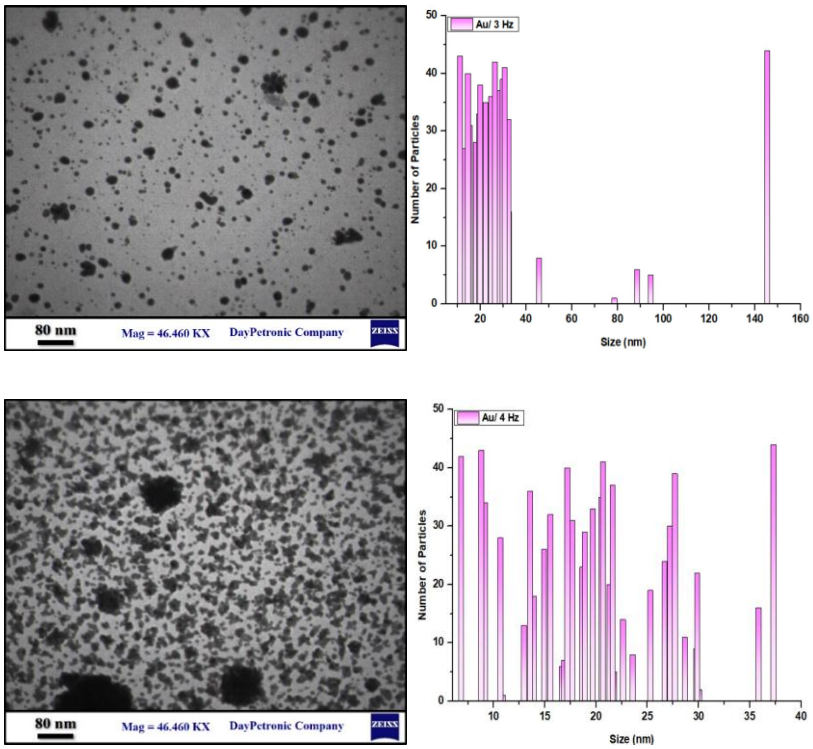


Figure 4. TEM image of AuNPs at frequency (3 and 4) Hz when 1200 pulse

Table 2. Average nanoparticle size values

Frequencies (Hz)	Pulse	Average Size (nm)
3	800	92
	1000	75
	1200	69
4	800	71

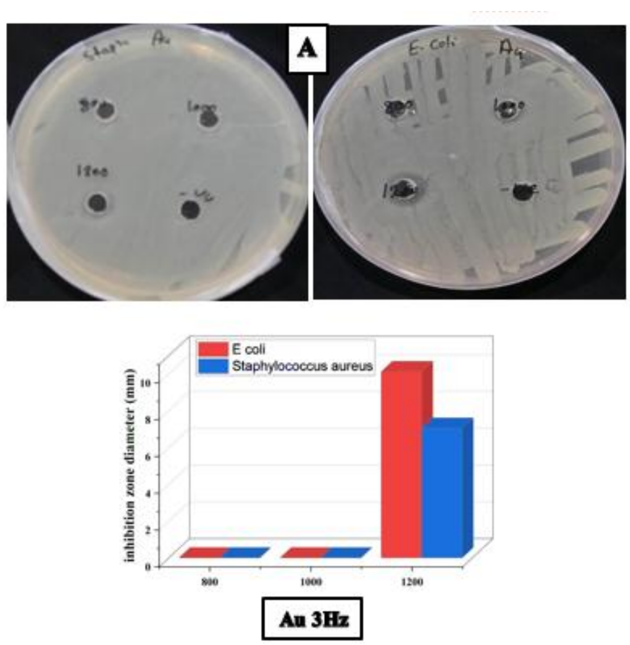
	1000	60
	1200	38

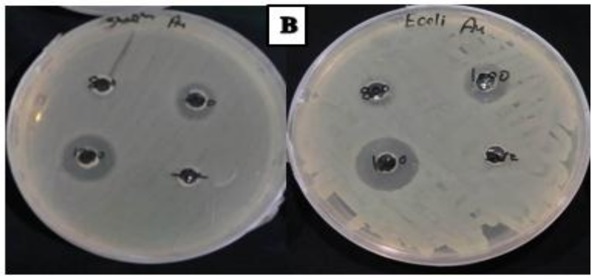
Table 3. Average nanoparticle size values

Method	Frequencies (Hz)	Pulse	Au(nm)
PLAL	3	1200	31
	4	1200	21

D. Measurement of bacterial actvity

The antibacterial activity of the AuNPs was evaluated against Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli. The assessment revealed a substantial antibacterial effect against both types of bacteria. Antimicrobial activity can be defined as a collective term for all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies, and may destroy microorganisms [20]. The inhibitory activity increased with the number of pulses and frequency , as illustrated in Figure 5 showed diameter of the zone of inhibition for AuNPs (*S.aureus*) and (*E. coli*) at frequency (3.4) Hz and summarized in Table 4 inhibitory activity (inhibition zone diameters) of gold particles against (S. aureus) and (E. coli) .





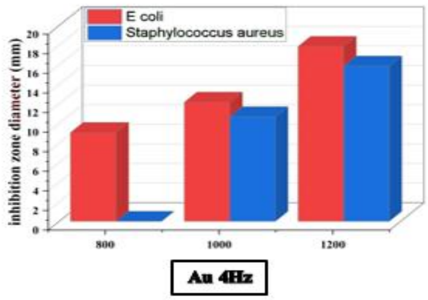


**Figure 5. Diameter of the zone of inhibition for AuNPs (*S.aureus*) and (*E. coli*).at A/frequency (3 Hz)
B/ frequency (4 Hz)**

Table 4. Inhibitory activity (inhibition zone diameters) of gold particles against (S. aureus) and (E. coli)

Frequencies (Hz)	Pulse	Inhibition zone diameter (mm) (S. aureus)	Inhibition zone diameter (mm) (E.coli)
3	800	0	0
	1000	0	0
	1200	7	10
4	800	0	10
	1000	11	12
	1200	16	18

IV. CONCLUSION

The absorption or direct transformation of NPs increases with an escalation in the number of pulses. By altering the number of pulses and frequency, the size and dispersion of the NPs can be controlled. Specifically, as the frequency and pulse count increase, the particle size decreases more rapidly. However, at pulse counts of 800 and 1000, no bacterial activity was observed against Gram-positive and Gram-negative bacteria. The size and morphology of AuNPs are directly related to their antibacterial activity. The present review shows that the antibacterial activity of AuNPs has been studied against a small number of bacteria, so it is recommended that their antibacterial activity against important pathogenic bacteria should be considered.

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