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Antibacterial Silver Nanoparticles produced by Nd:YAG Laser

Zainab I. Abd-Alwahab
Laser and Optoelectronic Engineering Department
College of Engineering, Al Nahrain University,
Baghdad, IRAQ

Bassam G. Rasheed
Laser and Optoelectronic Engineering Department
College of Engineering, Al Nahrain University,
Baghdad, IRAQ
dr_bassamghalib@yahoo.com

Abstract.

Q-switched Nd-YAG laser of wavelengths (1060, 532 and 322 nm) with energy in the range 200 to 1000 mJ and 1 Hz repetition rate was employed to synthesis silver nanoparticles using pulse laser ablation in liquid. Effect of laser wavelength has been studied for the fundamental, second and third harmonic generation. The experimental UV-Vis absorbance data were fitted with theoretical Mie-Gans model. It is found that the smaller silver nanoparticle of 12 nm capable to terminate both Staphylococcus and Streptococcus bacteria.

Keywords: Silver nanoparticles, Laser ablation, Antibacterial activity

Introduction .

Nanomaterial's display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications [1]. Due to the reduced size of their constituent elements nanostructured materials have electronic, magnetic and chemical properties, which differ considerably from those of the corresponding bulk materials. For example, nanostructured materials have been found to exhibit increased strength and hardness, higher electrical resistivity, enhanced diffusivity, reduced density, etc. compared to the bulk due to quantum confinement effect [2]. Hence, these materials are promising candidates for a variety of applications, which include heterogeneous catalysis, gas sensor technology, microelectronics, nonlinear optics, etc [3].

Discoveries have led to the observation that new properties exist when the size of materials is on the nanoscale due to electronic confinement in semi-conductors and surface effects in metals. The significance of nanoscale quantum confinement of the electrons provides visualization of the shift in the characteristics of the material depending on the size of the nanoparticles [4]. Noble metal nanoparticles such as Ag and Au NPs have been a source of great interest due to their novel electrical, optical, physical, chemical and magnetic properties [5]. The main characteristics of metallic NPs are large surface energies, specific electronic structure, plasmon excitation and quantum confinement [6].

Moreover, silver nanoparticles are being used in numerous technologies such as; desirable optical, conductive, and antibacterial properties. These applications can be categorized to diagnostic applications, conductive applications, optical applications and antibacterial applications [7].

Silver is a nontoxic, safe inorganic antibacterial agent capable of killing about 650 types of diseases causing microorganisms. Silver has ability to exert a bactericidal effect at minute concentrations. It has a significant potential for a wide range of biological applications such as antibacterial agents for antibiotic resistant bacteria, preventing infections [8]. Previous studies [7, 9-11] have proposed three mechanisms of the antimicrobial activities of AgNP: (i) Could attach to cell membrane and disrupt the permeability and respiration functions of the cell and thus kill the cells; (ii) Reactive oxygen species (ROS) can be generated on the surface of nanoparticles and cause damage of DNA by exerting oxidative stress; (iii) Silver ions released from AgNP's can also cause disruption of ATP production and DNA replication.

Aim of this work focused on synthesis of silver nanoparticles using three wavelength and estimate the size, shape and particles distribution. Moreover examine the AgNP's experimentally prepared on some types of bacteria.

1. Experimental Procedure

The AgNP's were prepared via PLAL (Pulse Laser Ablation in Liquid). The experimental setup for laser ablation of solid metal target immersed in water or aqueous solution is shown in Fig (1). The setup includes: Nd-YAG laser with three wavelengths 1064nm, 532 nm and 355 nm wavelengths were used for laser ablation process. The Nd-YAG laser beam was focused by a lens of 10 cm focal length onto a metallic target to achieve energy density 1o J / cm^2. The laser is fixed by a holder on the target at the bottom of container as shown in figure below. The silver plate was fixed in a container filled with 5 ml deionized distilled water. The liquid depth was 6 mm above the silver surface. After the laser ablation process is advanced, smoke-like colloids above the metal plate was observed

Figure 1: The experimental setup of the laser ablation process.

2. Result and Discussion

The laser wavelength is one of the most affective parameter on the formation of the silver nanoparticles. Figure (2) shows the absorption spectra of silver nanoparticles synthesized by pulse laser ablation technique. Laser ablation was carried out by 500 pulses and the solution gradually turned to be colored with the increase of the number of pulses. An interesting phenomenon is observed that is the color of the suspension is changed faster for the laser wavelength of 1064 nm than 532 nm at the same laser energy

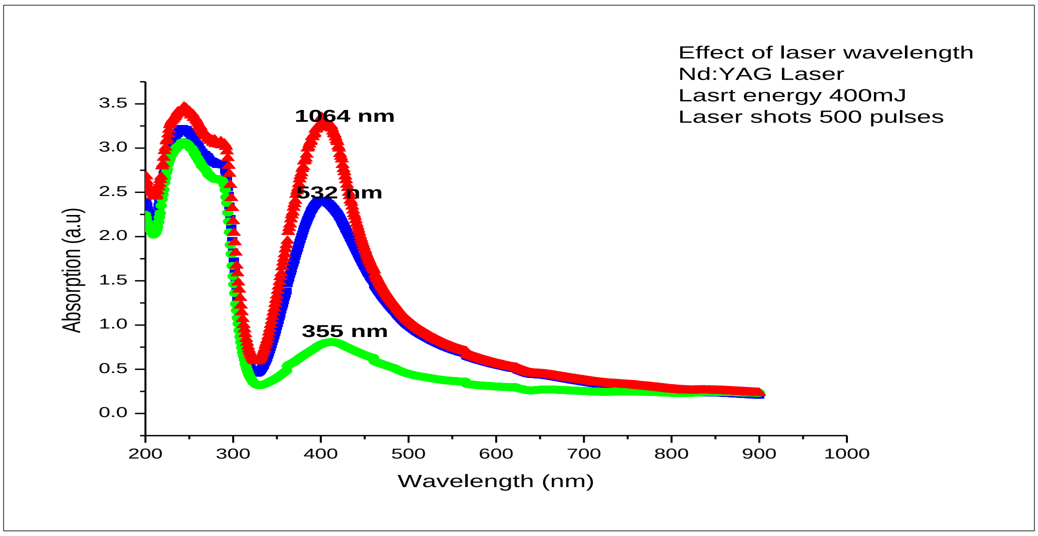

Figure 2: The surface plasmon spectra of Ag NP's prepared by laser ablation of metal plates immersed in DDDW. The laser energy 400 mJ and number of pulses 500 at different wavelengths 1064 nm, 532nm and 355 nm.

Table 1: the absorption peak and FWHM of silver nanoparticles prepared by PLAL at different laser wavelengths.

Laser Wavelength (nm)	Absorption Peak	Full Width at Half Maximum(FWHM)
1064	3.28	95 nm
532	2.3	110 nm
355	0.79	140 nm

Table (1) shows that the absorption peak of sample synthesized by 1064nm wavelength is higher than that synthesized by 532nm and 355nm, which is related to higher concentration of nanoparticles synthesized by 1064nm than the samples synthesized by 532nm and 355nm. Therefore, one could conclude that the particle densities of samples prepared at 1064nm laser wavelength are better than other. So that the laser wavelength of 1064nm is more efficient in fabricating nanoparticles in distilled water, also the laser wavelength of 532nm is more efficient than 355nm to produce silver nanoparticles .

Mie-Gans fitting model [12] has been used to calculate the size of silver nanopartices. This model was used for the evaluation of the average size of silver nanoparticles based on the fitting the experimental data of UV-Vis spectra by Mie-Gans model. The model gives good results using a calibration of the dumping frequency of the surface Plasmon resonance and accounting for the presence of nonspherical AgNP's in solution by the Gans model for spheroids. Moreover, the fitting model provides other information not available from TEM such as the concentration of AgNP's in the sample and the fraction of nonspherical nanoparticles since it is involve the volume of nonsphericeal particles where there are two axes. Thereby, the model is particularly useful for measuring aggregation processes. This can be estimate using Mie-Gans fitting model.

The dielectric constant can be corrected for size using size-dependent relaxation frequency $\Gamma(R)$ by introduction of the following relationship [2]:

$$\Gamma(R) = \Gamma_\infty + A\frac{\upsilon_F}{R} \quad ….. (1)$$

Where Γ (R =0.25∗10^{14} s^{-1} is the bulk metal value, υF=1.39*108 cm/sec is the Fermi speed, A is an empirical parameter and R is the radius of Ag NP's.

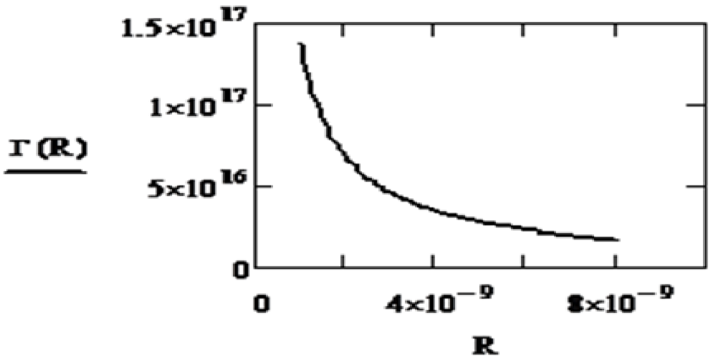

Figure 3: Relaxation frequency of prepared silver nanoparticles produced by PLAL.

On the assumption that only the free electron behavior is affected by the size of nanoparticles, $\varepsilon(\omega,R)$ can be expressed in the following way [12]:-

$$\varepsilon(\omega,R) = \varepsilon_\infty(\omega) + \left[\omega_P^{\,2}\left(\frac{1}{\omega^2+\Gamma_\infty^{\,2}} - \frac{1}{\omega^2+\Gamma(R)^2}\right)\right] + i\left[\frac{\omega_P^{\,2}}{\omega}\left(\frac{\Gamma(R)}{\omega^2+\Gamma(R)^2} - \frac{\Gamma_\infty}{\omega^2+\Gamma_\infty^{\,2}}\right)\right] \quad ….. (2)$$

Where $\varepsilon_\infty(\omega)$ is the dielectric function of bulk silver

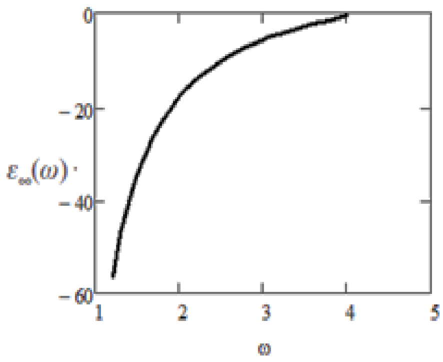

Figure 4: Dielectric function of the Bulk silver**.**

And ω_P is the plasma frequency:

$$\omega_P = \sqrt{\frac{Ne^2}{\varepsilon_0 m_e}} \quad \text{….. (3)}$$

Where N=5.86*10^{28} (atom/$m^{3)}$ is the conduction electron density *e* is the electron charge, *me* is the electron effective mass and ε_0 is the vacuum dielectric permittivity.

Since aggregation can involve two or more particles and most isolated particles with nonspherical shape basically are low aspect ratio spheroids, one can account for the contribution of small aggregates and non-spherical particles to the overall extinction spectra using the Gans model that is the Mie theory Extension to particles with spheroidal shape. Therefore, the fitting of experimental UV-Vis spectra was completed including the contribution of nonspherical particles by means of the Gans model. The σ_{ext} averaged over all possible orientation in space is [2].

$$\sigma_{ext} = 9\frac{\omega}{c}\varepsilon_m^{3/2} V \frac{\varepsilon_2(\omega,R)}{[\varepsilon_1(\omega,R)+2\varepsilon_m]^2+\varepsilon_2(\omega,R)^2} \quad \text{…. (4)}$$

Where *v* is the frequency of incoming photons, εm is the matrix real dielectric constant, $V = (4\pi/3)\, ab^2$ is the volume, and *e* is the eccentricity of the spheroid.

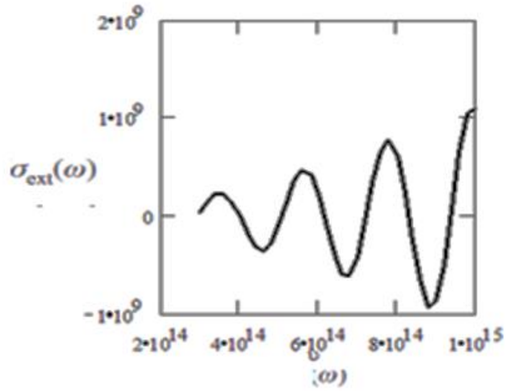

Figure 5: The extinction cross section of AgNP'S**.**

Figure (5) reveals that the extinction cross section grows up as a function of the incident light frequency due to the effect of quantum confinement compared with that of bulk.
The size distribution N(R) is

$$N(R) = \frac{e^{\frac{[-(R-R0)]}{2\theta^2}}}{\sqrt{2\theta\pi}} \quad \text{….. (5)}$$

Where R is size of AgNPs , R_o=4*10^{-9} and θ is the standard deviation. Despite the size and shape distribution contained in each sample, the Mie-Gans (MG) models with the above-discussed assumptions allowed the optimal fitting of experimental UV-VIS spectra using only three parameters: (i) the average radius of the nanoparticles (*R*), (ii) the standard deviation (θ) of Gaussian distribution, and (iii) the fraction of spherical to spheroidal silver nanoparticles.

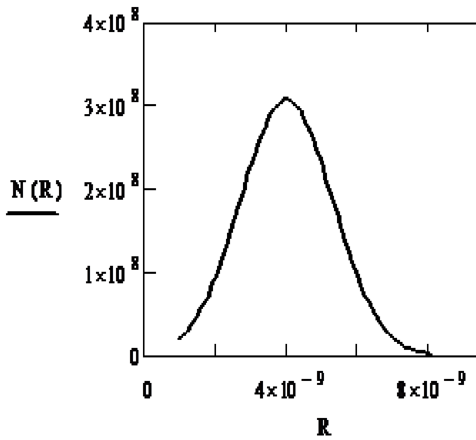

Figure 6: Size distribution of AgNP's prepared by PLAL

Scaling the values of the calculated spectra to the experimental ones allows determination of the concentration of the nanoparticles according to [13]:

$$\mathrm{Abs}(\omega, R) = A * \log \sigma(\omega) * N(R) \quad \text{…..} \quad (6)$$

Where A = Avogadro number = 6.022 x 10^{23}, N(R) the size distribution and σ(ω) is extinction cross section

Figure (7) shows the experimental UV-Vis data for three wavelengths fitted with theoretical Mie-Gans model .While table (2) gives the estimated size of silver nanoparticle obtained from fitting. These values indicate that the smaller silver nanoparticle can be obtained from shorter laser wavelength 355 nm compared with these of 1064 and 532 nm. One can also observe narrow size distribution which correlates to the standard deviation. Moreover, it is also found that the absorbance intensity for larger wavelength (1064 nm) is higher than others and that is due to the silver nanoparticles concentration where higher number of particle leads to increase the absorption of the incident light.

(A)

(B)

(C)

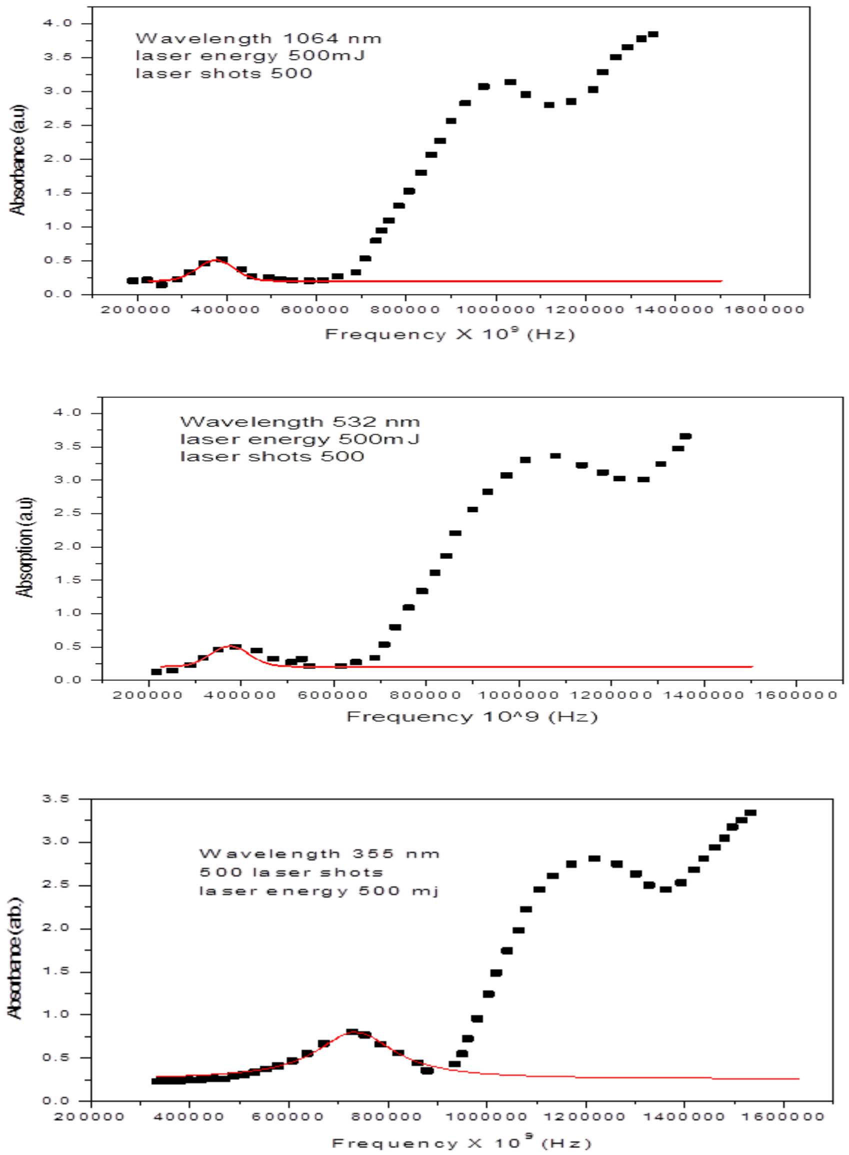

Figure 7: Mie-Gans fitting model of Ag NP's prepared by PLAL by using Nd:YAG laser at (A) 1064 nm (B) 532 nm (C) 355 nm. The dash line is the experimental data while the solid line is the theoretical model.

Table 2: The fitting parameters of the theoretical model and their experimental data.

Wavelength (nm)	L_0(size in nm)	Θ(standard deviation in Å)	P.P (Peak Position in Hz)
1064	25	2.4	$3.6*10^{14}$
532	17	1.5	$4.2*10^{14}$
355	12	1.2	$7.5*10^{14}$

Antimicrobial ability is a well-known property of AgNP's . AgNP's can inactivate abroad spectrum of microorganisms. The antibacterial activity of silver nanoparticles has been examined via the inhibition zone (IZ) and minimum inhibition concentration (MIC).

Figure 8: The inhibition zone of silver nanoparticles of Streptococcus**.**

Table 3: The inhibition zone (IZ) for some types of bacteria for AgNPs of (30µg/ml).

Bacteria Type	**DIZ** (mm)
Staphylococcus	10
Streptococcus	5

Table (3) shows the activity of silver nanoparticle with concentration (30µg/ml) for some types of bacteria. (30µg/ml). Staphylococcus and Streptococcus were sensitive to AgNPs

Table 4: Minimum Inhibitory Concentration (MIC) of some types of bacteria for AgNPs.

Bacteria Type	**MIC** (µg/ml)
Staphylococcus	3.75
Streptococcus	0.85

As given in table (4) Streptococcus was more sensitive to AgNPs than Staphylococcus. The interactions of silver nanoparticles with biosystems are just beginning to be understood, and these particles are increasingly being used as microbicidal agents. It may be speculated that silver nanoparticles with the same surface areas but with different shapes may also have different effective surface areas in terms of active facets. This explains how the surface areas of different nanoparticles influence their killing activity or to relate the bacterial killing capacity of silver nanoparticles with their effective surface areas, In addition the size of AgNPs which plays important effective for killing bacteria.

3. Conclusions

Laser ablation of pure silver sheet in liquid could be employed to produce silver nanoparticles when proper laser characteristics are used. Shorter laser wavelength produced smaller silver nanoparticles with lower concentration. While the fundamental laser wavelength (longer wavelength) can be used to prepare larger sizes with higher concentration, Moreover low laser energy leads to produce larger sizes of elliptical shape. These nanoparticle have antibacterial activity against some types of bacteria like staphylococcus and streptococcus. It is found that Nd:YAG laser is sufficient to produce silver nanoparticles. Best laser parameters for synthesis smaller nanoparticles of 12 nm were laser wavelength of 355 nm, nergy 400mJ, energy density of 10 j / cm^2 and number of pulses of 500 pulse. Moreover, it is also found that 6 mm liquid depth above the silver sheet gives better ablation efficiency.

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دقائق الفضة النانوية المضادة للبكتريا المحضرة بليزرNd: YAG

زينب عصام عبد الوهاب
قسم هندسة الليزر والبصريات/ كلية الهندسة / جامعة النهرين

بسام غالب رشيد
قسم هندسة الليزر والبصريات/ كلية الهندسة / جامعة النهرين

الخلاصة :

يعرض هذا العمل طريقة لتوليد جسيمات الفضه النانوية باستخدام ليزرالنديميوم-الياك النبضي ذو أطوال موجية مختلفة (1060, 532, 355 nm) طاقة متغيرة من 100 الى 1000 ملي جول ومعدل تكرارية 1 هرتز بطريقة التبخر الانفجاري. تمت دراسة تاثير هذه الاطوال الموجية على حجم وشكل جسيمات الفضة المحضرة, كما تم استخدام طريقه جديدة لمعرفه حجم هذه الدقائق اعتمادا على Mie-Gans Model بالأستعانة ببعض نتائج من الجانب العملي التي تم قياسها باستخدام جهاز UV-Vis Absorption ومن خلالها تم الاستنتاج ان الجسميات الاصغر حجما حضرت باستخدام ليزر الياك النبضي ذو الطول الموجي الاص , 355 nm كما تم الاستنتاج ايضا ان جسيمات الفضه بحجم اصغر من nm12 لها القابليه على قتل نوعين من البكتريا التي تعيش على جسم الانسان وهي بكتريا (Staphylococcus Streptococcus).