Silver Nanoparticles Synthesized By Nd: YAG Laser Ablation Technique: Characterization and Antibacterial Activity

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Keywords

Laser ablation, AgNPs, Nanotechnology, Plasmon resonance, Antibacterial activity

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RESEARCH PAPER

Silver Nanoparticles Synthesized by Nd: YAG Laser Ablation Technique: Characterization and Antibacterial Activity

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Abstract

The pulsed laser ablation in liquid (PLAL) technique can produce high purity nanoparticles, it is a top-down physical method based on the principle of dividing metal ion bulk precursors into metal atoms, this method was used in this work to synthesize silver nanoparticles (AgPNs) by using Nd: YAG laser with two wavelengths (355 nm) and (532 nm) at energies (500 mJ) and (600 mJ) respectively, with the number of pulses (500, 600, 700, 800, and 900 pulses) for each wavelength. The properties of the prepared nanoparticles were investigated by UV–Vis, XRD, SEM with EDX, AFM, and FTIR analysis and then tested for antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus mutans*) and two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) isolated from the oral cavity. The findings demonstrated that AgNPs generated via the PLAL method have antibacterial activity and could be employed to kill dangerous and pathogenic bacteria.

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1. Introduction

Nanotechnology is a relatively new technology that has led to significant advances in a variety of fields [1,2]. It is the nanometer-scale modification of atomic materials to achieve the desired shape and properties for the application [3]. Nanoparticles are extremely useful due to their unique properties and applications, particularly in biotechnology, medical imaging, and catalysts [1]. A metallic particle must be between (1–100 nm) in size to be considered “nano”. Nanoparticles (NPs) have a large specific surface area but a small metal mass. Metal NPs are typically created by reducing metal salts in a solution or by forming metal atom aggregates by heating or vaporizing a metal in a vacuum or inert gas [4]. They've recently gained a lot of attention because of their non-toxicity, insensitivity to air and light, size-related electronic properties, good conductivity,

chemical stability, magnetic properties, and potential biological applications [5]. Because of their magnetic, electronic, and optical properties, colloidal metal NPs are of great interest to researchers from a variety of disciplines, including materials science, physics, engineering, and chemistry [6]. Surface plasmon resonance (SPR) is one of the distinct properties of metallic NPs [4]. When exposed to specific light energies, free electrons collectively oscillate on the metal surface, resulting in wavelength-dependent absorption and scattering; metal NPs can highly absorb light in the visible spectrum by producing collective vibration of conduction bands in powerful resonance with fixed light frequencies [7]. Excitation of the plasmon raises the electromagnetic field in the surrounding environment and causes measurable changes in NPs that respond in the optical field [8]. Metal-based nanoparticles with anti-oxidant and

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antimicrobial properties have been incorporated into biomaterials used in human health care [9]. Among the various metallic NPs studied, the noble metal silver and its oxides have a prominent position in nanomaterial research due to their unique properties that can be applied in a wide range of fields and applications such as sensing, optoelectronics [10,11], catalysis, and drug delivery [2,12]. Silver nanoparticles (AgNPs) have piqued the interest of many researchers and scientists due to their unique properties in physical, chemical, and biological fields when compared to their macroscale counterparts [2,13]. Bacteria, as we all know, are capable of causing infectious diseases in both animals and humans due to their diverse species, wide distribution, and variable medical resistance. As a result, it is critical to detect and inhibit pathogenic bacteria [14]. AgNPs have the potential to be used in biomedical applications due to their high surface plasmon resonance (SPR) and anti-microbial properties, as well as being less toxic than the bulk form. They have demonstrated a wide range of applications in everyday consumer life, such as nanosilver coated medical device surfaces to reduce hospital-related infections, footwear, bandages, and a slew of anti-microbial household items. They have an unrivaled ability to fight infectious diseases and to inhibit the growth of bacteria, mold, and germs [15]. They are a common inorganic antibacterial agent that has broad antibacterial activity against bacteria and fungi [14]. While the threat of biological and bacterial attacks has increased dramatically in the last decade or so, particularly in human consumption sectors such as food, food packaging, and water, scientists are motivated to develop new inorganic antibacterial nanoparticle substances that are free of side effects and easy to use [16]. Researchers recently discovered how to use a laser to activate the bactericidal effect of AgNPs, which represents a promising advancement in the fight against antibacterial resistance. It has the potential to be engineered as a drug delivery tool due to its strong interaction with light. Because of their potential applications in diagnostics and therapeutics, AgNPs have received a lot of attention [1,7]. Antimicrobial activity is mediated by binding to the negatively charged bacterial cell wall, resulting in destabilization of the cell envelope and altered permeability [17]. Previous studies have shown that the antibacterial activity of AgNPs is proportional to their specific surface area and size, with smaller particles exhibiting greater antibacterial activity. This difference in efficacy due to size is due to their high surface area to volume ratio, which allows for efficient bonding with the bacterial surface [7]. Several

methods for controlled synthesis of AgNPs have been reported to date, including evaporation condensation, chemical reduction, sol–gel, laser ablation, electron irradiation, gamma irradiation, successive ionic layer deposition (SILD), microwave processing, electro-chemical, photochemical, bromide-mediated polyol method, green synthesis, and biosynthesis [18]. The laser ablation method, which can generate high purity nanoparticles without using a toxic chemical mixture, employs a simple and low-cost synthesis process [13], which is a top-down physical method based on the principle of dividing metal ion bulk precursors into metal atoms [5,19]. Laser ablation is a promising method for producing metal colloids and NPs. It offers the critical benefit for biological applications of producing NPs with a surface free of reactant residue ions, as well as very low processing setup costs [20]. The power of the laser beam, as well as the temperature of the beam, influence the response of materials to light. Continuous laser irradiation of material causes a chain of reactions such as heating, melting, boiling, and plasma formation. The response of materials to a laser beam in terms of thermal effects such as melting, boiling, vaporization, and nucleation phase-explosion, as well as some mechanical effects such as deformation and stress. Figure 1 depicts the process of absorption within matter as a function of increasing power.

The position of the plasmon band in an Ag colloidal solution depends on the type of liquid

Fig. 1. Absorption process within matter as a function of increasing power.

Fig. 2. Schematic diagram of PLAL for NPs synthesized.

environment used during laser ablation. Under the same laser beam parameters, the plasmon peak of AgNPs is affected not only by the type of liquid but also by the chemical concentration of the liquid, the plasmon band can also be changed by changing the wavelength of ablation laser light [21]. SPR is caused by AgNPs' lower intrinsic loss of plasmonic energy at visible frequencies [22]. In this study, we prepare AgNPs using the laser ablation method and investigate their physical properties and antibacterial activity.

2. Materials and methods

AgNPs were prepared using the pulsed laser ablation technique in liquid (PLAL) and a pure silver plate with dimensions (1, 1, & 2 mm) and placed in (5 ml) of (99.99%) purity ethanol in a beaker and at a distance (5 cm) from the laser source, where Q-switched Nd: YAG laser was applied, rate of repetition (6 Hz), wavelengths (355, 532 nm) and energies (500, 600 mJ) respectively, as it's schematic shown in fig. 2, and the number of pulses (500, 600, 700, 800, and 900 pulses) for each wavelength. After that, the samples were structurally and optically

analyzed. The antibacterial activity of the solution was then tested on two types of Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus mutans*) and two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) isolated from the oral cavity using the drilling method by placing (10 μ L) the solution in the hole. The diffusion method in agar was used to assess the effect of AgNPs on the bacteria types used. This method entails spreading bacteria on agar plates and then waiting a few minutes. Then, using the heads of sterile micropipette tips, holes were punched into the agar and a mixing solution was poured into the holes. After incubation, the inhibitory potency was determined by measuring inhibition zone diameters from various directions multiple times with a ruler.

The mentioned bacteria were used after they were obtained ready (isolated, classified, and grown), where these steps can be summarized as collecting them from infected people, isolating and classifying them in standard ways to know their types, and growing them on nutritious culture media, taking sterile methods and maintenance conditions into account in all steps, then freezing them until use. The prepared particles were then tested for their ability to inhibit these bacteria by planting them in an agar medium in Petri dishes, using the drilling method, placing the solution containing the particles in the holes, and then incubating the dishes in the basin for (24) hours.

3. Results & discussion

3.1. UV–Vis absorption analysis

It is one of the most widely used techniques for the identifying of various substances such as transition metal ions, organic compounds, and biological molecules. The first evidence of a synthesis

Fig. 3. UV–Vis absorption spectra at 355 nm.

Fig. 4. UV–Vis absorption spectra at 532 nm.

Fig. 5. XRD patterns of AgNPs.

reaction; the color of silver colloidal solutions changes from colorless to yellow color and then darker as the number of pulses increases as a result of the silver ablation process. UV–Vis analysis was used to perform the initial characterization of AgNPs and their oxides. It was noticed as in [fig. 3](#) when using the (355 nm, 500 mJ) of the Nd: YAG laser that the maximum absorption was observed at (406, 406, 410, 414, and 406 nm) for the number of pulses (500, 600, 700, 800, and 900 pulses) respectively, whereas when using the (532 nm, 600 mJ) the maximum absorption was observed at (405, 403, 403, 399, and 398 nm) for the number of pulses (500, 600, 700, 800, and 900 pulses) on straight as in [fig. 4](#), where the maximum absorption represent the SPR of AgNPs.

These figures show that the shorter wavelength of the laser has produced a higher absorbance value, indicating the high concentration of synthesized NPs in solutions, which is consistent with previous

studies such as in [\[6\]](#), Furthermore, the shorter wavelength led to more uniformity in the width of the absorption peaks by increasing the number of pulses, and the higher SPR peak with an increase in the pulses is attributed to a more efficient redistribution of nanoparticles due to the longer period and the accumulation of an effective electric bilayer around the NPs, as in [\[23\]](#).

3.2. XRD analysis

[Figure 5](#) represents the XRD patterns obtained of the synthesized AgNPs; one corresponds to the sample prepared at the parameters (355 nm–500 mJ – 900 Pulse) and the other two correspond to the samples prepared at (532 nm–600 mJ – (800 & 900) Pulse), and we notice in all the resulting patterns the appearance of a number of intensities and peaks that belong to AgNPs with a cubic crystal structure (the fitted XRD pattern was compared to the standard

Fig. 6. SEM images of AgNPs at 355 nm-500 mJ-900 P zoom (A) 10 μ m (B) 200 nm.

Fig. 7. SEM images of AgNPs at 532 nm-600 mJ-800 P zoom (A) 10 μm (B) 200 nm.

Fig. 8. SEM images of AgNPs at 532 nm-600 mJ-900 P zoom (A) 10 μm (B) 200 nm.

pattern (COD Card Number [96-901-3054])) and another section refers to AgNPs with a hexagonal crystal structure (COD Card Number [96-150-9146]). We also notice the appearance of a several peaks representing Ag oxides NPs, including (Ag_3O_4 – COD Card Number [96-151-0026]), (Ag_2O_3 – COD Card Number [96-150-9693]), and (Ag_2O_2 – COD Card Number [96-901-3054]). The apparent crystal

systems, angles, and Miller's index are depicted in this figure.

The effect of laser-induced oxidation is an unavoidable consequence of the laser effect on metals in the presence of air. This effect occurs as a result of the high temperatures experienced by the metal directly exposed to the laser; heat dissipation to the surroundings quickly turns in, allowing heat to

Fig. 9. EDX analysis at 355 nm-900P.

Fig. 10. EDX analysis at 532 nm-800P.

Fig. 11. EDX analysis at 532 nm-900P.

propagate to the vicinity, oxidizing a metal area beyond the directly exposed and ablated channel. The results show that the chemical composition changes on metal surfaces as a function of net delivered fluency. The general trend can be seen as a rapid increase in oxygen content at lower laser fluencies, followed by an asymptotic increase at higher fluencies, as in [24]. Some studies stated that the formation of silver oxides NPs was caused by the interaction of Ag atoms with oxygen atoms or radicals during their decomposition in liquid. They also stated that the type of oxide formed is affected by on the temperature and oxygen partial pressure, as in [3].

3.3. SEM analysis

SEM images of the synthesized AgNPs were made to determine the morphology of the NPs. Figures 6–8 shows that the synthesized particles ablated in ethanol are between nanospheres and nanofibers, so the particle size variation is widely distributed. Also from the particle size distribution, it indicates that the NPs sizes generally vary between (36 and 50) nm.

The elemental compositions of AgNPs are shown in figs. 9–11 based on EDX analysis, with a lengthy peak showing the presence of silver in the prepared samples.

3.4. AFM analysis

The samples created at higher pulses are substantially more uniform and dense, as seen in figs. 12–14. The surface topography analysis revealed that increasing the number of pulses of the laser causes an increase in grain size, as the grain size of the prepared AgNPs at (532 nm–600 mJ – 800 P) is (64.87 nm), while increasing the number of pulses at (532 nm–600 mJ – 900 P) causes an increase in grain size to (79.07 nm), and at (355 nm–500 mJ – 900 P) the grain size is equivalent to (72.32 nm).

Fig. 12. AFM analysis at 355 nm-500 mJ-900 P (A) 3D image, (B) Granularity cumulative distribution.

Fig. 13. AFM analysis at 532 nm-600 mJ-800 P (A) 3D image, (B) Granularity cumulative distribution.

Fig. 14. AFM analysis at 532 nm-600 mJ-900 P (A) 3D image, (B) Granularity cumulative distribution.

3.5. FTIR analysis

FTIR absorption spectra can reveal the chemical change of functional groups in a composite. Figures 15 and 16 represent FTIR analysis of AgNPs obtained at (355 nm–500 mJ) and (532 nm–600 mJ) respectively, where this analysis is utilizable for characterizing the surface chemistry of nanoparticles.

Spectra FTIR in both figures showed absorption peaks corresponding to the group of hydroxyl (O–H), alkane (C–H), and aliphatic amines (C–O) stretching vibrations, in addition to appearing carboxylic acid (C=O) stretching group in the synthesis of NPs process in fig. 16, also, in both figures the band which below (600 cm⁻¹) is in charge of the AgNPs forming and its oxides. It can be seen from the two figures that the number of pulses affects the value of the absorption peaks, as well as the appearance and disappearance of some of them, as the number of pulses affects the concentration of solutions.

3.6. Energy gap (Eg)

As we noticed from the analysis of XRD, beside AgNPs, silver oxides NPs were formed in the samples prepared in this work, and to calculate the energy gap (Eg) for these oxides we plot absorption as a function of wavelength as figs. 17 and 18 and use the following equation [25]:

$$E_g (\text{eV}) = 1240 / \lambda_g (\text{nm}) \quad (1)$$

where λ_g is the absorption edge calculated as the intersection of the absorption curve's tangent and the abscissa coordinate [26]. In general, we find that Eg increased by increasing pulses, and this uniform increase appears clearly in the samples prepared at the wavelength (355 nm; (1.68–3.1 eV)) more than when prepared at (532 nm; (1.63–3.88 eV)), and this result is similar to the behavior that we observed in the absorption spectrum.

Fig. 15. FTIR analysis at 355 nm-500 mJ.

Fig. 16. FTIR analysis at 532 nm-600 mJ.

3.7. Antibacterial activity

The antimicrobial activity of silver nanoparticles against types of bacteria Gram-positive and negative bacteria was investigated by agar plate. The results of our study showed that the AgNPs prepared did not have significant inhibiting activity on some of the four mentioned bacteria (*S. aureus*, *S. mutans*, *E. coli*, and *Pseudomonas*), this may be due to increased bacterial resistance proven by antibiotic sensitivity test (not found in this research). Also, the lower bacterial sensitivity to AgNPs is primarily due to the thickness of the peptidoglycan layer, which may prevent the nanoparticles from passing through the bacterial cell wall [27]. However, we can observe the inhibition regions of AgNPs prepared

by laser ablation for both wavelengths and all mentioned pulse setups as shown in [figs. 19 and 20](#).

We can see that AgNPs affected bacteria in varied quantities, and that particles prepared at (355 nm) were more effective because they affected all species, whereas bacteria (*S. mutans*) were not affected by particles prepared at (532 nm) because they did not show inhibition zones.

Because the bacterial cell membrane contains so many sulfur-containing proteins, silver nanoparticles can react with sulfur-containing amino acids both inside and outside the cell membrane, reducing the viability of the bacteria. It's also been suggested that Ag ions generated by AgNPs can bind with phosphorus moieties in DNA, causing DNA replication to be inactivated, or react with

Fig. 17. Energy gap of Ag oxides NPs at 355 nm-500 mJ.

Fig. 18. Energy gap of Ag oxides NPs at 532 nm-600 mJ.

Fig. 19. Antibacterial activity at 355 nm-500 mJ] (a) *S. aureus*, (b) *S. mutans*, (c) *E. coli*, (d) *Pseudomonas*.

sulfur-containing proteins, causing enzyme functions to be inhibited, resulting in cell viability and, eventually, cell death [15]. The diameters of the bacteria lining areas were calculated as in the two figs. 21 and 22.

In many situations, it's still unclear whether a single mechanism or a combination of processes was responsible for the death. One of them could be due to the interaction of AgNPs with the bacterial cell wall as well as the simultaneous penetration of Ag + ions inside the bacterial cells [27]. In general, the antibacterial activity of NPs is primarily dependent on the electrostatic attraction between positively charged NPs and negatively charged bacterial cell surfaces, and this attraction is critical for NPs' activity as a bactericidal agent [28]. Cell death is caused by mitochondrial damage and the upregulated expression of several apoptotic proteins, and the current result, likes previous studies, show that NPs led to mitochondrial and DNA damage, resulting in cell death. This is due to the cellular toxicity of NPs, and added to the growing

body of evidence regarding the potentially damaging effects of nanoparticles, indicating that caution should be exercised in their widespread usage [29].

4. Conclusions

This work investigated the ability to synthesize AgNPs using the physical method that is PLAL, and one of its biomedical applications is its antibacterial activity. The results demonstrated the possibility of obtaining AgNPs using two wavelengths of Nd: YAG laser (355 nm and 532 nm) in the process of ablation and with different numbers of pulses by changing the color of the solution and analyzing the absorption spectrum as a preliminary proof of the nanosynthesis of the material, knowing that this laser treatment led to the formation of silver oxides NPs as well, and the energy gaps were calculated for them, and it was found that they increase with the increase in the number of pulses in general. In addition, FTIR analysis was performed, which

Fig. 20. Antibacterial activity at 532 nm-600 mJ (a) *S. aureus*, (b) *S. mutans*, (c) *E. coli*, (d) *Pseudomonas*.

revealed that the effective groups present in the prepared samples are (O–H, C–H, C–O, and C=O). The structural analysis included XRD, SEM with EDX, and AFM for selected samples, which

were prepared with the highest number of pulses and gave the highest antibacterial activity for both wavelengths used, as XRD, FTIR, and EDX assays proved the formation of Ag and its oxides NPs, the

Fig. 21. Histogram of antibacterial activity at 355 nm-500mJ data table show that the inhibition zoons are calculated in mm.

Fig. 22. Histogram of antibacterial activity at 532 nm-600mJ data table show that the inhibition zoons are calculated in mm.

sizes of which were detected by SEM and AFM. Under the conditions of this work, it can be stated that the prepared samples at the wavelength (355 nm) are the most effective. Laser-ablated AgNPs demonstrated antimicrobial activity as well as cytotoxicity in vitro. As a result, more research is required to fully characterize these particles' toxicity mechanisms. It is critical that biological applications utilizing AgNPs be given special consideration for anticancer potentials while also warranting toxicity against normal cells. The results demonstrated that PLAL is an easy and fast method for metal nanosynthesis and it can be a good alternative to other methods, and the NPs prepared with it have biomedical efficacy, however, more studies may be required to change the laser parameters and investigate other applications.

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