Preparation of silver nanoparticles and study the optical and antibacterial properties

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Abstract:

In this work preparation and characterization of Silver nanoparticles which were synthesized by pulsed (Q-switched, 1064 and 532nm doubled frequency-Nd: YAG) laser ablation of silver metal immersed in double distilled and deionized water DDDW, without any chemical additives and studied the optical,and antibacterial properties by using different techniques. The influence of laser parameters such as number of pulses as well as laser fluence on the properties of nanoparticles elucidated. It has been observed that laser energy and number of laser pulses have a control over the size of the nanoparticles. Increasing laser energy and number of pulses shows a clear blue shift in the absorption peak of fabricated nanoparticles indicating that the average size of the particles decreases with increasing laser energy and number of pulses. Ablation for longer period reduces the average size of nanoparticles. The Antibacterial of synthesized silver NPs against four pathogens: streptococcus, staphylococcus, proteus and Enterobacter were studied .

Key woeds:Ag nanoparticles , optical properties , antibacterial agent

تحضير دقائق الفضة النانوية ودراسة خواصها البصرية و خواصها كمضادات للبكتيريا

الخلاصة:

في هذا العمل تم تحضير دقائق الفضة النانوية ودراسة خواصها البصرية والمضادات البكترية حيث تم تحصيرها بأستخدام ليزر نبضي بطول موجي 1064nm و 532nm بمضاعفة التردد لليزر الأندياك . التذرية بالليزر تمت بغمر معدن الفضة بماء مقطر لا ايوني وبدون اية اضافات كيمياوية وكذلك تمت دراسة خواصها البصرية والمضادة للبكتيريا بأستخدام مختلف التقنيات و تأثير عوامل الليزر مثل عدد نبضات الليزر وطاقة الليزر على تلك الخصائص .

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تمت ملاحظة بأن طاقة الليزر وعدد النبضات يتحكمان بحجم الدقائق النانوية وان زيادة طاقة الليزر وعدد النبضات يؤدي الى ظهور الأزاحة الزرقاء في قمة طيف الأمتصاص وهذا يعطي مؤشر بأن معدل حجم الدقائق النانوية يقل مع زيادة طاقة الليزر وعدد نبضاته وان التذرية لفترة طويلة تقلل من حجم الدقائق . التأثير المضاد البكتيري لدقائق الفضة درس ضد اربعة انواع من البكتيريا وهي ستربتوكوكس ، ستافيلوكوكس ، بروتس و انتيروباكتر.

INTRODUCTION:

Nanoparticles have very special properties compared to bulk materials or even micron size particles. These special properties encompass thermodynamic, chemical, and optical behavior [1]. Metal nanoparticles are built by a minimum of three metal atoms, of which each one is chemically bound to at least two other ones [2]. The Nanotechnology collectively describes technology and science involving Nano scale particles that increases the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems. It can be employed as an efficient tool to explore the finest processes in biological processes and biomedical sciences. Besides this, NPs play an indispensable role in drug deliver, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering [3]. Materials at the nanometer dimension are not new. They are common in nature, for example, life depends on many nanoscales objects, including proteins, enzymes and DNA, and nanosized particles occur naturally in the atmosphere [4]. Nanotechnology is currently employed as a tool to explore the darkest a venues of medical sciences in several ways like imaging, sensing, targeted drug delivery and gene delivery systems and artificial implants. Hence, nanosized organic and inorganic particles are finding increasing attention in medical applications due to their amenability to biological functionalization [5]. Metal NPs have strong light absorption that can be used for localized photothermal therapy. NPs can be conjugated with antibodies to target cancer tumor sites for localized photothermal treatment of cancer and drug delivery [6].The nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics [7]. It is known that metal nanoparticles have novel magnetic, electronic and optical properties, which vary on the basis of their size, shape and composition. It has been known for long time that silver ions are highly toxic to a wide range of bacteria [8]. The synthesis of metal NPs has been published, among the synthetic procedures studied clean, nontoxic, and eco-friendly methods have received considerable attention from biologists and chemists. Different prokaryotic microbial and eukaryotic cells, i.e., fungi, bacteria, algae, and plants, have been tested for this purpose and many synthesis protocols have been developed for the fabrication of metal NPs [9]. Nanoparticles of noble metals have recently become the focus of research because of their unique properties, which are different from those of bulk materials [10]. Silver nanoparticles (AgNPs) are subjected to new engineering technologies with extraordinarily novel resultant morphologies and characteristics. Silver is engineered into ultra-small particles (normally between 1 to 100 nm) [11]. Nanoparticles (AgNPs) have sparked intense excitement in nanotechnology and biotechnology due to their high catalytic activity and excellent antimicrobial activity [12]. AgNPs in particular are considered as non-toxic, show inhibitory and bactericidal effects and have found numerous applications as antibacterial agents in food processing industries, clothing, sunscreens and cosmetics

[13]. Sharp surface plasmon absorption of silver nanoparticles makes them very attractive for bioscience and biotechnology [14]. Nanoparticles of metallic silver, as a rule, have a shape close to spherical, and are capable of forming large aggregates therefor obtaining stable dispersions of Nano silver in water or organic medium, use is often made of stabilizers-surfactants or polymers (e.g. polyvinyl pyrrolidone).The field of application of silver nanoparticles is quite expansive: coatings for absorption of solar energy, catalysts of chemical reactions, antimicrobial and disinfectant means, component of food wrappings [3]. The low toxicity of elemental NPs makes them excellent materials for application in medicine and veterinary sciences [9]. The most important specific properties of silver nanoparticles that made as an antibacterial we can summarized in the following points:

- Unique properties, which are different from those of bulk materials.
- Silver nanoparticles have many applications, among them antibacterial applications and Nano composite fabrications are some of the more important applications [10].
- Though silver nanoparticles are cytotoxic but they have tremendous applications in the field of high sensitivity bimolecular detection and diagnostic, antimicrobials and therapeutics, catalysis and micelectronics.
- Biologically synthesized silver nanoparticles have many application like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials [15].
- Silver nanoparticles have many applications, among them antibacterial [16].

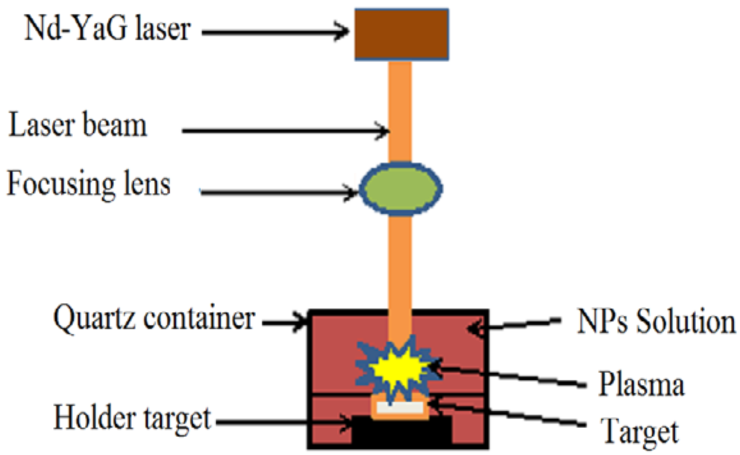
The antibacterial effects of silver (Ag) salts have been known for over 2000 years, but they have only been in common use as an antimicrobial since the 19th century. Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds [17]. Silver nanoparticles (AgNPs) are very widely used currently in industry, mainly because of their anti-bacteria properties, with applications in cosmetics and as bactericides in fabrics and other consumer products [18]. Because of their antibacterial properties, the Ag NPs attract nowadays great interest for applications in nanomedicine. Their usage in combination with antibiotics enhances the antimicrobial effect. Silver nanoparticles (Ag NPs) represent a new generation of antimicrobials. Ag NPs have a very broad range of antimicrobial activity and kill both Gram negative and Gram-positive bacteria, including proteus. Escherichia-coli, Staphylococcus aureus and Streptococcus [19-21]. An antimicrobial refers to a substance that kills or inhibits the growth of microorganisms. Since the discovery of antimicrobial drugs in the 1960s, many infectious diseases have been overcome. Typically, antimicrobials kill bacteria by binding to some vital compounds of bacterial metabolism, thereby inhibiting the synthesis of functional biomolecules or impeding normal cellular activities [22].

Experimental Procedure:

Laser Ablation System

The experimental setup for pulse laser ablation (PLA) (Fig .1) of a solid metal target is fixed on the bottom of a quartz chamber and immersed in pure de-ionized water or aqueous solution. The system of Nd:YAG laser device, type HUAFEI providing pulses of

1064nm and/or 532 nm (frequency doubled) wavelength with energy per pulse of 800 mJ, pulse width of 10 ns, repetition rate of 1Hz and effective beam diameter of 5mm, with a convex lens with (100 mm) focal length is used to achieve high laser fluence



Figure(1): Experimental setup for nanoparticles synthesis by pulse laser ablation liquid (PLAL) process.

Samples preparation

Silver plate, was supplied from Fluka company, with high purity listed of(~99.99%). The plates were cutting to pieces with dimensions 10 mm×10 mm to suite the experimental arrangement and polished, and then washed in ethanol and double distill deionized water (DDDW) for several times to remove the impurity from its surface. The plates were placed in cleaned glass vessel containing 5mL of DDDW in such a way that the plates were fully immersed in water.

Results and Discussion :

The average size, shape and the homogeneity of nanoparticles can be qualitatively described by the peak position and shape of the absorption spectrum. Immediately after fabrication, the particles in water. And a sample was poured into a quartz cell (10 mm × 10 mm × 50 mm in size) for spectral characterization. A UV-VIS spectrophotometer was used for obtaining the absorption spectra. and we were studied the effects of different parameters on optical properties as follows:

Effects of number of Laser Pulses.

Figure. 2 shows the Surface Plasmon Extinction (SPE) spectra of silver nanoparticles solution, synthesized by pulsed laser ablation. The liquid depth was selected 8 mm above the target for silver which was irradiated by focused energy of 370, 300 mJ/pulse and 1064 nm Nd: YAG laser. The beam spot diameter at the metal surface was 1.25mm. The number of pulses applied for the silver target ranged from 20 to 80 pulses as shown in figure 2. When the laser pulse struck the metal surface immersed in liquid; it created a spark plume with a strong shockwave that propagated in all directions. The spark emitted light and cracking noise, which were followed by a visible cloud of metal particles oozing out of the metal surface and dispersed slowly in all directions floating in liquid, easily noticed by naked eye. The color of solution was changed and the intensity was increased when advancing in the laser shots. The Surface Plasmon Extinction (SPE) peaks in visible region are the characteristic metals NPs formation. The optical properties of Ag Nanoparticles formed via Liquid Phase- Pulsed Laser Ablation (LP-PLA) at different number of pulses with constant laser fluence 370, 300 mJ/pulse at 1064nm laser wavelength. And can be shown in the same figure that the silver nanoparticles it is expected the maximum position of optical extinction spectra is located around 400 nm. This is typical for Plasmon absorption of Ag nanoparticles. So when the particles were subjected to 400 nm laser excitation the position of the Plasmon peak shifted to the blue region. The silver nanoparticles, was faint yellow in color.

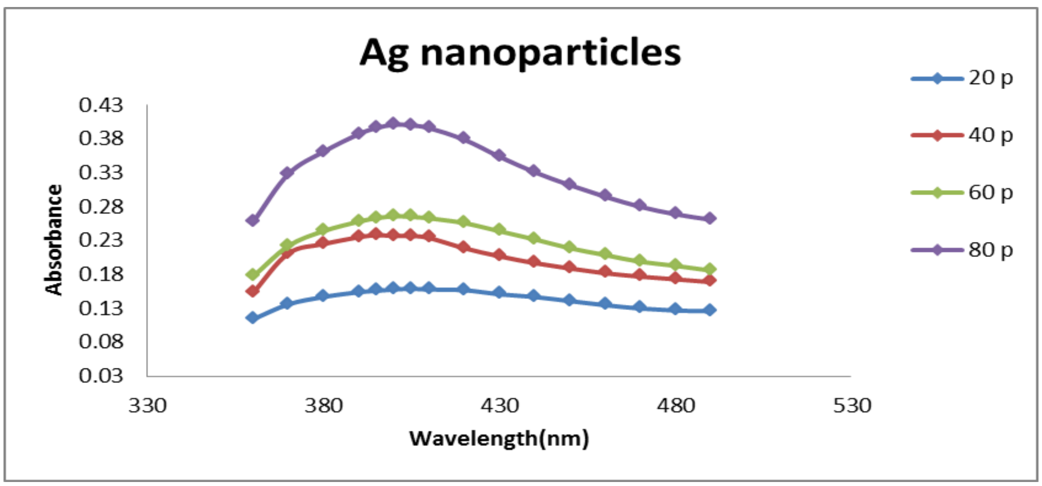


Figure (2): The Surface Plasmon Extinction (SPE) spectra of the Plasmon band of Ag NPs at range of 20-80 pulses, energy of 370, and λ=1064 nm.

Figure (3A, B) represented the concentration and absorbance as a function of Pulses Number. From the slope of Fig. (3A) curves can be noticed that the increasing rate of the particle abundance gradually increased with the number of laser pulses at all wavelengths. It should be noted that the particle abundance obtained by shorter wavelength laser light was lower than that obtained by longer wavelength laser light in the entire laser pulses. This finding indicated that the effect of the self-absorption on the shorter wavelength laser light was brought by a very low concentration of colloidal particles. A possible explanation for such efficient absorption is due to the localized particles in the vicinity of the surface of the target. It may be possible that the concentration of the particles at the ablation spot may be higher than that estimated by absorbance of the colloidal solution. Furthermore, the ablation efficiency at shorter wavelengths is still lower than that at longer wavelengths even in the very low particle concentration, the result agrees with that the increases of number of laser pulses lead to increasing of concentration of nanoparticles in solution. Figure (3B) shows the absorbance of the Ag colloidal solution at 400 nm as a function of the number of laser pulses. The concentration of produced Ag nanoparticles of 40 μg/ml was estimated by Atomic Absorption Spectroscopy (AAS). The absorbance at the wavelengths of the laser light for Ag colloidal solutions as a function of the laser pulses. The absorbance of the solution at 400 nm obtained with 1064 nm laser light was lower than that at 400 nm obtained with 532 nm laser light in the entire pulses. This finding indicated that the efficiency of the inter-pulse self-absorption should be lower for 1064 nm laser light than that for 532 nm laser light, which is inconsistent with ablation efficiencies observed. Thus, the influence of the intra-pulse self-absorption should be taken into account to explain the efficient absorption of the shorter wavelength laser light. Because the intra-pulse self-absorption will occur in definite area and will not be concerned with the entire concentration of the solutions, the absorption of shorter wavelength laser light can occur even at a very low entire concentration.

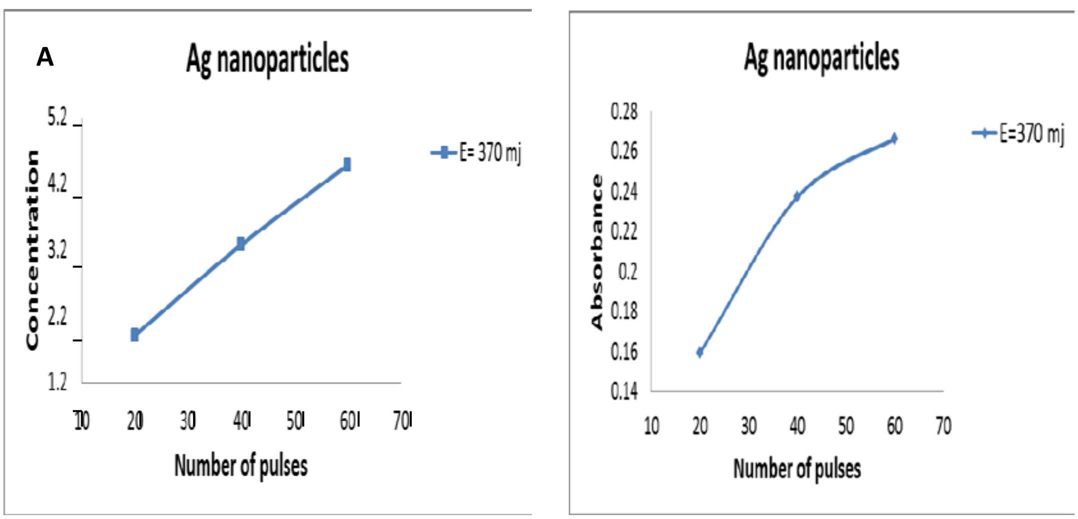
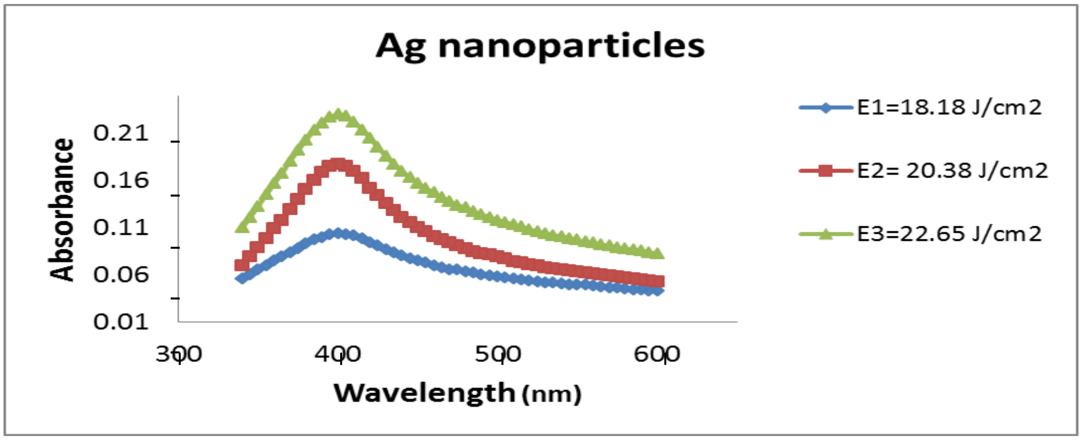


Figure (3) A: the concentration as a function of number of pulses for Ag NPs., B: the absorbance as a function of number of pulses for Ag NPs.

Effects of Laser fluence

Another parameter having an important effect on the formation of metal nanoparticles is the laser fluence. Fig. 4 shows the absorbance of Ag colloidal solution as a function of the laser fluence. Surface Plasmon resonance spectra of Ag samples, prepared at different laser ablation fluence.. The laser fluence at the target surface was (18.18,20.38,22.65) J/cm^2 , and the beam was focused to have a diameter near the outer edges of the target of 1.5 and 2.5 mm. and the smoke like colloids above the metal plate was observed. The plate was located at 8 mm from the liquid surface for all samples. Laser ablation listed for 40, 30 pulses, and the solution gradually turned to colored with the increase of the number of laser pulses. We measure a sensible increase in the SPR intensity; accompanied by a slight change in bandwidth and maximum wavelength, when laser fluence increasing. This enhancement in intensity can be explained by the increase in the concentration of metals nanoparticles formed in solution during the ablation process. The height and the width of the SPR peaks were found also to be dependent upon the laser fluence. Fig.4 gives the absorption spectra of silver colloids, and shows that the number of silver nanoparticles in the colloid increases with increasing laser fluence. The extinction spectrum shows characteristic features of silver nanoparticles in water, which has a narrow peak around 405 nm. The prominent single peak at 405 nm is consistent with the fact that the nanoparticles present in the solution are predominantly spherical in shape. The average size of the particles for three different ablating laser fluence at (18.18, 20.38, 22.65) J/cm^2 respectively. This is in agreement with results from UV-VIS spectra that particle size decreases with increasing energy of the ablating laser pulse. As a result the average particle size reduces with increasing of ablating laser fluence.

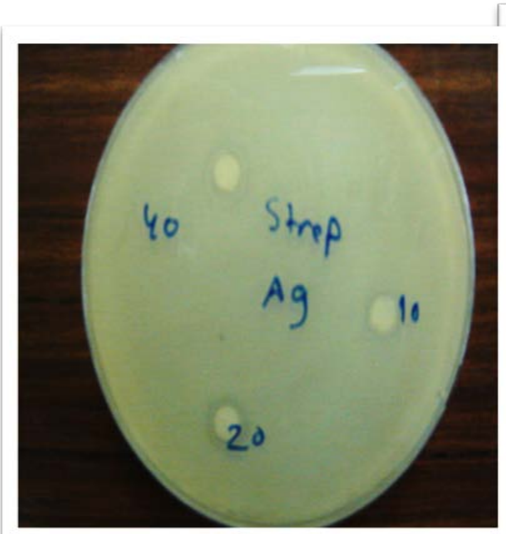


Figure(4): Absorption spectra of colloidal solution of silver nanoparticles at different laser fluence.

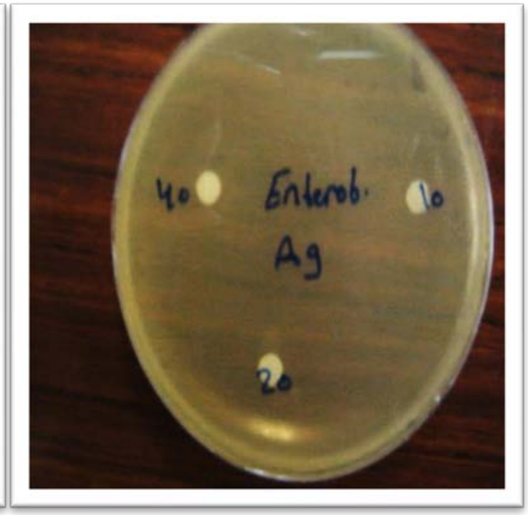
Antibacterial Effects of Silver Nanoparticles

Ag nanoparticles bacterial activity was studied against four different pathogenic bacteria *Streptococcus, Staphylococcus Aureus, Proteus* and *Enterobacter*.

The antibacterial activity of different solutions of nanoparticles was tested by the disc diffusion agar method as shown in table 1. The presence of an inhibition zone represented the greater antibacterial effect of silver nanoparticles. The size of inhibition zones were different according to the types of bacteria, the size and the concentration of nanoparticles solution as compared with antibiotic disc which being more effective as shown in table 1. The effect of different concentrations of Ag NPs like-10µg/ml, 20µg/ml and 40 µg/ml of silver nanoparticles on bacteria were performed. Figure 5 shows that the increased of the concentration of Ag nanoparticles the antibacterial activity of Ag nanoparticles were increased. Antimicrobial activity of the nanoparticles solution was investigated using the Kirby Bauer Disc diffusion method 40-41. The diameter of the zone of inhibition was calculated. Larger the diameter of zone of inhibition, greater is the antibacterial activities. The inhibition zones produced by different antibacterial against the same bacteria vary in size due to differences in antibacterial molecular structures. A larger zone of inhibition is produced by an antibacterial that diffuses more slowly. Bacteria strains sensitive to the antibacterial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. Microbial organisms isolates investigated were Streptococcus, Staphylococcus aureus, Proteus and Enterobacter. The nanoparticles solution was administered at 50µl per disc at a concentration of (40, 20, 10) µg/ ml. Gram-negative bacteria such



as Proteus and Enterobacter being most sensitive than Gram-positive such as streptococcus and staphylococcus. This is may due to the differences in cell wall structures of Gram-negative and Gram- positive bacteria. AS is known, the cell wall in Gram-positive bacteria is composed of a thick layer of peptidoglycan, which consists of linear polysaccharide chains cross-linked by short peptides, thus forming a 3D rigid structure. In Gram-negative, the layer of peptidoglycan is thinner with an external layer of polysaccharides which are not rigid. The presence of silver nanoparticles sites in relation to the Gram-negative cell and its ability to penetrate the wall cell is a better and more effective compared to antibiotics.



Figure(5): Images of antibacterial activities of discs different concentration of Ag NPs on Streptococcus, Staphylococcus, Proteus and Enterobacter respectively.

Table (1) shows the Zone of inhibition of Antibacterial test of Ag NPs.

Bacteria pathogen	**Ag concentration**		
	10µg/ ml	**20µg/ ml**	**40µg/ ml**
Streptococcus	6.5 mm	7 mm	8.5 mm
Staphylococcus	6 mm	7 mm	9 mm
Proteus	7 mm	7 mm	7 mm
Enterobacter	Nil	Nil	Nil

Conclusions:

The absorbance of the solution at 400 nm obtained with 1064 nm laser light was lower than that at 400 nm obtained with 532 nm laser light in the entire pulses, and the efficiency of the inter-pulse self-absorption should be lower for 1064 nm laser light than that for 532 nm laser light, which is inconsistent with ablation efficiencies observed . The prominent single peak at 405 nm is consistent with the fact that the nanoparticles present in the solution are predominantly spherical in shape at different ablating laser fluence. Bacteria strains sensitive to the antibacterial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. Microbial organisms isolates investigated were *Streptococcus, Staphylococcus aureus, Proteus and Enterobacter*.

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