Antibacterial Activity of Silver Nanoparticles Produced by Laser

Bassam G.Rasheed
Applied sciences department, University of Technology, Baghdad , Iraq
e-mail address: dr_bassamghalib@yahoo.com

Abstract:
Q-switched Nd:YAG laser has been employed to prepare colloidal solutions of silver nanoparticles. SEM investigations as well as the surface Plasmon of UV-Vis spectra reveal formation of silver nanoparticles. Those nanoparticles were exhibited antibacterial activity on some bacteria which live in human body. It is found that higher concentration of silver nanoparticles of 50 μg/ml and small nanoparticles (less than 25 nm) would increase the inhibition zone around the implanted bacteria and reach 15 mm for *Proteus*. Remarkable increment of antibiotics when silver nanoparticles added to improve the antimicrobial effect against gram-negative and gram-positive bacteria. It is also found that the most sensitive bacteria for silver nanoparticles was Proteus and the minimum inhibition concentration of 15 μg/ml was obtained for Staphylococcus.

Keywords: Laser ablation, Silver Nanoparticles, Antibacterial activity

1. Introduction

Silver and gold nanoparticles have unique features and extensive applications in diverse fields [1]. Studying these particular features have always been of great interest to many scientists [2]. In fact, nanoparticles display completely unique properties in comparison with their bulk size counterparts [3,4]. A large number of materials which were considered to be safe develop toxicity at nano size ranges which is mainly related to the increased specific surface area and high reactivity of nano size materials [5]. The broad antibacterial properties of silver nanoparticles encourage this material to be used in biomedical applications [6]. The bactericidal of silver nanoparticles is attributed to the presence of electronic effects due to the changes of the electronic structure of the surface because of size reduction. The antibacterial activity of silver nanoparticles can be used in medicine to reduce infections as well as prevent bacteria colonization on prostheses [7]. The advantages of using these inorganic oxides nanoparticles as antimicrobial agents are their greater effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance [8,9].

2. Theoretical background

The laser ablation process of pure silver sheet in liquid ambient is currently considered as the most significant prospective strategy to produce silver nanoparticles[10]. This process has been activated due to potential applications of silver nanoparticles especially in antibacterial and human health care [11]. Many works have been carried out on using lasers to synthesize silver and gold nanoparticles [12-16] and Nd–YAG laser has a dominant role in precision processing via laser ablation. This technique offers a great possibility of controlling the nanoparticle features since we can easily control the experimental conditions, such as nanoparticle host ambiance and laser parameters [17]. Controlled synthesis of nanoparticles in liquid media could produce a final product in the form of a stable colloid of nanoparticles. The colloidal suspension of silver nanoparticles consists of different silver nanoparticle sizes and this colloidal suspension has the following properties: Chemically stable, Nontoxic and contamination, Very easy to handle, Resizing and reshaping can also be done through melting and fragmentation technique.

There are very broad band of bacteria live in the human body and causes different types of diseases, infections and some time lead to fatal illness. There are famous types of bacteria which commonly affected the human health. These types are *Staphylococcus aureus, Escherichia coli, Bacillus subtilis Pseudomonas aeruginosa, S. epidermidis, Proteus* and *Streptococcus.*

As several pathogenic, bacteria have developed multi-resistance properties against various antibiotics. Therefore, great demands were emerged to enhance the antibiotics activity and overcome the bacteria resistance.

Microscopic examination remains the initial diagnostic test in the processing of specimens in the clinical microbiology laboratory. The timely report of a Gram stain result gives the physician important information about the presence and cause of infection. The Gram stain has a broad staining spectrum and classifies bacteria as either Gram-positive or Gram-negative.

Aim of this paper is to prepare colloidal suspensions of silver nanoparticles by laser ablation in liquid medium and study their antibacterial activity, moreover, improve the antibacterial activity of some familiar antibiotics when both silver nanoparticles and antibiotics mixed together.

3. Experimental Setup

The experimental procedure of the work includes the following stages; Silver nanoparticles preparation, bacteria inoculation and cultivation and finally culture incubation.

Silver nanoparticles could be synthesized by pulsed laser ablation of a piece of silver metal plates (ounces: 99.999%) placed on the bottom of quartz vessel containing 1ml of double deionized distilled water (DDDW). Q-switched Nd:YAG laser (Hufi Co., Taiwan) of variable energy in the range 100 – 1000 mj) was used. The laser beam was collected by 10 cm focal length focusing lens to obtain laser spot of 1.2 mm diameter as shown in figure (1). This range of laser energy and spot size provide suitable laser energy density in the range (1-20 J/cm^2) to ablate

silver nanoparticles [10]. The silver sheet is fully immersed in the DDDW and liquid depth of 8 mm above the sheet is adjusted. When the laser pulse strikes the silver surface, spark plume is emerged and strong shockwave is propagated in all directions. This spark plume is followed by visible cloud of silver particles which is expanded and dispersed slowly within the liquid. This process can be recognized by naked eyes.

Silver nanoparticles solution was prepared in this work in concentration of about 36 µg/ml. The antimicrobial activity of this solution was examined. Different concentrations of (18, 9, 4.5 µg/ml) were also prepared from this stock solution (36 µg/ml). The absorption spectra were investigated using Uv-Vis spectrophotometer (Biotech Co., UK). The colloidal solutions were also tested against different pathogens (*Staphylococcus*, *Streptococcus*, *Proteus*, *Candida albicans* and *Enterobacter*) as compared with the effect of some antibiotics: amoxicillin(25 µg), chloramphenicol (30 µg)and streptomycin (10 µg).

Disk diffusion method was used to examine the antimicrobial activity of each solution by adding 50µl to a sterilized filter paper and then, it was placed in an incubator (Uni medica, China) at 37^{0}C (the average temperature of human body) until dry. Oven (Suarez, Brazil) to supply this temperature was used. It is found that Agar diffusion method was also used for all concentrations and bacteria types. Generally bacteria require a relative humidity of 70 to 80%.

Liquid specimens are usually inoculated by transfer with a sterile pipette or syringe and needle. After implantation of the inoculums, a wire loop is flamed and cooled, or a sterile plastic disposable loop is selected.

The antimicrobial effect of different antibiotics (Amoxicillin and Penicillin) were tested using the Kirby-Bauer method and the effect of combination of Ag nanoparticles with these antibiotics were also tested to examine the effect of adding Ag nanoparticles on antibiotic action (40 µl of Ag nanoparticles in concentration of 50µ g/ml was added to each antibiotic disk).

4. Results & Discussion

Remarkable change of colloidal solution color from yellow to brown can be recognized when the number of laser pulses increased from 100 to 500 to prepare silver nanoparticles as shown in figure (2). It is found that these sets of silver nanoparticles colloidal solutions prepared by pulsed laser ablation are chemically stable for long time (months). It is also found that Ag NPs prepared with high laser energy and low concentration (low number of laser pulses) exhibit weak antibacterial activity on different types of bacteria compared with antibiotics effect on the same type of bacteria and no considerable differences in the antimicrobial effect between the concentrated and diluted solutions, therefore, there was a need to use silver nanoparticles colloidal solutions of higher concentration greater than 36 µg/ml. Furthermore, silver nanoparticles with different shapes and smaller sizes could have different antibacterial activity.

Effect of silver nanoparticles concentration in the solution on *Staphylococcus*, *Streptococcus*, *Proteus*, *Candida albicans* and *Enterobacter* were also studied for 500 and 1000 laser pulses. The density of silver nanoparticles (concentration) could be easily distinguished by naked eye. The dark brown color in figure (3) represents high concentration of Ag NPs prepared by 1000 laser pulses. Since high laser energies (greater than 600 mj) were not shown effective activity for different types of bacteria in our investigation, lower laser energy of 400 mj was used to prepare silver nanoparticle colloidal solution. This energy was employed to produce silver nanoparticles of smaller sizes compared with higher laser energy as shown by the morphological investigation by SEM as shown in figure (4). Figures (5) and (6) show the surface Plasmon spectra of silver nanoparicles colloidal solutions prepared by different laser energies and number of laser pulses, respectively.

It is observed that some bacteria (*Staphylococcus* and *Enterobacter*) are greatly affected by silver nanoparticles while others like *Streptococcus* and *Proteus* are not affected by Ag NPs and became resistant to Ag NPs as given in table 1, while these bacteria are highly affected by antibiotics as shown in figure (7). Therefore, combination of Amoxicillin (25 µg) and silver nanoparticles of 50 µg/ml concentration prepared with 400 mj was examined on different types of bacteria. Table (1) explains that the diameter of the inhibition zone varied between 17 to 40 mm and the antimicrobial effect has improved for *Staphylococcus* from 20 mm to 28 mm. Figure (8) illustrate the inhibition zone in agar diffusion dish around silver nanoparticles of 50 µg/ml concentration prepared with 400 mj laser energy for *Staphylococcus* and *Proteus* with and without silver nanoparticles.While tables (2) reveal that when Ag Nanoparticles was combined with *Streptomycin* antibiotic showed different effects depending on silver nanoparticles features, type of bacteria and the type of antibiotic used.

The antimicrobial effect of silver nanoparticles of higher concentration (60 µg/ml) has also studied. Table (3) gives the diameter of inhibition zone (DIZ) for different types of bacteria. Although Gram-negative bacteria (*Proteus* and *Enterobacter*) were affected by Ag-NPs more than Gram-positive bacteria (*Streptococcus* and *Staphylococcus*), this table reveal that all types of bacteria are affected with Ag NPs and the highest effect of Ag NPs was obtained on *Proreus* and it was 15 mm diameter which indicates that this kind is more sensitive to silver nanoparticles. Moreover, the antibacterial activity could also be determined by the minimum inhibition concentration (MIC). It is found that *Staphlycoccus* has minimum (MIC) of 0.46 µg/ml as given in table (4).

5. Conclusions

Some bacteria (*Staphylococcus*, *Streptococcus*, *Proteus*, *Enterobacter* and *Candida albicans*) which live in human body are affected by silver nanopaicles prepared with laser ablation in liquid. The antibacterial effect of silver nanoparticle depends on their size and concentration. The antibacterial activity of silver nanoparticles could be studied via inhibition zone and the minimum inhibitory concentration (MIC). The diameter of the inhibition zone was different according to the type of bacteria and the concentrations of Ag nanoparticles. It is found that *Staphlycocus* has minimum (MIC) of 0.46 µg/ml and *Proteus* is more sensitive to silver nanoparticles with (DIZ) 15 mm.

Silver nanoparticles exhibit positive effect on some antibiotic (synergism). It is found that Ag Nanoparticles improve the amoxicillin effect on Gram-positive bacteria like *S.aureus* and *Streptococcus* and also improve (S) effect on *S.aureus*, *Proteus* and *Enterobacter*, While Ag Nanoparticles increased the antibacterial effect of Amoxicillin on Gram-negative bacteria *like Enterobacter.*

Acknowledgment

I feel responsible to acknowledge CRDF and Arab Science & Technology Foundation (ASTF) for their support.

Table 1: The antimicrobial effect of Ag NPs 50 µg/ml concentration prepared with 400 mj using 1000 pulse (Ag). The antimicrobial activity is measured in presence and absence of *Amoxicillin* (AX) (25 µg).

Pathogen	Ag	AX	Ag+AX
Staphylococcus	10	20	28
Streptococcus	R	29	36
Proteus	R	17	27
Enterobacter	10	41	40

Table 2: The antimicrobial effect of Ag NPs 50 µg/ml concentration prepared with 400 mj using 1000 pulse (Ag). The antimicrobial activity is measured in presence and absence of *Streptomycin* (S)(10µg).

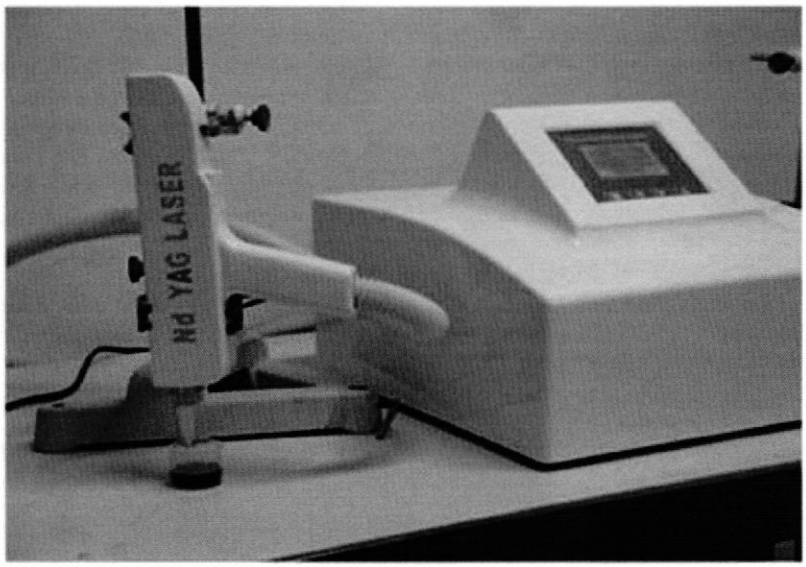
Pathogen	Ag	S	Ag+S
Staphylococcus	10	20	26
Streptococcus	R.	22	28
Proteus	R	10	16
Enterobacter	10	44	48

Table 3: The antimicrobial effect of silver nanoparticles solution at a concentration of 60µg/ml.

Pathogen	(DIZ) in mm
Streptococcus	10
Staphylococcus	8
Proteus	15
Enterobacter	11
Candida albicans	8

Table 4: The minimum inhibitory concentration (MIC) of silver nanoparticles.

Pathogen	(MIC) µg/ml
Streptococcus	12
Staphylococcus	0.46
Proteus	7.5
Enterobacter	15
Candida albicans	3.75



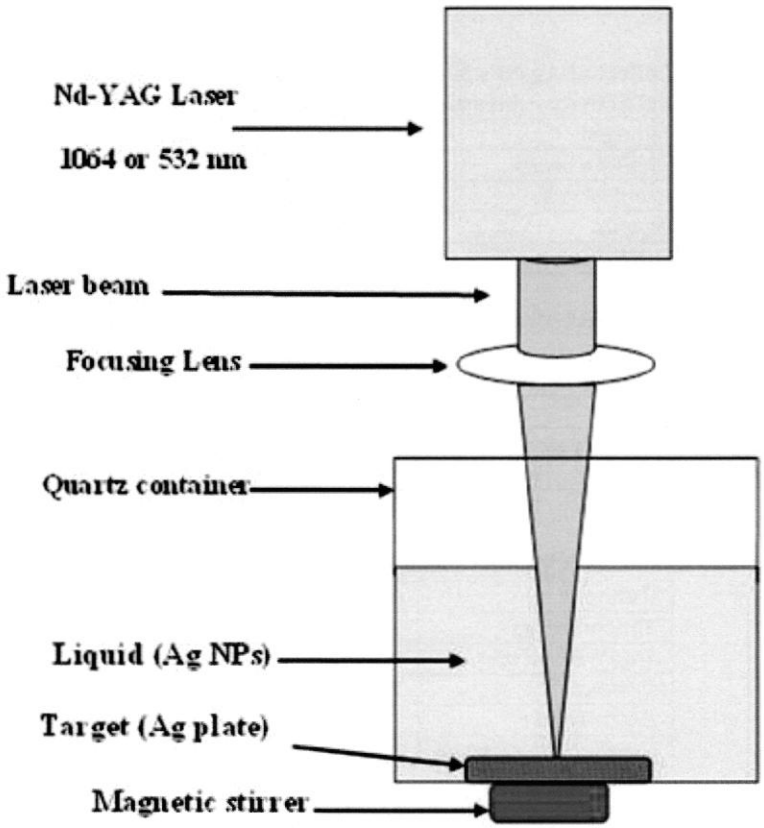


Figure (1): (Top) Q-switched Nd:YAG laser used to prepare silver nanoparticles. (Bottom) schematic diagram ofthe experimental set up

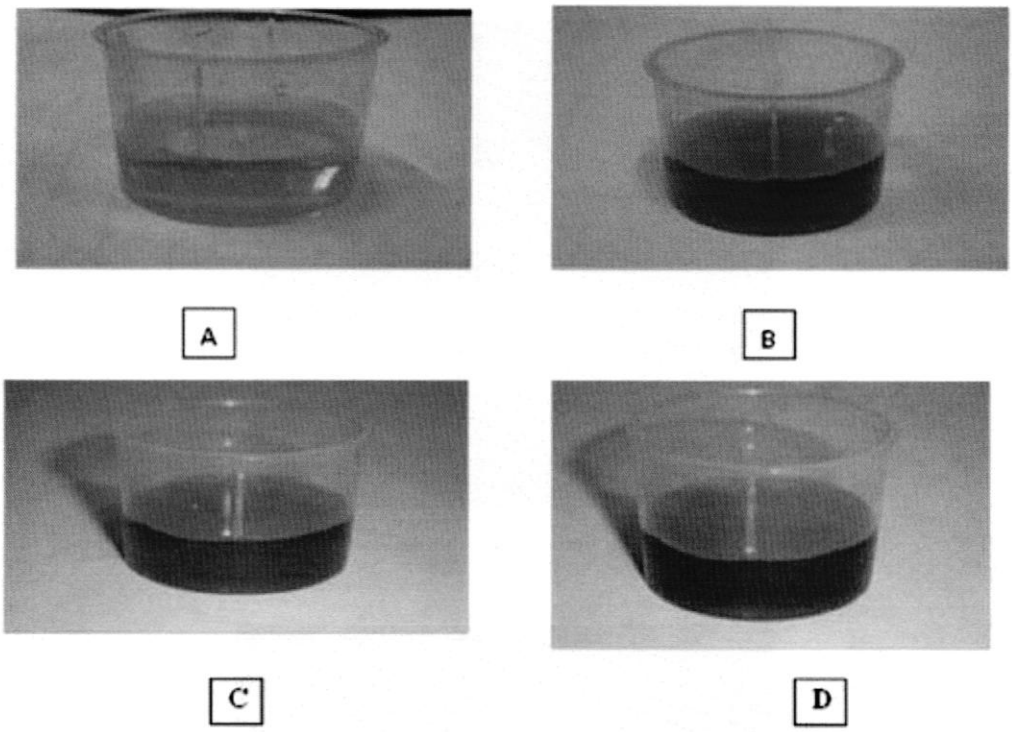


Figure (2): Effect of laser number of pulses on silver nanopaarticle colloidal solution 100,200,300 and 500 in A,B,C and D, respectively prepared by Nd:YAG laser of 400 mj energy.

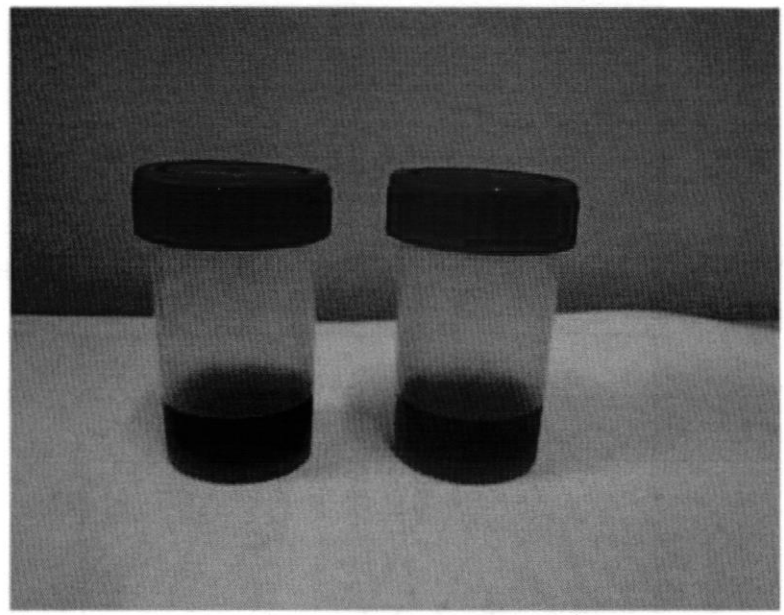


Figure (3): The colloidal solutions of silver nanoparticles prepared by Nd:YAG laser of 50J/cm^2, 1000 pulse and 400 mj laser energy (left) and 500 mj laser energy (right).

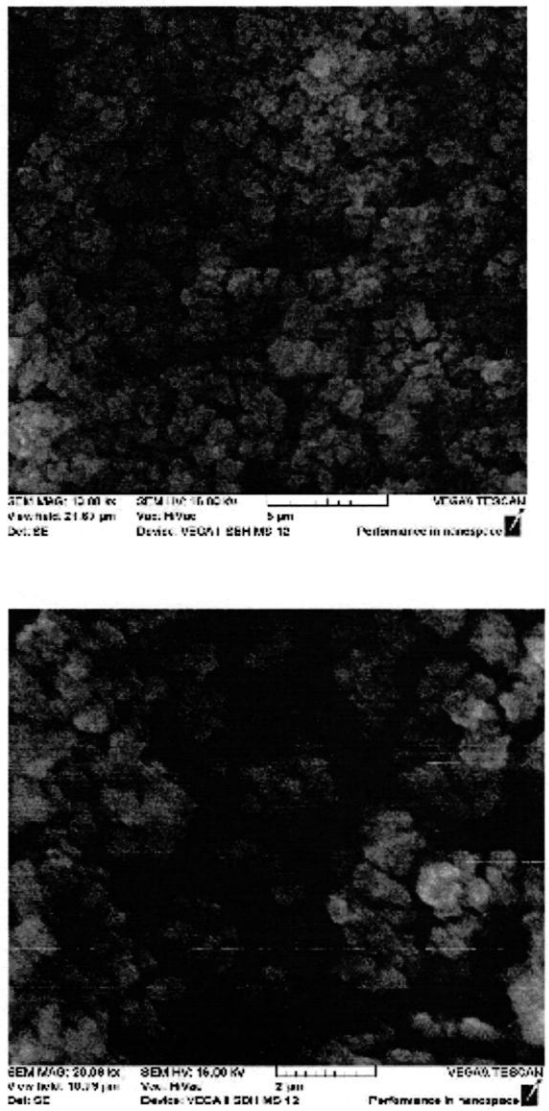


Figure (4): SEM of high concentrations silver nanoparticles 50 µg/ml (bottom) and 60 µg/ml (top) produced by Nd:YAG laser of 400 mj, 50 J/cm^2 and 1000 pulse.

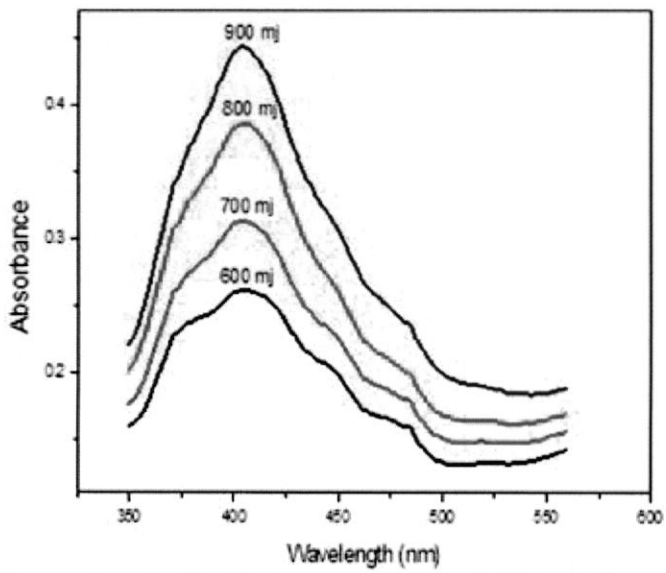


Figure (5): Effects of laser energy on the silver nanoparticles colloidal solutions prepared by Nd:YAG laser of 1000 pulses.

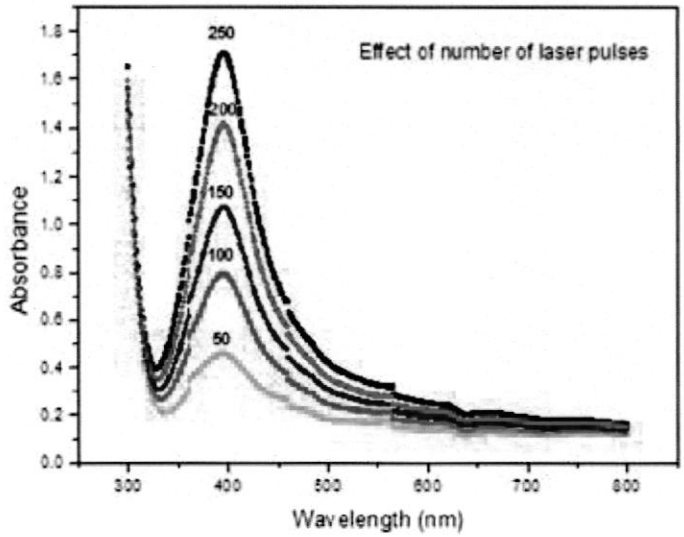


Figure (6): The absorbance spectra of silver nanoparticle colloidal solution prepared with 700 mj laser energy and different number of laser pulses.

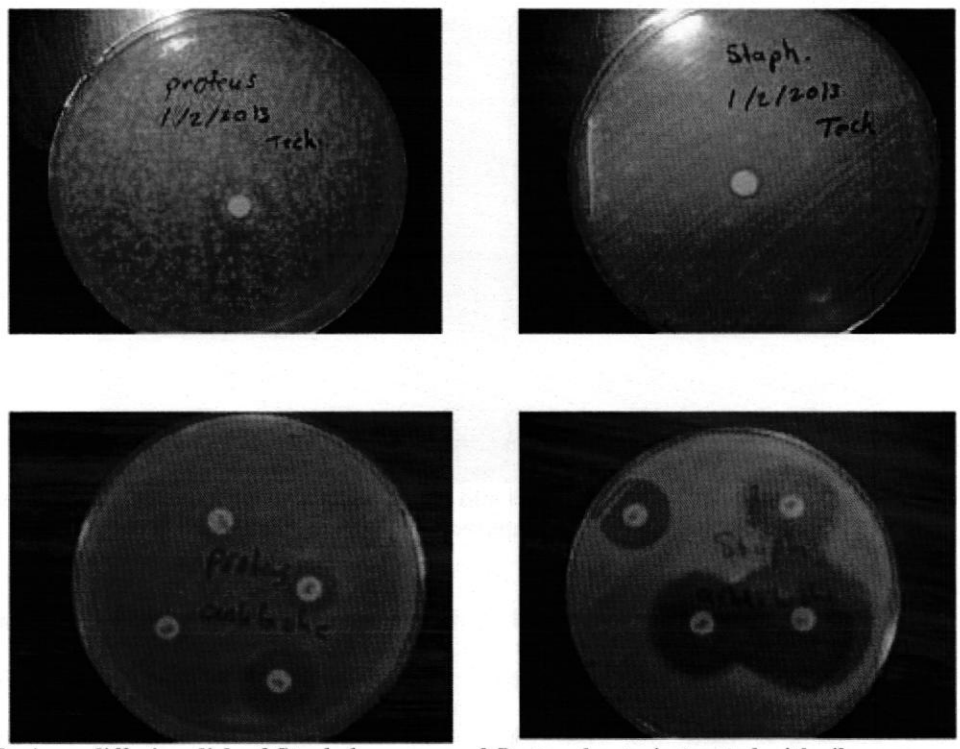


Figure (7): Agar diffusion dish of *Staphylococcus* and *Proteus* bacteria treated with silver nanoparticles of 50 µg/ml concentration prepared with 400 mj laser energy (top) and different types of antibiotics (bottom).

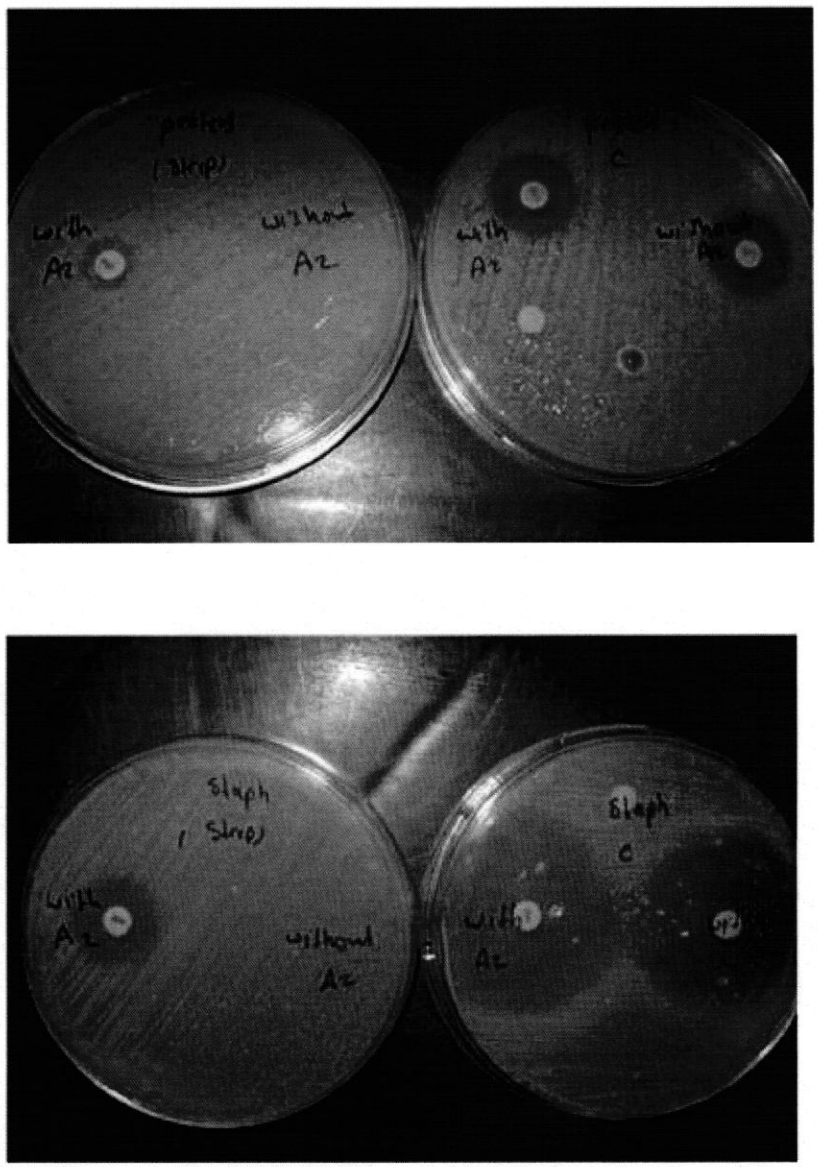


Figure (8): Agar diffusion dish of *Staphylococcus* and *Proteus* with and without silver nanoparticles of concentration 50 µg/ml prepared with 4500 mj laser energy

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الفعالية المضادة للبكتيريا لدقائق الفضة النانوية المحضرة بالليزر

بسام غالب رشيد

قسم العلوم التطبيقية ، الجامعة التكنولوجية ، بغداد ، العراق

الملخص

استخدم ليزر النديميوم –ياك ذو النبضات القصيرة لتحضير دقائق الفضة النانوية كعوالق في ماء مقطر لا أيوني. أن تحليل جهاز المجهر الألكتروني الماسح وقمم منحنيات أمتصاص هذه الدقائق تبين تكون دقائق الفضة النانوية. وجد بان لهذه الدقائق فعالية ضد بعض انواع البكتيريا التي تعيض في جسم الانسان. كما تبين بان دقائق الفضة النانوية تزيد من فعالية بعض المضادات الحيوية عند اضافتها لها. يعتمد تأثير دقائق الفضة النانوية في البكتيريا على تركيز هذه الدقائق في المحلول اضافة الى حجمها النانوي حيث اذا كانت هذه الدقائق بمعدل حجم اقل من 25 نانومتر وتركيز 50 مايكروغرام/مللترسيكون لها تأثير جيد على بكتريا من نوع غرام–موجب وغرام– سالب. تبين من خلال هذه الدراسة بان اكثر انواع البكتيريا التي تم دراستها تحسسا لدقائق الفضة النانوية هي (بروتياس) حيث كان قطر الامتناع لها هو (15 ملم) اما اقل تركيز قاتل للبكتيريا فكان لنوع (ستافلي كوكاس) وهو (0.4 مايكروغرام/مللتر).