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Antibacterial Activity of Gold and Silver Nanoparticles against Pathogen Species, *E. coli* and *S. aureus*

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Gold and silver nanoparticles (NPs) have been prepared by pulsed laser ablation in liquid (PLAL) operated at 532 nm wavelength with energy density about 17 J/cm^2 and 10 J/cm^2 for gold (Au) and silver (Ag), respectively, immersed in aqueous solvent with addition 5 mM sodium dodecyl sulfate (SDS) as surfactant. Transmission electron microscopy (TEM) revealed the spherical shape morphology of Ag nanoparticles and Au nanoparticles in the presence of surfactant and grain size of the Ag nanoparticles and Au nanoparticles was found to vary between 20 and 100 nm. Absorption spectra of Au and Ag colloidal confirmed the formation of nanoparticles and improve the addition of (SDS) as surfactant during PLAL method. Finally, the antibacterial activity of Au and Ag nanoparticles against bacterial species E. coli and S. aureus demonstrates that these nanoparticles were more active against Gram negative bacteria than Gram positive bacteria and this was attributed to change in the bacterial cell membranes composition.

***Keywords*:** Nanoparticles; Surface Plasmon Resonance; Pulsed laser ablation; Surfactant
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1. Introduction

Nanotechnology is considered as a new generation of technology that can have a great effect on economies through new consumer products, manufacturing methods and materials procedure [1]. Noble metals like silver and gold nanoparticles have taken great attention due to their good electrical, optical, chemical and magnetic properties attributed to their surface plasmon resonance (SPR) associated features that are basically useful in biology filed [2]. Pulsed-laser ablation in liquid (PLAL) is a good technique to synthesize nanoparticles in solutions with controlled shape and size. Besides, ablation of noble metals in water environment has attracted researchers because it represents a different technique to usual chemical reduction processes to obtain colloids of nanoparticles. So, this approach reveals environmentally friend green procedure with products that commonly without stabilized molecules or additional chemicals. The PLAL process can be applied in clean deionized water (DW) or in biological aqueous solvent and then establish protocols to improve the sensitivity of classic vibration spectroscope like in the case of surface enhanced Raman (SER) phenomena [3,4].

Moreover, controlling nanoparticle sizing of noble metals via laser ablation can be achieved by addition of particular molecules to aqueous solution, which physically or chemically interact with the surfaces of the formed particles. Ionic surfactants like cyclodextrins and sodium chloride were effectively used to decrease the particle size of noble metals [5]. As well, the accurate method to limit the increase of particles remain uncertain [6,7].

Besides, sodium dodecyl sulfate (SDS) represents the most qualified surfactant to limit average size of Au and Ag down to 10 nm through laser ablation in aqueous solution. Recently, laser ablation process permits to synthesize nanoparticles with less contaminant using reduced agent, but coagulation development of atoms leads to broaden size distribution of the nanoparticles and it is hard to control [4]. Since, a solution containing perfect metal nanoparticles can be efficiently produced, when laser ablation used in a liquid environment [8]. Moreover, the noble metals, such as silver, gold, tin and zinc, have been used for centuries as bactericidal agents due to their antibacterial properties against many types of bacteria. Besides, silver and gold nanoparticles can interact with the functional groups on the cell membrane of bacteria which leads to inactive pathogen [9]. The silver nanoparticles possess high antimicrobial activity due to high specific area and high fractions of surface atoms of silver nanoparticles when compared with bulk silver metal. The antimicrobial effects of silver nanoparticles have been generally used for years in various medical applications. The gold nanoparticles are significantly used in the medical field as they are easily tacking up by membrane of bacteria. The toxicity of gold nanoparticles against various cell kinds depends on their sizing. Furthermore, the capability of pathogenic bacteria to resist antimicrobial agents was emerging in latest years and representing a main health trouble [10-14].

Therefore, the present study aims to synthesize silver and gold nanoparticles using pulsed laser ablation in liquids and study the structural changes with 5mM sodium dodecyl sulfate (SDS) in aqueous solvent. Also, the antibacterial activities of silver and gold nanoparticles against *Escherichia coli* and *Staphylococcus aureus* are investigated.

2. Materials and Methods

Silver and gold nanoparticles were synthesized by irradiating the metal target plate using pulsed laser, as shown in Fig. (1). The noble metal plate with thickness of 1 mm was located on the bottom of quartz container containing deionized water (DW) and SDS surfactants of 5mM concentration at ambient temperature. The noble metal plate was irradiated with a focused output of the second harmonic Nd:YAG laser at a repetition rate of 5 Hz and pulse width of 9 ns. The laser flounce was set at 17 and 10 J/cm^2 for gold and silver targets, respectively, with a positive lens of 10 cm focal length.

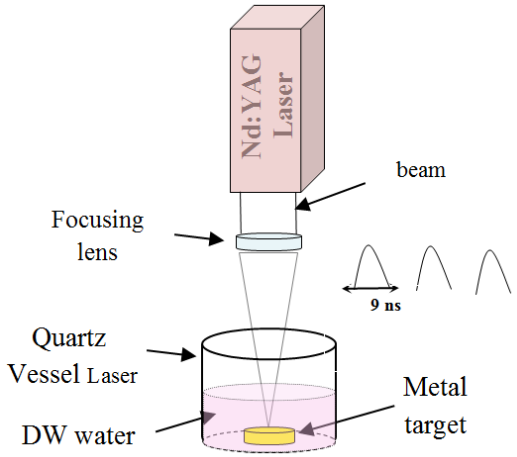

Fig. (1) Schematic diagram of laser ablation setup

The morphology and size distribution of gold and silver nanoparticles were confirmed by EM10 C-100KV Carl Zeiss (Germany) transmission electron microscope (TEM). A SHIMADZU UV-1800 UV-Visible spectrophotometer was used to determine absorption spectra of the formed nanoparticles colloidal in the wavelength range 190-1100nm and the speed of the wavelength scan is up to 2500 nm/min.

Antimicrobial activity was performed with standard strains by using *S. aureus* and *E.coli*. The antibacterial test is performing by using disk method. First, a suspension of bacteria is provided by preparing the saline solutions of isolating colonies chosen from nutrient agar plate. Then the agar plates are grown for 18 hours. A sterilized nutrient broth of 5ml is inoculation with a loop full of test organism and incubation for 24 hours. Then, 0.2 ml from overnight culture of organism are distributed into 9ml of sterile nutrient broth and incubation for 3 hours to make standard McFarland turbidity using the spectrophotometer of 600nm and production of the culture with concentration of 10^6 CFU/ml. Surface of Mueller-Hinton Agar (MHA) was absolutely inoculated using a clean cotton swab in all directions and rotating the plate. The sterile paper discs (8mm) are soaking in gold and silver nanoparticles solvents, respectively, for few minutes then left to dry in a clean circumstance for test. Then, the soaking discs are placed on the inoculation agar and incubating at 37°C for 24 hours. After incubation, the zones of clearing around the disk are measured.

3. Results and Discussion

Figure (2) shows the absorption peaks of silver and gold nanoparticles synthesized by laser ablation technique in 5mM of SDS at wavelengths of 415 and 532 nm, respectively, due to SPR of gold and silver nanoparticles affected by SDS concentration. The results of UV-visible analysis reveal the absorption peaks of gold nanoparticles (dashed line) and silver nanoparticles (solid line) depending on SDS concentration of 5mM. In Fig (2), the peaks of absorbance tend to increase by addition of 5mM SDS.

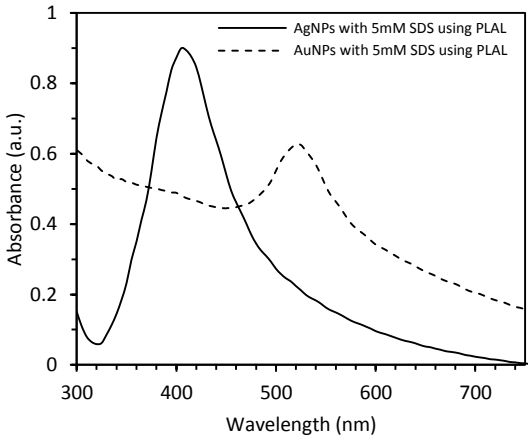

Fig. (2) Absorption spectra of (a) Ag nanoparticles and (b) Au nanoparticles synthesized by pulsed laser ablation technique in 5mM SDS

Obviously, the surfactant (SDS) plays a significant role in determining the stability of the gold and silver nanoparticles as the extinction of nanoparticles growth is controlled by the distribution and attaching rates of SDS on the nanoparticles. The particles were enveloped with surfactant since the distributed width of SDS surfactants becomes thin. Extremely negative-charge nanoparticles are repelling each other more efficiently and this allows the SDS surfactants covering them before the connection takes place. The kinetic consideration is predictable to bound coalescence of the formed clusters and hence resulting in lesser particles. The

surfactants, like SDS, contacting with nanoparticles prevent coalescence and agglomerated, which participate in stabilizing and successfully reducing the size of gold and silver nanoparticles.

The antibacterial activities of gold and silver nanoparticles were tested against standards of the *E.coli* and *S. aureus* by using disc diffusion test to determine the inhibition zones, as shown in Fig. (3).

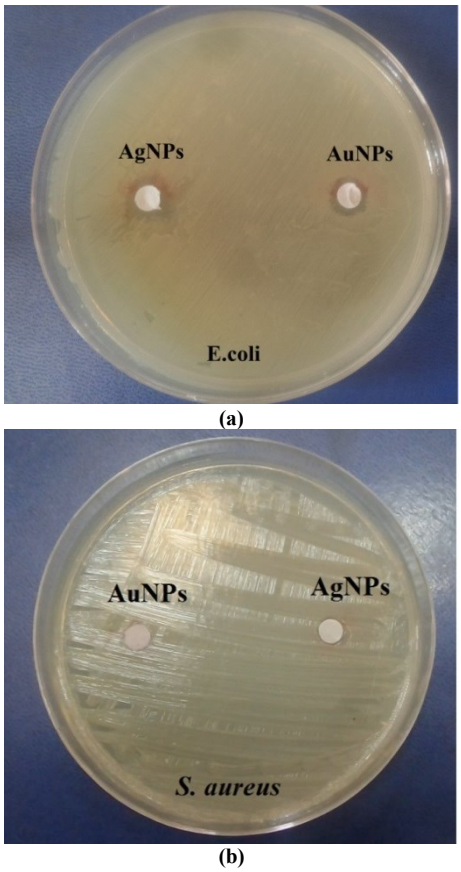

Fig. (3) Inhibition zone diameters in (mm) of silver nanoparticles (Ag NPs) and gold nanoparticles (Au NPs) against (a) *E.coli* and (b) *S. aureus* bacteria

The zone of clearance around the disk was measured by the diameter of the inhibition circle, as shown in Table (1). Therefore, our results show that the bacteria are susceptible to the silver and gold nanoparticles. Silver nanoparticles showed further activity on pathogens in comparison to gold nanoparticles. Besides, the colloidal of silver and gold nanoparticles reveals good antibacterial activity against tested pathogens as shown in Fig. (3). These results showed that the silver nanoparticles have higher antibacterial activity than gold nanoparticles due to the relatively inert chemical nature of gold. In Fig. (3a), the silver nanoparticles have higher activity against Gram negative bacteria than Gram positive bacteria and this was attributed to the change in the bacterial cell membranes composition. The gold nanoparticles are less efficient against the tested pathogens, as revealed in Fig. (3). The bactericidal mechanism of silver nanoparticles may consist of variation of thiol or sulfhydryl groups including biomolecules, like proteins, and electrochemical collapse that penetrates across the cell membranes of bacteria [11]. It is assumed that heavy metals like silver and gold release ions reacting with thiol or sulfhydryl groups (-SH) of proteins and inactivates them. Ionic silver and gold efficiently interact with thiol group of vital enzymes and inactivate bacteria. Ionic silver (Ag^{+}) replaces ionic hydrogen (H^{+}) of thiol groups and deactivates the proteins by decreasing the permeability which leads cell to die [14]. It is normal to state that attaching of silver or gold nanoparticles to the bacteria cell depends on the surface area presented for interaction. Nanoparticles have larger surface area presented for interactions, which improve bactericidal effect than the large size particles; therefore they convey cytotoxicity to the microorganisms [15]. The mechanism of penetrating bacteria using nanoparticles still not complete, but proposed metals are cytotoxicity and reacting with protein. As a result, the metals connect to protein molecules, metals efficiently interact with thiol group of vital enzymes and inactivate bacteria [16,17].

Table (1) Inhibition zone diameters in (mm) of Ag and Au nanoparticles impregnated against *S. aureus* and *E. coli* bacteria

The bacteria	*E.coli*	*S. aureus*
Inhibition zone diameter in (mm) of Ag nanoparticles	17mm	11mm
Inhibition zone diameter in (mm) of Au nanoparticles	14mm	10mm

The TEM analysis confirmed the nanosize of silver and gold nanoparticles prepared by laser ablation in 5mM SDS. The silver and gold nanoparticles have spherical shape, as shown in TEM images in Fig. (4). Besides, the influence of SDS is clearly seen in TEM images of samples. Both silver and gold nanoparticles are dispersed and mostly no aggregation was observed. The average particle size of gold nanoparticles is 20-30 nm while that of silver nanoparticles is 40-60nm. Since, it can be seen that addition of high concentration of SDS surfactant has reduced the nanoparticle size. These results reveal that charged particles of gold and silver repel each other because the SDS molecules are allowed to cover these nanoparticles during the reaction using pulsed laser ablation.

4. Conclusions

In conclusions, highly pure silver and gold nanoparticles have been prepared by pulsed laser ablation in liquid (PLAL) using 532nm Nd:YAG laser in 5mM SDS solution. Obviously, the results proved that the preparation of gold and silver nanoparticles is affected by SDS concentration as confirmed by UV-visible analysis with peak wavelengths of 532nm and 415nm, respectively, due to SPR of gold and silver nanoparticles. The

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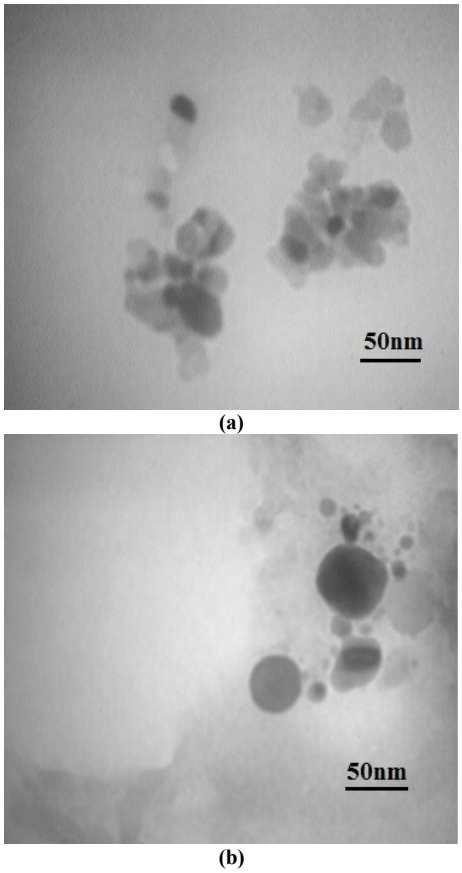
colloidal of silver and gold nanoparticles demonstrates good antibacterial activity against pathogens and becomes more active against Gram negative bacteria than Gram positive bacteria due to the composition of bacterial cell membranes. Since, bacterial inhibition depends on the effect of laser ablation conditions in synthesized silver and gold nanoparticles colloidal and addition of SDS surfactant.



(a)

(b)

Fig. (4) TEM images of (a) gold (Au) nanoparticles and (b) silver (Ag) nanoparticles, obtained by PLAL of metal plates immersed in DW with SDS surfactant solution using 400 pulses of Nd:YAG laser

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