Antibacterial Activity of Mono and Bimetallic Au:Ag Colloidal Nanoparticles Prepared by Pulse Laser ablation PLA

Adel H. Omran Alkhayatt[1] **Mahdi Husain Moheel[2]**

Mohammad Malik Abood[1]

University of Kufa, Faclty of Sciences, [1]Physics Department, [2]Biology Department, Najaf, Iraq

adilh.alkhayat@uokufa.edu.iq mahdialammar@yahoo.com

janamohammadmalik@gmail.com

Abstract:

Au, Ag mono and Bimetallic Au:Ag colloidal nanoparticles were synthesized in two steps using pulsed laser ablation in liquid technique (pure Au, Ag metal plates of immersed in deionized water). The pulse laser (Q-switched, Nd: YAG)1064 nm with energy 700 mJ, frequency 5 Hz and 200 laser pulses at room temperature have been used. Absorbance (SPR band), size, surface morphology and structure of the synthesized Nps have been studied and investigated by UV-Vis spectrophotometer, transmission electron microscopy, and x-ray diffraction. The absorbance spectrum of Au, Ag and Au:Ag nanoparticles shows sharp and single peaks around 513 nm, 398 nm and 523 nm respectively. This gives indicate that the production of pure and spherical Au and Ag NPs with average size in the range 16 - 100 nm. The particles size measurements were confirmed by TEM. The Au:Ag nanoalloy appears to be nearly spherical with an average size ranging from 29 to 72 nm and the SPR band at (450 nm). The XRD result showed very low crystallinity structure. The antibacterial activity of Au, Ag and bimetallic Au:Ag NPs were evaluated for NPs obtained by PLAL using applied pulses (100,150,200) in DI water, on the Gram-positive isolate (Staphylococcus aureus) and Gram-negative isolate (Escherichia coli). The results and images of inhibition zones diameter showed that the NPs have synergistic effects on the studied bacteria up to 15 mm and 4 mm at the highest and lowest values and it increase with the increase of laser pulses number.

http://dx.doi.org/10.31257/2018/JKP/100102

Keywords: Au, Ag mono and Bimetallic nanoparticles; pulsed laser ablation in liquid; SPR band; antibacterial Activity.

النشاط المضاد للبكتريا للجسيمات النانوية الغروية أحادية وثنائية المعدن للذهب والفضة المحضرة بالتشظية بالليزر النبضي

عادل حبيب عمران الخياط١ مهدي حسين محيل٢ محمد مالك عبود١

كلية العلوم جامعة الكوفة ١ قسم الفيزياء ٢ قسم علوم الحياة

الخلاصة

الجسيمات النانوية الغروية أحادية وثنائية المعدن للذهب والفضة تم تحضيرها بخطوتين باستخدام تقنية التشظية بالليزر النبضي في السائل (ألواح معدنية من الذهب والفضة النقية مغمورة في الماء منزوع الأيونات). تم استخدام الليزر النبضي ندميوم - ياك ١٠٦٤ نانو متر وبطاقة ٧٠٠ ملي جول، وتردد ٦هيرتز و ٢٠٠ نبضة ليزر في درجة حرارة الغرفة. تم دراسة الأمتصاصية (حزمة الرنين البلازمون السطحي)، الحجم ، مورفولوجية السطح والتركيب البلوري للجسيمات النانوية المحضرة بأستخدام مطياف الأشعة المرئية- فوق البنفسجية، المجهر الألكتروني النفاذ وحيود الأشعة السينية. طيف الأمتصاص للجسيمات النانوية Au, Ag و Au:Agأظهر قمم حادة ومنفردة في حدود ٥١٣ ، ٣٩٨ و ٥٣٢ نانومتر على التوالي. وهذا يعطي مؤشر لأنتاج جسيمات نانوية نقية وكروية للذهب والفضة وبحجم نانوي في المدى ١٦ - ١٠٠ نانومتر. قياسات حجم الجسيمات النانوية انجز بالمجهر الألكتروني النفاذ. السبيكة النانوية ذهب:فضة تظهر جسيمات كروية تقريبا وبمتوسط حجم تقريبا ٢٩الى ٧٢ نانومتر وحزمة رنين البلازمون السطحي عند ٥٤٠ نانومتر. أظهرت نتائج حيود الأشعة السينية تركيب منخفض البلورية جدا. الفعالية المضادة للبكتريا للجسيمات النانوية الذهب والفضة ووالجسيمات النانوية ثنائية المعدن ذهب:فضة المحضرة بطريقة التشضية بالليزر النبضي في السائل وبعدد نبضات ١٠٠، ١٥٠و ٢٠٠ نبضة في الماء اللاأيوني قدرت على البكتريا موجبة وسالبة الغرام ستافيلوكوكس وايكولاي على التوالي. أظهرت نتائج وصور قطر منطقة التثبيط أن الجسيمات النانوية لها تأثير تثبيطي فعال على البكتريا تحت الدراسة وبقيم تتراوح بين ١٥ ملم و ٤ ملم كأعلى وأوطأ قيم وتزداد الفعالية بزيادة عدد نبضات الليزر.

كلمات مفتاحية: جسيمات الذهب والفضة أحادية وثنائية المعدن; التشظية بالليزر النبضي في السائل; الرنين البلازموني السطحي; النشاط المضاد للبكتريا.

1. Introduction

Nanoparticles are of large scientific advantage as they are effectively a bridge between bulk materials and atomic or molecular structures. Bulk materials must have constant physical properties (size independent) whereas, at the nanoscale, the physical properties of the nanomaterials are size dependence such as quantum size effect and quantum confinement in semiconductor nanoparticles and surface Plasmon resonance in some metal nanoparticles [1]. According to the novel physical, chemical and magnetic properties of Noble metal nanoparticles, Ag and Au nanoparticles have been a source of great applications [2, 3], such as catalytic [4], biomedical applications [5], surface-enhanced Raman scattering (SERS) [6], drug delivery [7], antibacterial [8], biosensing [9]. Gold and silver nanoparticles are chemically stable and typically exhibit surface enhanced Raman scattering SERS in the visible wavelength range, where they may cause a tremendous increase in various optical cross-sections. In parallel with the NPs of pure metals, bimetallic NPs particularly have attracted large interest, as a way for developing new materials [10]. The specific properties of bimetallic NPs are improved and enhanced because of the synergistic effects of the two distinct metals [10, 11]. The resonance frequencies strongly depend on particle shape and size as well as on the optical properties of the material within the near-field of the particle [12]. They were very likable for biophysical, biochemical, and biotechnological applications due to their special physical properties, mostly due to their sharp plasmon absorption peak at the visible region. Another important advantage is that Ag and Au nanoparticles prepared by Pulsed Laser Ablation in Liquid process were stable for a period of months. Additionally, gold and silver nanoparticles are chemically stable and typically exhibit surface enhanced Raman scattering SERS in the visible wavelength range, where they may cause a great increase in various optical cross-sections. The resonance frequencies strongly depend on the particle shape and size as well as on the optical properties of the material within the near-field of the particle [12]. One of the most important methods of manufacturing nanoparticles is the pulsed laser ablation method in liquid (PLAL). If is currently explored as a prospective top-down (dispersion method) strategy of metals nanoparticles preparation. PLAL include focused laser pulsed irradiation of a bulk metal target in a liquid. Due to the absence of chemical precursors, and it is environmentally friendly, and has an easy experimental set-up [13]. The antibacterial activity of the nanoparticles can be attributed to their total surface area, as a larger surface to volume ratio of nanoparticles provides more efficient means for enhanced antibacterial activity [14, 15].

This work aimed at the synthesis of Au, Ag and Au: Ag bimetallic NPs using PLA technique in DI water. Then, the synthesized NPs were characterized by using different techniques. The antibacterial activities prepared NPs were used to study against human pathogenic bacterias of Staphylococcus aureus and Escherichia coli as references for Gram-negative and Gram-positive bacteria, respectively.

2. Experimental

The Au and Ag nanoparticles were produced by the pulsed laser ablation. The focused energy was 700 mJ /pulse and 1064 nm wavelength of Nd: YAG laser and the number of applied pulses 200 pulses, pieces of pure (99.9 %) gold and silver plates were placed on the bottom of Pyrex vessel containing 2ml of ultra-pure deionized water and the distance between the target and laser source is 5 cm. The bimetallic alloy (Au:Ag) was produced in two steps, 1. the piece of high purity Gold plates were placed on the bottom of Pyrex vessel containing 2ml of ultrapure deionized water and the distance between the target and laser source is 5 cm to prepared Au colloidal Nps. 2. Then the pure silver piece was placed in the gold colloidal Nps previously formed and applied the variables mentioned above to synthesis Au:Ag bimetallic Nps. Characteristics optical and structural properties of metallic and bimetallic

NPs were recorded using UV–Vis spectrophotometer Mega 2100 and XRD–6000 SHIMADZU x-ray diffractometer. The nanoparticles prepared samples were identified by the transmission electron microscope TEM type CM10 pw 6020, Philips-Germany. The TEM carbon grids were loaded into the sample. The images were obtained at an accelerating voltage of 60 kV, with maximum magnification of 64000x-180000x.

Both of *Staphylococcus aureus* and *Escherichia coli* clinical isolates were used as bacterial model for evaluating the antibacterial activity of Au, Ag and bimetallic Au:Ag nanoparticles. Antibiotic susceptibility was carried out for all isolated bacteria.

1. The tips of 4-5 isolated colonies of the bacteria were used to test tube containing 5 ml of sterile normal saline in a cell density equivalent to turbidity of McFarland tube No. (0.5) which approximately equal to bacterial cells density of $1.5x10^{8}$cells/ml.

2. A sterile cotton swab was dipped into the standardized bacterial suspension. The excess fluid was removed by rotating the swab firmly against the inside of the tube above fluid level. The swab was then streaked onto the dried surface of a Muller-Hinton plate in 2 different planes to obtain an even distribution of the inoculums.

3. The plate lids were replaced and the inoculated plates were allowed to remain on a flat and level surface undisturbed for 3-5 min to allow for the absorption of excess moisture.

4. With the sterile forceps, the selected discs were placed on the inoculated plate and pressed gently into the agar. Within 15 min the inoculated plates were incubated at 37 $^{\circ}$C for 18 -24 hr in an inverted position.

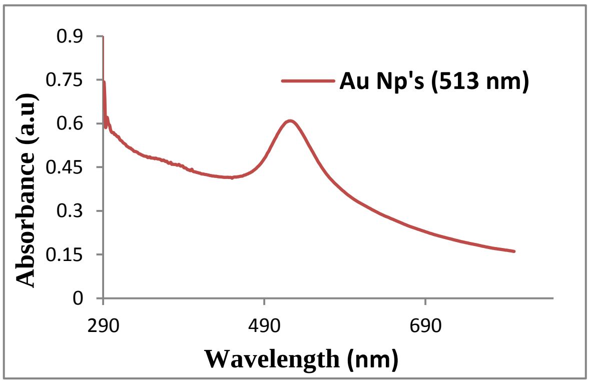
5. After incubation, the diameters of the complete inhibition zone were noted and measured using reflected light and a ruler. The end point, measured to the nearest millimeter, was taken as the area showing no visible growth.

6. The results were interpreted, the critical diameters and to the leaflet of antibiotics manufactures.

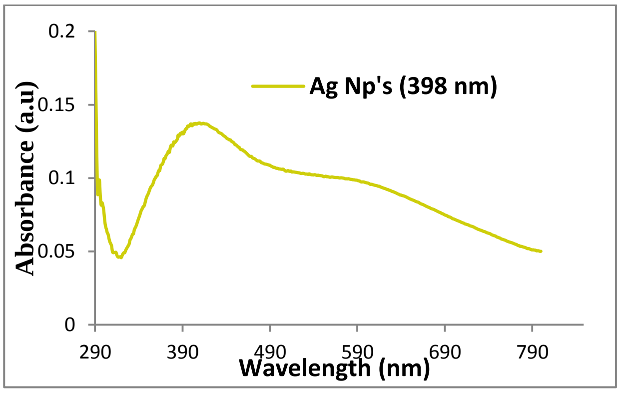
3. Result and Discussion:

3.1 Absorbance (SPR band)

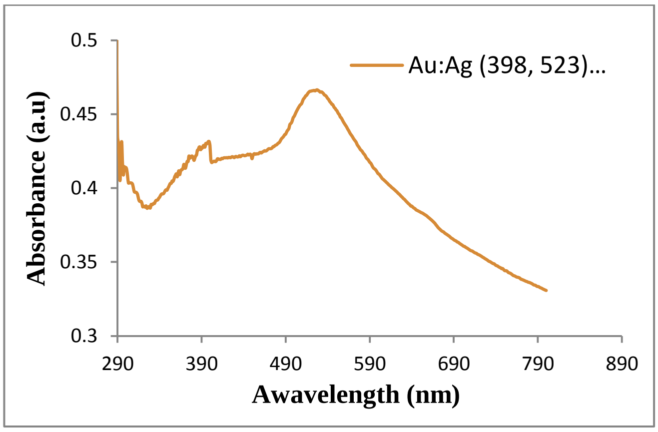
The surface Plasmon resonance was investigated by UV–Vis absorption spectra for Au, Ag and Au:Ag bimetallic NPs synthesis by PLAL, using Nd: YAG1064 nm with energy 700 mJ, frequency 5 Hz and 200 laser pulses at room temperature.



a



b



c

Figure 1: Optical Absorbance spectra (SPR band) of a. Au,b. Ag and c. Au:Ag NPs.

Gold (Au) metallic colloidal NPs were synthesized by pulsed laser ablation at room temperature. The absorption band of Au NPs has been reported in the range of 517- 575 nm [16, 17]. From Fig.(1a) it is noted that the absorbance spectra and the SPR band of Au Np's are shifted towards the short wavelength (blue shifted), and the absorbance (SPR band) for Au was in the wavelength 513 nm. This means that the size is very small in the range from 16 to 20 nm [17].

Silver (Ag) metallic colloidal NPs were produced by the pulsed laser ablation at room temperature. The absorption band (SPR band) of Ag Nps has been reported in the range 398 - 400 nm [6, 8]. From Fig.(1b) the absorbance spectra of silver NPs and the SPR band are shifted towards small wavelength. It can be noticed that the absorbance edge spectra for Ag is in 398 nm .This means that the size is small Ranging from 25 to 100 nm [17]. Au and

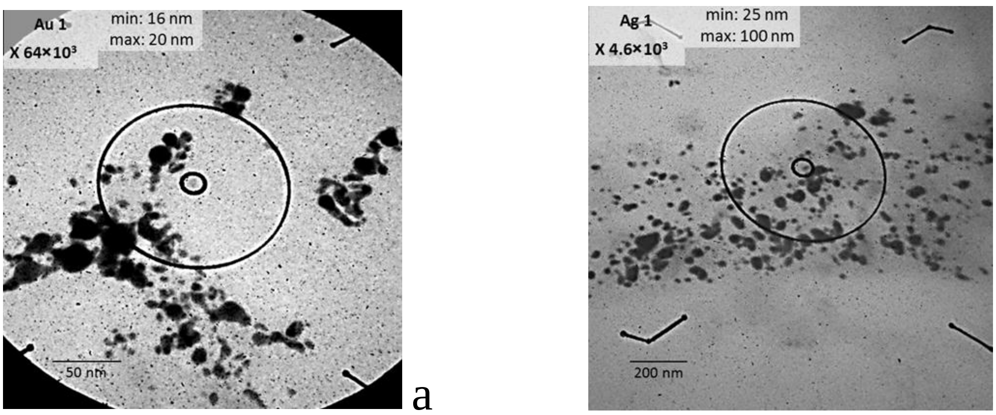
Ag NPs exhibit characteristic SPR peaks at about (400 nm and 520 nm) [6, 16].

Bimetallic (Au:Ag) colloidal NPs absorbance band (SPR band) reveals double resonance peaks (398 and 523) nm which indicating the presence of pure Ag (398 nm peak) and (Au 523 nm peak) structures as presented in the figure (1c). The results matched well with the composition alloy NPs [18]. All of these results confirm the formation of mono dispersed NPs in the current study.

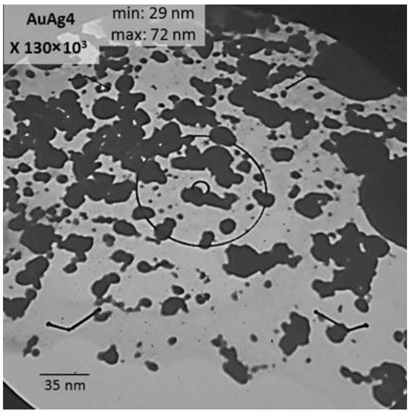
The presence of the single surface Plasmon peak implied that the formed nanoparticles were nearly spherical. In the case of ellipsoidal particles, the absorption spectrum would have two Plasmon peaks. The height and the width of the SPR peaks were found to be dependent upon the laser shots. The gold nanoparticles were faint pink in color due to Plasmon absorption .The losses in the ablation of Au compared with Ag are attributed to the large reflectivity from the metal surface [19].

3.2 TEM studies

Fig. (2a) Shows the TEM image of Au nanoparticles synthesis by laser ablation of gold plate immersed in 2 ml of DI water, at laser shots of energy 700 mJ and 200 pulses. The particle size of the synthesized NPs was calculated to have the average diameters between 16 and 20 nm. However, the laser irradiation of the nanoparticles can stimulate further changes of their morphology or can change the rate of their aggregation. The spherical shape of the produced Au Np's is clearly seen in TEM image presented.



b



c

Figure 2: TEM image of a. Au, b. Ag and c. Au:Ag NPs.

Fig. (2b) Shows TEM image of silver nanoparticles produced by the laser ablation of silver plate immersed in 2 ml of DI water, at laser shots of 700 mJ and 200 pulses. The prepared nanoparticles size was calculated to have the average diameters between 25 and 100 nm. It can be notice from the figure that the Ag NPs has a spherical shape.

Fig. (2c) shows the TEM image of colloid obtained after the laser irradiation. The clusters appear to be nearly spherical with size ranging from 29 to 72 nm for bimetallic (Au:Ag) NPs. The result was good agreement with those reported in literature [20] indicating the producing of bimetallic (Au-Ag) NPs core-shell Nano alloys [21] instead of separate Ag and Au nanoparticles.

3.3 XRD studies

The synthesized colloidal NPs were deposited on glass substrate by drop casting method. The structure property of prepared NPs films was investigated by X-ray diffraction. The XRD pattern of Au NPs illustrated in Fig.(3) which indicate that it has very low crystallinity and very low intensity peaks of crystal planes (111) and (200) referred to the face-centered cubic structure as compared with (JCPDS) data (card no.02–1095). Th**e** very low crystallinity of the deposited Au NPs film can be attributed to the low concentration from the number of laser pulses which lead to low film thickness [22].

The same result have been absorbed for Ag NPs film where the very low crystallinity and very low peaks intensity along (111) and (200) plans compared with the (JCPDS) data (card no. 04-0783) as shown in fig. (8). The very low crystallinity of the deposited Ag NPs film can be attribute to the low concentration from the number of laser pulses which lead to low film thickness**.**

The crystalline nature of bimetallic alloy was investigated by XRD analysis. Similar lattice constants of Au, Ag and the complete miscibility of these metals make their lattice planes so close and hard to distinguish. The XRD pattern of bimetallic Nps illustrate in fig. (9) the patterns shows very low crystallinity and low intensity peaks along (111) |and (200) lattice planes referred to the face centered cubic (FCC) structure compared with (JCPDS) cards data no.02–1095 and 04-0783 corresponding to Au, Ag [23].

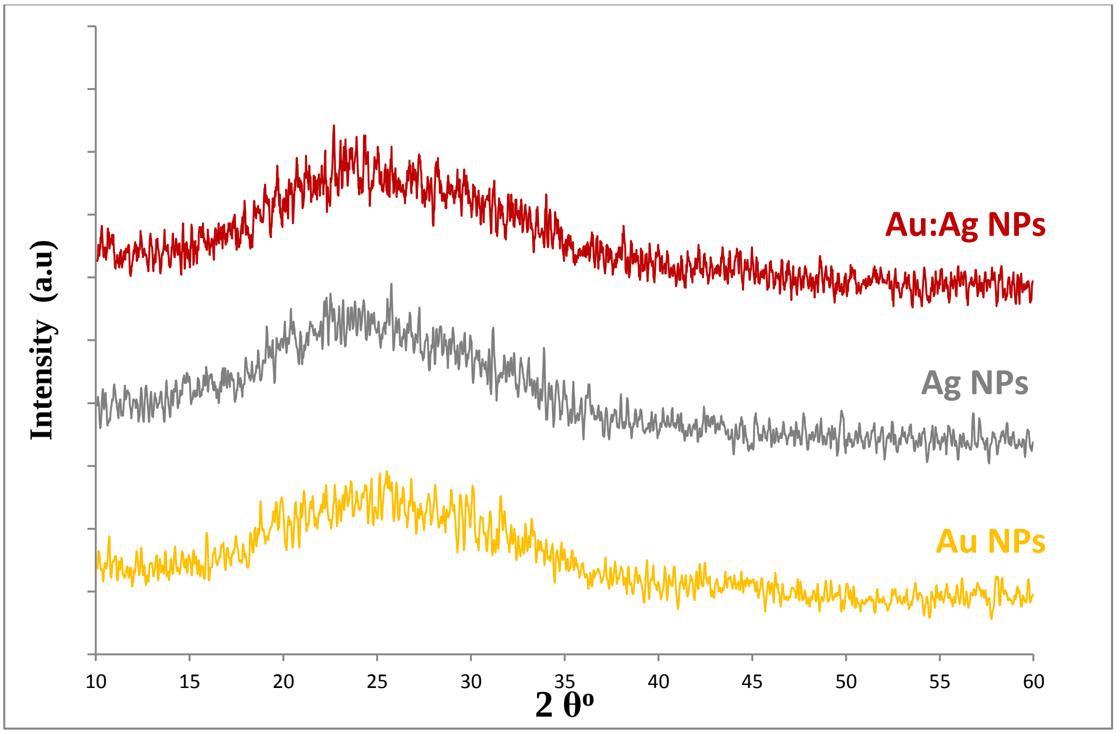
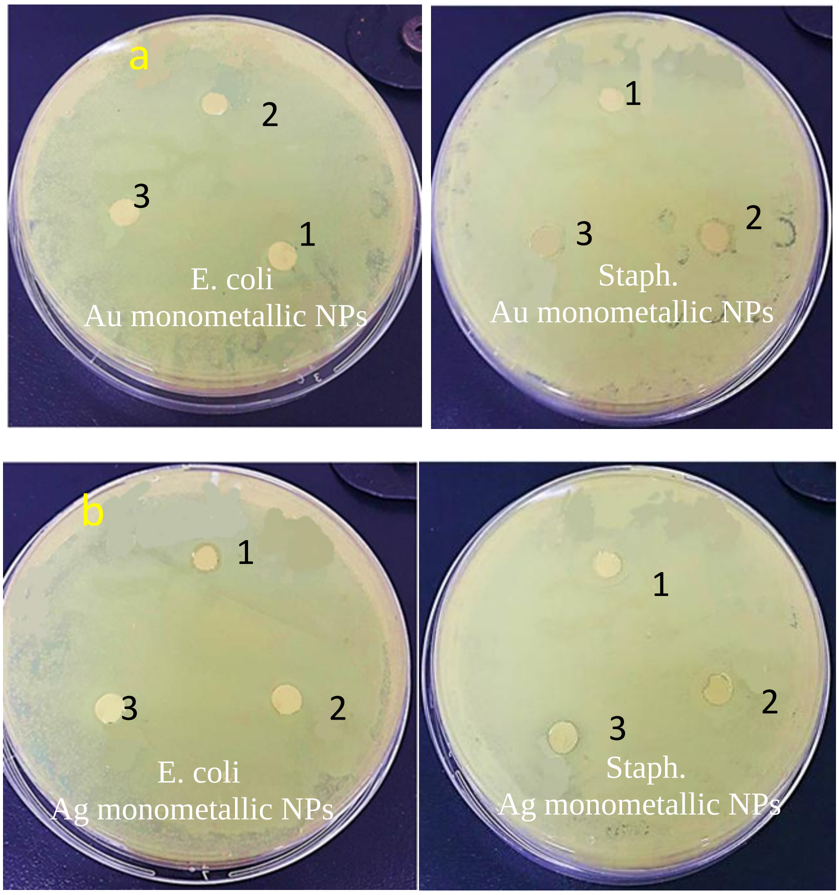


Figure 3: XRD pattern of Au, Ag and Au:Ag NPs.

3.4 Antibacterial activity

Antibacterial activities of Au, Ag and bimetallic (Au:Ag) nanoparticles were studied against Escherichia coli Gram-negative bacteria and Stphylococcus aureus Gram- positive bacteria . The nanoparticles are synthesized using laser energy of (700) mJ pulse, wavelength 1064 nm of Nd: YAG laser and the number of applied pulses (100, 150 and 200) pulses which they have relatively small sizes. They applied is to be tested against Staphylococcus aureus and Escherichia coli isolates which were illustrated. The results and images of the inhibition zones showed that the nanoparticles have synergistic effects on the studied Gram-positive isolate (Staphylococcus aureus) and on the Gram-negative isolate (Escherichia coli) that have low specific concentrations and relatively large sizes. When the number of laser shots increases, the concentration of the atoms ejected in solution increases, whereas the ejection rate decreases. The inhibition zone depends on several factors: increase in time of exposure and increase in the nanoparticles concentration in DI water. These factors and the concentration of bacteria and external conditions in the laboratory directly affect the inhibition zone. The Figures 10, 11 and 12 shows the antibacterial activity experiment results for Escherichia coli and Stphylococcus aureus. Table.1 illustrate the diameter zone of inhibition for E.coli and staghe bacteria by using different colloidal nanoparticles

From the table it can be seen that the diameter zone of inhibition for E.coli and staghe bacteria was increased by increasing the number of laser pulses. The largest diameter of the inhibition zone was 1.5 cm for Ag NPs synthesized at 200 pulses on E.coli bacteria and 1.2 cm on staghe bacteria. For bimetallic nanoparticles the largest diameter of the inhibition zone was 0.9 cm for Au:Ag NPs synthesized at 200 pulses on E.coli bacteria and 0.7 cm for Au:Ag NPs synthesized at 200 pulses on staghe bacteria .



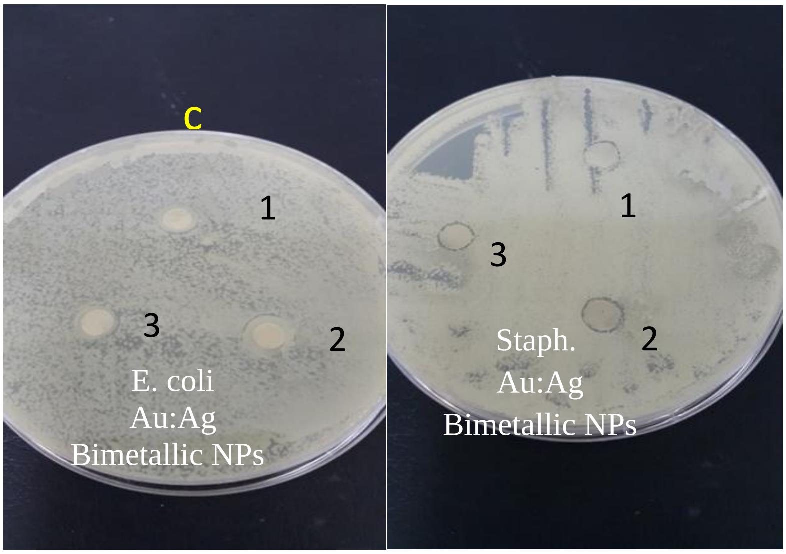


Figure 4: Antibacterial Activity of a. Au, b. Ag and c. Au:Ag NPs on Escherichia coli and Stphylococcus aureus with applied; 1. 100, 2. 150 and 3. 200 pulses.

Table 1: Diameter zone of inhibition for E.coli and Staghe bacteria by using different colloidal nanoparticles.

Metals Nanoparticles	E.coli diameter zone of inhibition (cm)			Staghe diameter zone of inhibition (cm)		
	100 Pulses	150 Pulses	200 Pulses	100 Pulses	150 Pulses	200 Pulses
Au	0.9	1	1.1	0.4	0.7	1
Ag	0.8	1	1.5	0.6	1.1	1.2
Au:Ag	0.6	0.8	0.9	0.6	0.7	0.7

4. Conclusions

Pulse laser ablation method is a simple and controllable process with a low cost. The present work confirms the success of the ablation in liquid method to synthesized gold, silver, suspension, in deionized water solution. Bifunctional Au:Ag NPs have been successfully synthesized in two steps.The optical absorbance SPR band, morphology, particles size and structure properties of the synthesized NPs were investigated. The results and images of inhibition zones show that nanoparticles have synergistic effect on the studied Gram-positive isolate (*Staphylococcus aureus*) and on the Gram-negative isolate (*Escherichia coli*) that have low specific concentrations and relatively large sizes. The largest diameter of the inhibition zone was absorbed for the NPs synthesized at highest number of laser pulses (200 pulses).

Acknowledgments

The authors would like to acknowledge the assistance offered by the Department of Physics and Department of Biology in the Faculty of Science, University of Kufa/Iraq.

5. Reference

[1] U. Kreibig, M. Vollmer, Optical Properties of Metal Clusters, Springer, Berlin, 1994.

[2] P. Sen, J. Ghosh, A. Abdullah, P. Kumar, Vandana (Preparation of Cu, Ag, Fe and Al nanoparticles by the exploding wire technique) Proc. Indian Acad. Sci. (Chem. Sci.) 115, (2003) 499–508.

[3] A. R. Siekkinen, J. M. McLellan, J. Chen, Y. Xia (Rapid synthesis of small silver nanocubes by mediating polyol reduction with a trace amount of sodium sulfide or sodium hydrosulfide) Chemical Physics Letters 432 (2006) 491–496.

[4] D. Dorranian, S.Tajmir, F. Khazanehfar (Effect Of Laser Fluence On The Characteristics Of Ag Nanoparticles Produced By Laser Ablation) Soft Nanoscience Letters, 3, pp.93-100 (2013).

[5] P. K. Jain, K. S. Lee, I. H. El-Sayed, , And M. A. El-Sayed(Calculated Absorption And Scattering Properties Of Gold Nanoparticles Of Different Size, Shape, And Composition: Applications In Biological Imaging And Biomedicine) J. Phys. Chem. B 2006, 110, pp.7238-7248.

[6] M .J. Haider, M. S. Mehdi(Effect Of Experimental Parameters On The Fabrication Of Silver Nanoparticles By Laser Ablation) Tech.Journal, Vol. 32,Part (B), No.4, (2014).

[7] A.K .Khan, R. Rashid, G. Murtaza and A. Zahra (Gold Nanoparticles: Synthesis And Applications In Drug Delivery) Tropical Journal Of Pharmaceutical Research July 2014; 13 (7): pp.1169-1177.

[8] T. M. Al-Nori (Antibacterial Activity Of Silver And Gold Nanoparticles Against Streptococcus, Staphylococcus Aureus and E.Coli) Al- Mustansiriya J. Sci Vol. 23, No 3, 2012.

[9] L. F. Hamza, I.M.Ibrahim(Preparation Of Silver Nanoparticles By Pulsed Laser Ablation In Liquid Medium) International Journal Of Engineering And Computer Science Volume - 3 Issue -9 September, 2014 pp. 8261-8264.

[10] S. Petrović, B. Salatić, D. Milovanović, V. Lazović, L.j. Živković, M. Trtica and B. Jelenković, Agglomeration in core-shell structure of Cu-Ag nanoparticles synthesized by the laser ablation of Cu target in aqueous solutions, J. Opt. 17 (2015) 025402 (6p).

[11] M. Vinod and K.G. Gopchandran, Bimetallic Au-Ag nanochains as SERS substrates, Current Applied Physics 15 (2015) 857-863.

[12] J. Prikulis, F. Svedberg, M. Kall (Optical Spectroscopy of Single Trapped

Metal Nanoparticles in Solution) Nano Letters 4 (2004) 115-118.

[13] Gerardo Palazzo, Gabriele Valenza, Marcella Dell'Aglio and Alessandro De Giacomo, On the stability of gold nanoparticles synthesized by laser ablation in liquids, Journal of Colloid and Interface Science 489 (2017) 47–56.

[14] Kohler, N.; Sun, C.; Wang, J.; Zhang, M. Langmuir 2005, 21, 8858- 8864.

[15] Zhen-Zhen Ma and Ren-Cheng Tang, Preparation of silver nanoparticles by using the hydrolyzates of poly (lactic acid) and their application for the antibacterial functionalization of poly (lactic acid) non-woven fabric, Mater. Res. Express 4 (2017) 035009.

[16] X. Huang, M. A. El-Sayed (Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy) Journal of Advanced Research 1 (2010) 13-28.

[17] Stephan Link and Mostafa A. El-Sayed, Size and Temperature Dependence of the Plasmon Absorption of Colloidal Gold Nanoparticles, J. Phys. Chem. B 1999, 103, 4212-4217.

[18] E. Messina, L.D' Urso, E. Fazio, C. Satriano, M.G. Donato, C.D'Andrea , O. M. Marag, P.G. Gucciardi, G. Compagnini and F. Neri, Tuning the structural and optical properties of gold/silver nano-alloy sprepared by laser ablation in liquids for optical limiting, ultra-sensitive spectroscopy, and optical trapping, Journal of Quantitative Spectroscopy & Radiative Transfer, 113 (2012) 2490-2498.

[19] Abdulrahman, A. , K., ("Preparation of Ag and Au Nanoparticles by Pulsed Laser Ablation in Liquids ". PhD Thesis the University of Technology (2010).

[20] P. Y. Lim, R. S. Liu, P. L. She, C. F. Hung, H. C. Shih (Synthesis Of Ag Nanospheres Particles In Ethylene Glycol By Electrochemical-Assisted Polyol Process) Chemical Physics Letters 420 ,pp.304-308, (2006).

[21] Barbic, M.; Mock, J. J.; Smith, D. R.; Schultz, S. J. J. Appl. Phys.2002, 91, 9341.

[22] Haoming Bao, Yingying Wang, Hongwen Zhang ,Qian Zhao, Guangqiang Liu, Weiping Cai (Ultrathin tin oxide layer-wrapped gold nanoparticles induced by laser ablation in solutions and their enhanced performances) Journal of Colloid and Interface Science 489 (2017) 92-99.

[23] Zahra Mohammadpour, Afsaneh Safavi, Seyyed Hossein Abdollahi(Chlorine triggered de-alloying of AuAg@Carbon nanodots: Towards fabrication of a dual signalling assay combining the plasmonic property of bimetallic alloy nanoparticles and photoluminescence of carbon nanodots) Analytica Chimica Acta 959 (2017) 74-82.