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Effect of Colloidal Gold Nanoparticles Preparation Conditions on Viability of MCF-7 Breast Cancer Cells

Israa Ali Hameed[1*], Ammer Ayesh Habeeb[2], J. Al-Zanganawee[2] and Esam H. Hummadi[3]**

[1]Diyala General Direction of Education – Diyala – Iraq
[2]Department of Physics – College of Science – Diyala University – Diyala – Iraq
[3]Department of Biotechnology – College of Science – Diyala University – Diyala – Iraq

[*]Israa-ali32@yahoo.com
[*]jasimmansoor13@gmail.com

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Abstract

This study investigates the effects of changes of laser ablation energy parameters on the structural and optical properties of colloidal gold nanoparticles (AuNPs) prepared using pulsed Nd:YAG laser system at wavelength 1064 nm of highly purified solid targets of Gold noble elements plate in distilled water and studying the effects of colloidal AuNPs on the viability of MCF-7 cancer cell lines. Different laser ablation energies (500, 600, 700 and 800) mJ at a fixed number of laser pulses of (100) pulses were used to prepare colloidal AuNPs nanoparticles. The optical and surface morphological characteristics of AuNPs were studied using UV-Vis Spectroscopy, and Field Emission Scanning Electron Microscopy (FE-SEM). The results of UV-Vis absorption spectra showed an individual absorption peak in the visible region for the colloidal AuNPs solutions which appeared at (523-529) nm. Also, UV-Vis Spectroscopy results showed an increase in intensity of the absorption peaks and the concentrations of the materials.

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The color of AuNPs solution was also changed from light to dark red with the increase in energy of the laser ablation. The results showed that the peaks of the surface Plasmon resonance peaks were shifted towards the longer wavelengths (converted to the red color). FE-SEM and TEM images analysis showed that most nanoparticle diameters were within the nanoscale. An increase in the average nanoparticle diameter was obtained with the increase of the laser ablation energy. The morphology of nanoparticles was almost spherical and homogenous with some agglomeration. The colloidal AuNPs showed *concentration-dependent manner* on the growth of MCF-7 breast cancer cells with IC_{50} value of about 12.98 mg/L.

Keywords: laser ablation, gold nanoparticles, MCF-7 Cancer cells, FE-SEM.

تأثير ظروف تحضير جسيمات الذهب الغروي على نمو خطوط خلايا سرطان الثدي MCF-7

اسراء علي حميد[1] ، عمار عايش حبيب[2]، جاسم محمد زنكنة[2]* و عصام حامد حمادي[3]

[1]المديرية العامة للتربية في ديالى – ديالى – العراق
[2]قسم الفيزياء – كلية العلوم – جامعة ديالى
[3]قسم التقانة الاحيائية – كلية العلوم – جامعة ديالى

الخلاصة

تضمن هذا العمل دراسة تأثير معلمات طاقة استئصال الليزر المتغيرة على الخواص التركيبية والبصرية لجسيمات الذهب النانوية الغروية (AuNPs) التي تم تحضيرها باستخدام نظام الليزر النبضي (Nd: YAG) ذي الطول الموجي nm (1064) على هدف من لوح عنصر الذهب النقي جدا داخل الماء المقطر ومن ثم دراسة تأثير جسيمات الذهب النانوية المحضرة على تثبيط خطوط الخلايا السرطانية MCF-7 . تم استخدام طاقات الليزر المختلفة للاستئصال mJ (500، 600، 700، 800) ونبضات ليزر ثابتة مكونة من (100) نبضة لتحضير الجسيمات النانوية AuNPs الغروية. درست الخصائص المورفولوجية الضوئية والسطحية لـ AuNPs باستخدام التحليل الطيفي للأشعة فوق البنفسجية والمجهر الإلكتروني الماسح بمجال الانبعاث (FESEM). أظهرت نتائج أطياف الامتصاص المرئي وفوق البنفسجي قمم امتصاص فردية في المنطقة المرئية للمحلول الغروي للذهب AuNPs، وتراوحت بين nm (523-529). كما أظهرت نتائج الطيف المرئي وفوق البنفسجي زيادة في شدة قمم الامتصاص وفي تركيزات المحلول الغروي وكذلك تغير لون محلول AuNPs إلى اللون الأحمر الداكن مع زيادة طاقة الاستئصال بالليزر وكانت ازاحة قمم رنين بلازمون السطح (SPR) نحو الأطوال الموجية الاكبر

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(الازاحة إلى اللون الأحمر). أظهر تحليل الصور FE-SEM و TEM أن معظم أقطار الجسيمات متناهية الصغر كانت في حدود المقياس النانوي. اوضحت النتائج زيادة في متوسط قطر الجسيمات النانوية مع زيادة طاقة الاستئصال بالليزر. وفضلا عن ذلك، فأن شكل الجسيمات النانوية كان في معظمها جسيمات كروية الشكل تقريبا، متجانسة مع وجود بعض التكتل. درس تأثير تركيز AuNPs على نمو خطوط خلايا سرطان الثدي MCF-7 واثبتت نتائج هذه الدراسة زيادة واضحة في معدل تثبيط الخلايا والتي تزامنت مع زيادة تراكيز جسميات الذهب الغروية في المحلول مع قيمة IC_{50} والتي تبلغ 12.98 mg/L.

الكلمات المفتاحية: الاستئصال بالليزر، جسيمات الذهب النانوية، خلايا سرطان MCF-7، المجهر الإلكتروني الماسح بمجال الانبعاث.

Introduction

Nanotechnology is a modern field of science that plays a dominant role in our life. Nanotechnology deals with the production and manipulation of a particle structure ranging from about 1 to 100 nanometers [1]. Nanotechnology has many applications in medical chemistry, atomic physics and other fields [2]. For example, the use of nanotechnology in health care is a promising branch of medical treatment due to nanoparticles' practical and cost-effective applications [3]. The nanoparticles of noble metals such as gold and silver are particularly versatile for a variety of biomedical applications including their highly sensitive diagnostic tests [4]. The reason why nanoparticles of noble metals are distinguished is because of their plasmonic frequency in the visible region that gives the colors and interesting optical properties and their unique interaction with light [5]. Gold nanoparticles (AuNPs) are used in treatments because of their distinctive characteristics of small size, high aspect ratio, high interaction with living cells, good stability at high temperatures and conveyance into the living cells [6]. The prepared nanoparticles are generally characterized by varying size and shape and are initially examined using visible ultraviolet spectroscopy [7].

Laser ablation of a solid target immersed in a liquid can be considered as one of the most important top-down method for synthesis of nano-materials [8]. This method gives a unique opportunity to overcome the toxic effect of nanoparticles on cells prepared by chemical methods [9]. In the present study, the toxicity effect of gold nanoparticles prepared by the liquid

pulsed laser ablation technique on human breast cancer cells has been evaluated. Breast cancer is one of the most prevalent malignant cancers in females causing high rate of mortality among women in the world [10]. This research aims to prepare and study the properties of colloidal gold nanoparticles by means of pulsed laser ablation in liquid (PLAL) and examine their effect on viability of breast cancer cell line.

Materials and Methods

a) Preparation of AuNPs Gold Nanoparticles

Gold nanoparticles were prepared by pulsed laser ablation in liquid (PLAL) of 99.9% high purity solid plate of gold target immersed in (8 mL) of distilled water (DW). The target was irradiated using a Q-switched ND: YAG laser with a wavelength of 1064 nm, 100 pulses and a frequency (1 Hz). The pulse duration was 10 nanoseconds and the distance between the laser source and the target was 8 centimeters. Different laser ablation energies (500, 600, 700, 800) mJ at fixed laser pulses of (100) pulse at room temperature were used to prepare the colloidal AuNPs. All the experiments were carried out at Department of Physics, College of Science, University of Diyala. Figure 1 illustrates the schematic diagram laser ablation system used in this study.

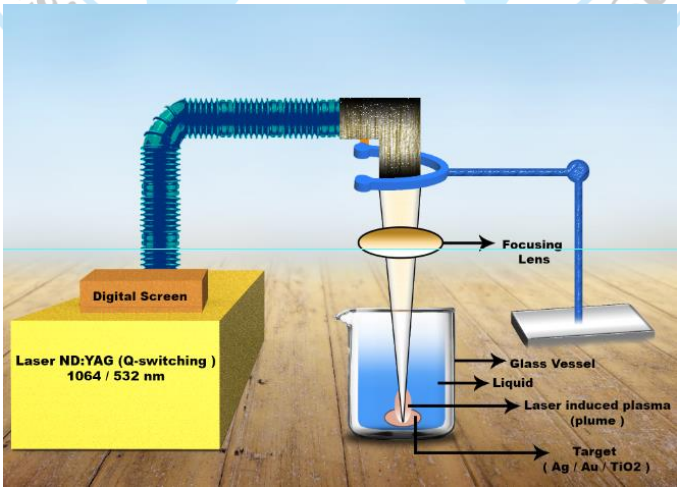

Figure 1: Schematic diagram of laser ablation in liquid system.

b) Characterization of Colloidal Gold Nanoparticles

The optical properties of the prepared colloidal AuNPs were studied using UV-Vis Spectroscopy. The shape and size of nanoparticles were tested by Field Emission Scanning Electron Microscopy (FE-SEM).

c) The Cytotoxicity Assay

The in vitro cytotoxicity of the colloidal AuNPs was tested on MCF-7 breast cancer cell line using MTT colorimetric assay. The MTT (methyl thiazolyl tetrazolium) is a rapid assay and widely used to examine cell growth rate and cytotoxicity of the tested compounds. Six concentrations of colloidal AuNPs ranging between 3.1 to 100 mg/L were prepared in RPMI 1640 medium with 10% fetal bovine serum. The concentrations of MCF-7 cells were adjusted to approximately 1×10^4 - 1×10^6 cells/well and transferred into a 96-well microplate and incubated in 5% CO_2, 95% air at 37°C for 24 hr. Then, 100 µL of each concentration of colloidal AuNPs solution was added into each well, and further incubated in 5% CO_2, 95% air at 37°C for 24 hr. A total of 10 µL of MTT solution (4 mg/ml) was added into each well and incubated again for 4 h. To stop the reaction and solubilize the formed formazan dye, 100 µL DMSO was added to each well and incubated again for 5 min. The absorbance of the microplate contents was measured by microplate reader at 570 nm (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Untreated cells were used as positive control. The growth inhibition percentage was calculated according to the following formula:

$$\boldsymbol{Inhibition\ rate}\ (\%) = (\boldsymbol{A_c} - \boldsymbol{A_s})/\boldsymbol{A_c} \times \mathbf{100}$$

Where, A_c is the absorbance value of the control and A_s is the Absorbance value of the tested NPs.

Results and Discussion

a) Spectroscopic analysis of Gold Nanoparticles

The optical property of nanoparticles is an important character for giving a particular information on some parameters such as particle shape, size, concentration and agglomeration state. At the surface, these particles interact with a specific wavelength of light and show maximum absorption at a given wavelength. As shown in figure 2, there is a wide range of *Surface Plasmon Resonance* (SPR) at (522-530) nm which is corresponding to the presence of gold particles with small dimensions in the solution which is in agreement with (Khalaf) [12]. The increase in ablation energy leads to increase the SPR peaks and absorbance spectrum of gold nanoparticles and this can be attributed to:

(1) Expulsion rate is low due to the absorption of the laser light by nanoparticles at the target surface which is considerably less efficient eradication.
(2) Alteration in the surface properties of the metal target by high laser pulses and thus the eradication efficiency is greatly reduced.
(3) The polarization of the solution was changed due to the presence of nanoparticles [13].

The color of colloidal solutions is determined by the size of the distribution and the state of the nanoparticle assembly. The red color of the colloidal gold indicates the *presence* of small particles while the dark red color indicates the *presence of* large aggregated nanoparticles [14]. Figure 3 illustrates the image of the gold nanoparticles samples prepared by ablation with different energies.

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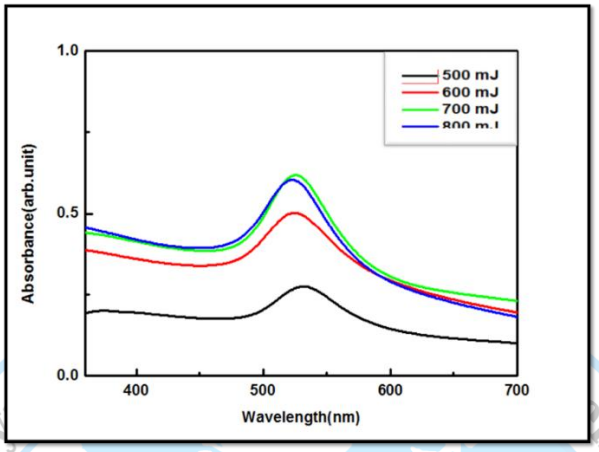

Figure 2: Absorption spectra of gold nanoparticles by laser ablation using different ablation energies.

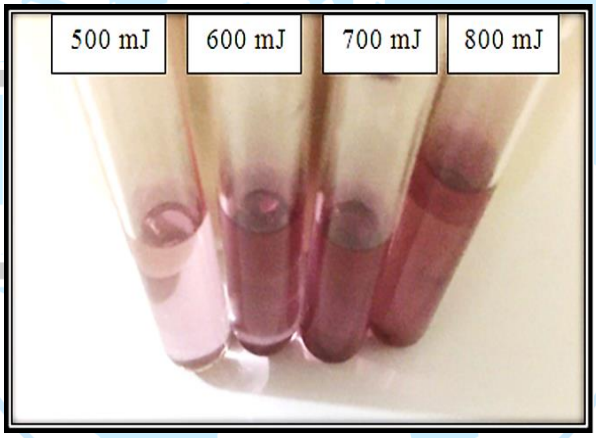

Figure 3: Gold nanoparticles solutions with different energies and constant number of pulses.

Table (1): Values of surface plasmon resonances (SPR) and the absorbance intensities of AuNPs at different Laser ablation energies.

Laser Energy (mJ)	SPR	Absorbance
500	0.291	529
600	0.511	523
700	0.648	524
800	0.631	524

b) Field Emission Scanning Electron Microscope (FE-SEM)

Figure 4 shows FE-SEM images of all samples obtained by laser ablation in distilled water at variable energies (500, 600, 700, and 800) mJ at number of pulses (100). The morphological

distribution of the nanoparticles can be observed in different sizes on the substrates. There was an increase in the size of the nanoparticles with the increase of laser energy. This can be due to the effect of laser energy on the plasma state during the laser ablation in clean water and this result is consistent with other research [15]. This is related to the overall process rather than the mechanism of nanoparticle formation. The data can be explained in terms of three mechanisms: (1) involves instantaneous start, short lifetime continuation, rapid plasma annihilation with high pressure and temperature; (2) the nucleation and species growth during and after annihilation, and (3) the oxidation and cooling of formed groups. From this it can be seen that the plasma state plays an essential and decisive role in such a situation of instantaneous formation. The morphology of the nanoparticles is controlled by laser energy which effects on the plasma condition, in particular, the age and density of the plasma [16]. All gold nanoparticles showed spherical shape.

c) Effect of gold nanoparticles on MCF-7 breast cancer cell line

The toxicity of colloidal gold NPs was examined at concentrations ranging between 3.1-100 mg / L on MCF-7 breast cancer cell line. As shown in Table 3, the cytotoxic effect of colloidal gold nanoparticles on the growth of MCF-7 cells was increased from 95.100% to 31.1% with increasing the concentration from 3.1 mg / L to 100 mg / L after 24 hr of incubation.

Table 2: Effect of different concentrations of colloidal gold nanoparticles on cellular growth of MCF-7 cell line

Dose (mg/L)	Viability % (Mean ± SM)
3.1	95.100 ± 1.659
6.2	85.918 ± 1.416
12.5	59.953 ± 2.039
25	46.759 ± 1.902
50	38.464 ± 3.136
100	31.134 ± 2.690

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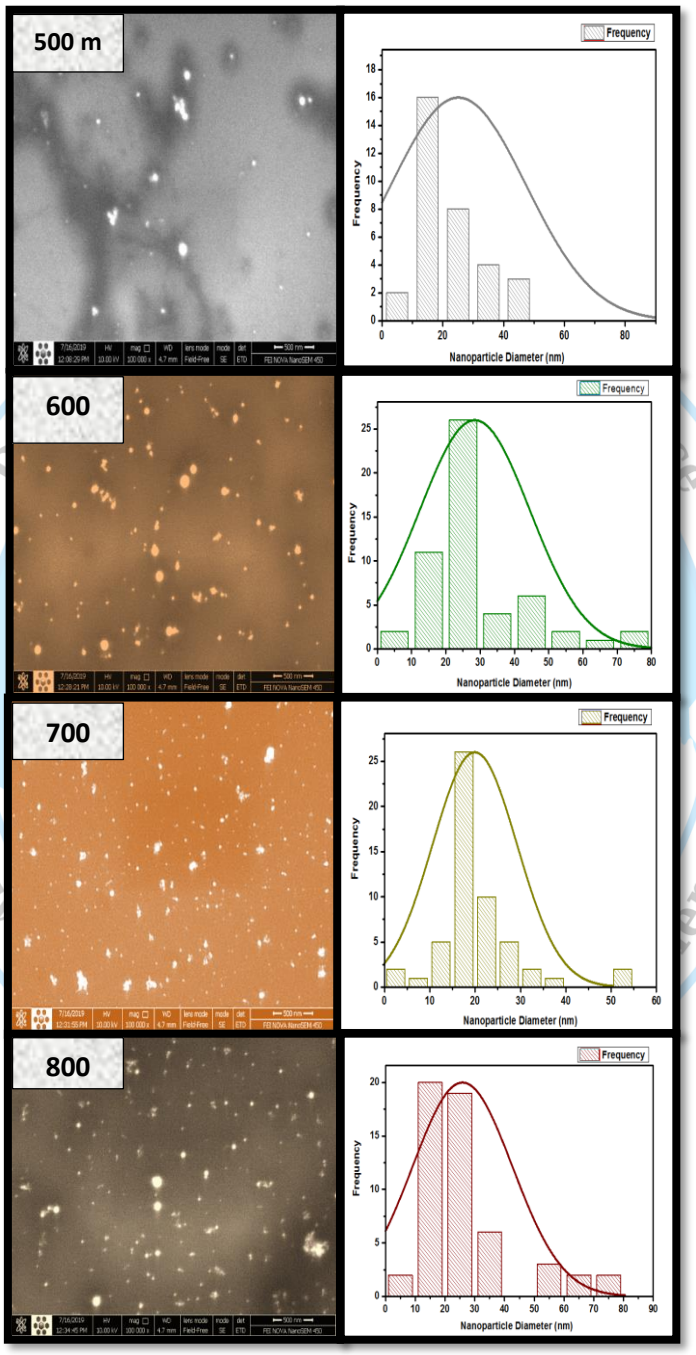

Figure 4: FESEM images and size distribution of gold nanoparticles at variable ablation energies and constant number of pulses

Figure 5 illustrates the effect of gold nanoparticles on the growth of breast cancer cell line. Colloidal gold nanoparticles showed concentration-dependent toxicity effect on cell line. The viability rate was decreased significantly from 100% to 0% at concentrations from 0.5 to 2 mg/L. The IC_{50} was found 12.98 mg/L.

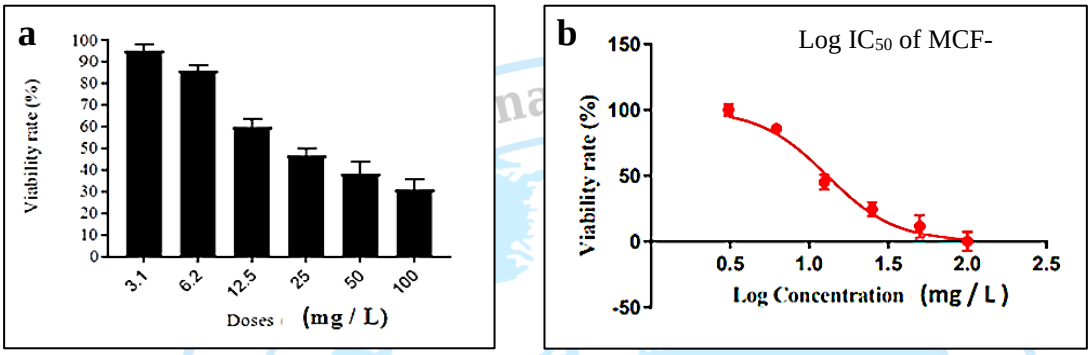

Figure 5: Concentration-dependent toxicity profile (a) and IC_{50} (b) of colloidal gold nanoparticles on MCF-7 cell line

Conclusion

The study showed the possibility to develop a method to target breast cancer cells by using colloidal gold nanoparticles.The different laser ablation energies resulted in synthesis of spherical and low agglomerated colloidal gold NPs, with moderate ranges of particles diameters distribution of prepared AuNPs at. Prepared colloidal AuNPs are able to pass through the cells by transport mechanisms such as diffusion around the center and edge of the culture wall or through its accumulation. This study suggests that colloidal gold nanoparticles can inhibit the growth of MCF-7 breast cancer cells.

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