Effects of Gold and Silver colloidal on Gama Glutamate Transferase Enzyme Activity in blood serum

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Received on: 26/1/2012 & Accepted on: 3/5/2012

ABSTRACT

Laser ablation of metal plate in water was performed in order to obtain gold and silver nanoparticles. Transmittance electron microscopy TEM and uv-vis spectrophotometer were conducted in order to determine the size and optical properties of the nanoparticles, respectively. The nanoparticles concentrations were also characterized by atomic absorption spectroscopy AAS measurement. The absorbance spectra show a sharp and peaks around 400 or 525 nm, indicating the production of silver and gold nanoparticles with average size of 25 nm, have been confirmed by TEM. Both Gold and silver nanoparticles show inhibition on the gama glutamate transferase (γ-GT) activity. The inhibition effect increase as a function of nanoparticles concentrations. Kinetic properties of (γ-GT) activity revealed (by nanoparticles) mixed type of inhibition.

تأثيردقائق الذهب والفضة النانوية على فعالية انزيم كاما كلوتاميت ترانسفريز في مصل الدم

الخلاصة

تم تحضير دقائق الذهب والفضة العالقة النانوية باستعمال تقنية الليزر النبضي بقصف هدف معدني بسمك مغمور في الماء المقطر. تم فحص الحجم والتوزيع الحجمي والخصائص البصرية للدقائق النانوية باستعمال تحليل مجهر الكتروني ماسح والمطياف على التوالي. وكذلك تم قياس تركيز الدقائق النانوية باستعمال طيف الامتصاص الذري .تم دراسة تأثير دقائق الذهب والفضة النانوية على فعالية انزيم كاماكلوتاميت الموجود في مصل الدم. اضهرت دقائق الذهب والفضة النانوية تأثير تثبيطي على فعالية الانزيم وزاد هذا التأثير بزيادة تركيز الدقائق النانوية.ومن دراسة الخواص الحركية للانزيم اضهرت تأثير تثبيطي من النوع المختلط.

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INTRODUCTION

Intensive investigations on metal nanoparticles suspended in solutions have been undertaken extensively because of their sizedependent characteristic properties. Laser ablation in liquid is one of the useful techniques to produce new materials. Recently, it has been reported that ablation of metal targets in water prepared colloidal solutions such as silver and gold nanoparticles [1–4]. Due to the unique photochemical and photophysical properties of nanoparticles differing from those of bulk, nanoparticles of metals are expected to be used as functional materials[5–8]. Moreover, it is also promising as a kind of 'green' process with little pollution of environment, as compared to chemical methods at the risk of using hazardous chemicals and/or releasing harmful byproducts [9–11].

Gama Glutamate Transferees (γ-GT) activity(EC 2.3.2.2) it's also called Gama Glutamate Transpeptidase (γ-GT) or (GGT) is found in kidneys and liver and catalyzes the transfer of a gamma-glutamyl group from glutathione (GSH)to an amino acid. GGT levels are increased in most forms of liver disease, especially cholestasis, a plasma membrane-bound enzyme, provides the only activity capable to effect the hydrolysis of extracellular glutathione, thus favoring the cellular utilization of its constituent amino acids [12].

The aim of this research to study the effects of nanoparticles of gold and silver on Gama Glutamate Transferase(γ-GT) enzyme activity ,and study the kinetics of the enzyme when the nanoparticels are found.

EXPERIMENTAL

Nanoparticles Preparation

Noble metal targets (Nilaco, 99.99%) were settled in a vesicle cell which contained 1 ml deionized water. The ablation was performed with the (1064 nm) of a Nd:YAG laser (HUAFEI), with a pulse width of 10 ns. Laser light was focused on the surface of the targets by using a quartz lens (10 cm). The solution was stirred during ablation with a magnetic stirrer. Observation of colloidal particles was performed by a TEM (CM10 pw6020, Philips-Germany). The solution was dropped onto a copper micro-grid, and dried in a decicator. This procedure was repeated until enough amounts of particles observed on the micro-grid, typically 2–3 times.. The size distributions were obtained by counting 100–200 particles in TEM images. Atomic absorption spectroscopy AAS (model GBS 933, Australia), was carried out to estimated the concentrations of prepared samples. UV–vis absorption spectroscopy measurements were carried out on a double beam, CECIL C. 7200 (France) spectrophotometer

Effect of Nanoparticles Au and Ag on Γ-Gt Activity

Kinetic colorimetric method for the determination of γ-GT activity was assayed by Persijn and Van Der Slik [13,14] . The principle of the method was the measurement of the 5-amino-2-nitro-benzoate form from reaction, which was absorbed at wave length 405 nm.

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L-γ-gluamyl-3-carboxy-4-nitroanilide +glycylglycine $\xrightarrow{\gamma\text{-GT}}$ L-γ-glutamyl - glycylglycine+5-amino-2-nitro-benzoate

A-.A stock solution (7 µg/ml) of nanoparticles of Ag was prepared and the following concentrations of (1,2,4,5,6,7) µg/ml were prepared, and a stock solution (7 µg/ml) of nanoparticles of Au was prepared and the (1,2,4,5,6,7) µg/ml concentrations were prepared by diluting with distilled water.The enzyme of γ-GT activity was measured in human serum by using the same method of this enzyme with replace 100µl of distilled water with 100µl of nanoparticles.

The inhibition percentage was calculated by comparing the activity with and without the nanoparticles and under the same conditions, according to the equation:-

% Inhibition = 100 -100 x activity in the presence of inhibitor/ activity in the absence of inhibitor.

B- To study the type of inhibition , a constant concentration of Ag nanoparticles (7 , 1) µg/ml and Au nanoparticles (7,1)µg/ml were used with different substrate concentrations of (0.4,0.8,1.2,1.6,2.0)mmol/L, (TRIS buffer pH 8.25 ,100 mmol/L) was used to prepare different substrate concentrations of γ-GT enzyme. The enzyme activity was determined with and without nanoparticles, by using the Lineweaver-Burk equation and plotting 1/v against 1/[s] were evaluated values [15] :-(a) ki, (b) Apparent vmax (v_{mapp}), (c) Apperent km (k_{mapp}), (d) Type of inhibition.

RESULTS AND DISCUSSION

Figure (1(A and B)) shows UV–vis spectra of colloidal solutions obtained by laser ablation of metal plates in water at various laser energy. The solution gradually turned to colored with the increase of the number of laser pulses. The absorption intensity of the plasmon band at 400 nm 526 nm indicating the formation of silver and gold nanoparticles, respectively[16,17]. The spectral shapes of the plasmon bands were almost identical among those colloidal solutions. For that reason, the increase in the absorption intensity of the plasmon bands implies that the formation efficiency of nanoparticles was increased. Moreover, all gold samples was around 520–530 nm, which was consistent with the presence of small 3–30 nm particles in the solution[18], which also confirmed by TEM.

. Figure 2 (A and B) shows TEM images and the particle size distributions of gold and silver nanoparticles, respectively. The nanoparticles thus produced were calculated to have the average diameters of 14 nm. It is observed that the average diameter and size distribution was increased with the increase of the laser energy. The silver nanoparticles prepared in water were more dispersed on the TEM grids than those gold nanoparticles. Upon laser ablation, various materials such as silver atoms, clusters, and droplets are emitted from the silver plate. Nanoparticles are formed via nucleation, phase transition, and crystal growth of these emitted substances[19]. On the other hand, when applied the colloidal solution on The biochemical samples of γ-GT enzyme, result revealed that the nanoparticles of Au and Ag caused inhibitory effects on the enzyme activity. The activity of enzyme as function nanoparticles concentrations were dedicated in Figures (1) and (2).

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Our results show that any increase in nanoparticles concentrations caused increase in enzyme inhibition. The greater inhibition of Au nanoparticles was 90% at concentration (7 µg/ml) and Ag nanoparticles was 75% at concentration (7 µg/ml), as shown in figures(3)and(4). Competitive, noncompetitive and uncompetitive inhibition can be easily distinguished with the use of double reciprocal plot of the Lineweaver-Burk plot. Two sets of rate determination in which enzyme concentration was held constant, were carried out. Varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzyme [20]. In the first experiment the velocity of enzyme without inhibitor was established, in the second experimental constant amount of inhibitor is included in each enzyme assay. Table(1) and figure (5) showed that the type of enzyme inhibitor using Lineweaver-Burk plot for Au nanoparticles on serum γ-GT activity. The Vmax and Km with (1 and 7) µg/ml of Au nanoparticles and without it, Km and Vmax without Au nanoparticles was 0.667 µg/ml, 20 U/L respectively. A liquate (1 and 7) µg/ml of Au nanoparticles were mix inhibition for enzyme activity. Mix inhibition changed the Km and the Vmax of the enzyme. When concentration of Au nanoparticles (1 and 7) µg/ml the Km were (0.77,0.91) µg/ml, Vmax were(0.33,0.72) U/L respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for compound which was studied in different concentration. The Ki of (1 and 7) µg/ml of Au nanoparticles were (7.14,2.17)µg/ml respectively.

Table(1) and figure (6) showed that the type of enzyme inhibitor using Lineweaver-Burk plot for Ag nanoparticles on serum γ-GT activity. The Vmax and Km with (1 and 7) µg/ml of Ag nanoparticles and without it , Km and Vmax without Ag nanoparticles was 0.667 µg/ml, 20 U/L respectively. A liquate (1 and 7) µg/ml of Ag nanoparticles were mix inhibtion for enzyme activity. Mix inhibition changed the Km and the Vmax of the enzyme. When concentration of Ag nanoparticles (1 and 7) µg/ml the Km were (2.0,2.5) µg/ml, Vmax were(0.92,9.09) U/L respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for compound which was studied in different concentration. The Ki of (1 and 7) µg/ml of Ag nanoparticles were (16.6,8.33)µg/ml respectively.

Moreover, The tripeptide glutathione (GSH) is used by cells to detoxify hydroperoxides, produced during oxidative stress, and is consumed in the process. Previous studies have indicated that cells can be protected against oxidative stress by extracellular GSH through its degradation catalyzed by the exoenzyme gamma-glutamyl transpeptidase (GGT) and its de novo synthesis within the cytosol[21]. Because of this, induction of GSH depletion has been proposed as a good strategy for sensitizing tumor cells to anti tumor agents[22] .

Heavy metales are toxic and react with proteins,therefore they bind protein molecules[8], heavy metales strongly interacts with thiol groups of vital enzymes and inactivates them [23,24] .We believed that the colloidal nanoparticles bind to functional groups of proteins,resulting in protein deactivation and denaturation [25,26,27].

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CONCLUSIONS

Certain pure colloidal nanoparticles can be formed by laser ablation in water. The formation rate, mean particle size and stability could be controlled by proper selection of the laser parameters such as laser energy and laser wavelength. Enzyme activity is related to human diseases. It is desirable to be able to inhibit enzyme activity and function. Nanoparticles incorporating enzymes also require that protein conformation is not altered. When used nanoparticles of Au and Ag in vivo for cancer detection and therapy, nanoparticles of Au and Ag inhibited (GGT) enzyme, therefore (GSH) would be increased as part of the adaptation of cells to oxidative stress. We suggest that nanoparticles colloidal interact with functional groups of GGT enzyme, resulting in protein denaturation and inactivate it, so nanoparticles of Au and Ag inhibited the enzyme. nanoparticles of Au was more interact with enzyme and inhibited it than nanoparticles of Ag , so we believe that used nanoparticles of Au for detection and therapy of cancer is better than nanoparticles of Ag.

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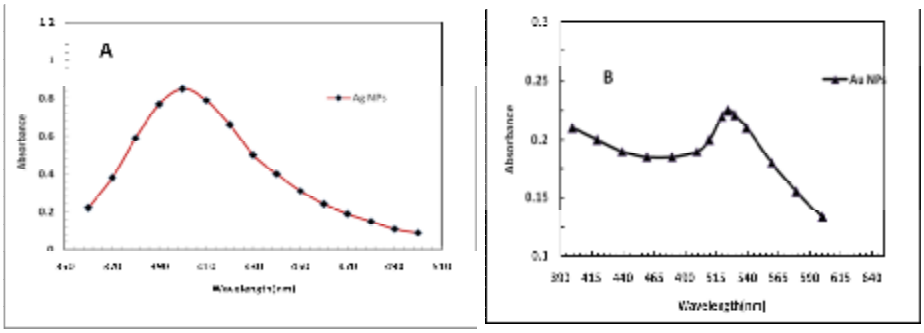


Figure (1): Absorbance spectra of silver nanoparticles (A), and gold nanoparticles (B), obtained by laser ablation of metal plates immersed in DDDW with laser energy of 600 mJ, laser shots of 15 pulses and wave length is 1064 nm of Nd-YAG.

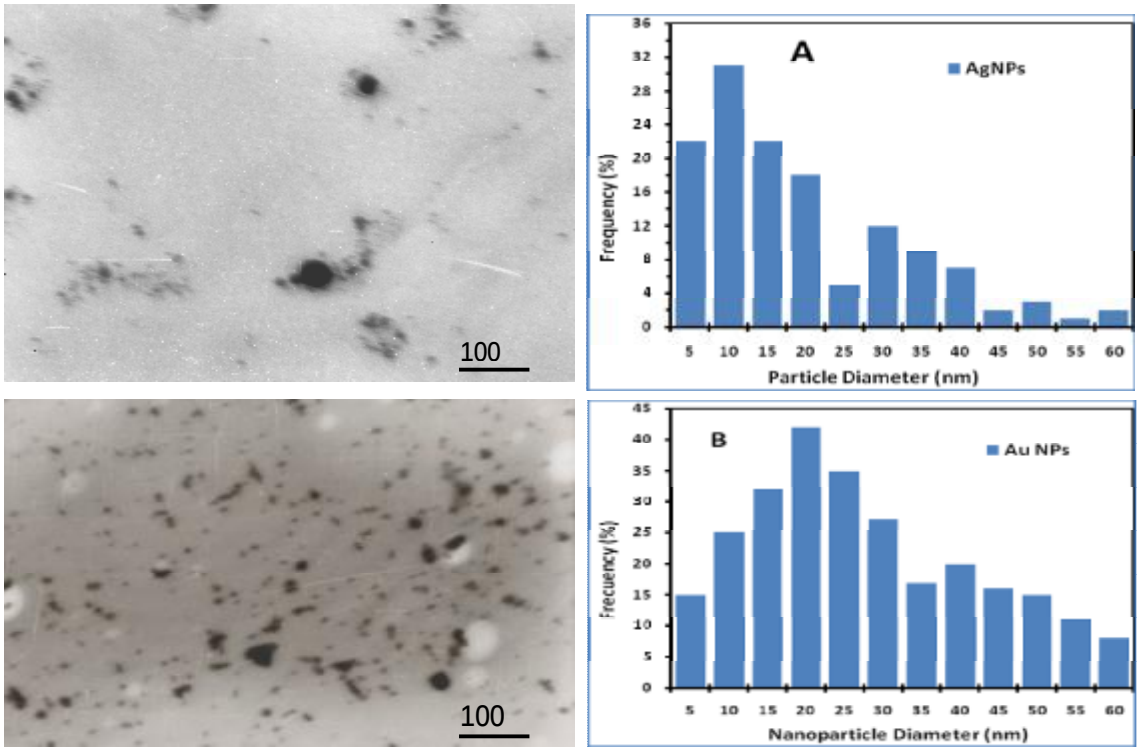


Figure (2): TEM images and size distributions of silver (A), and gold nanoparticles (B), produced by laser ablation of metal plats immersed in pure water, (λ=1064 nm and laser shots of 15 pulses).

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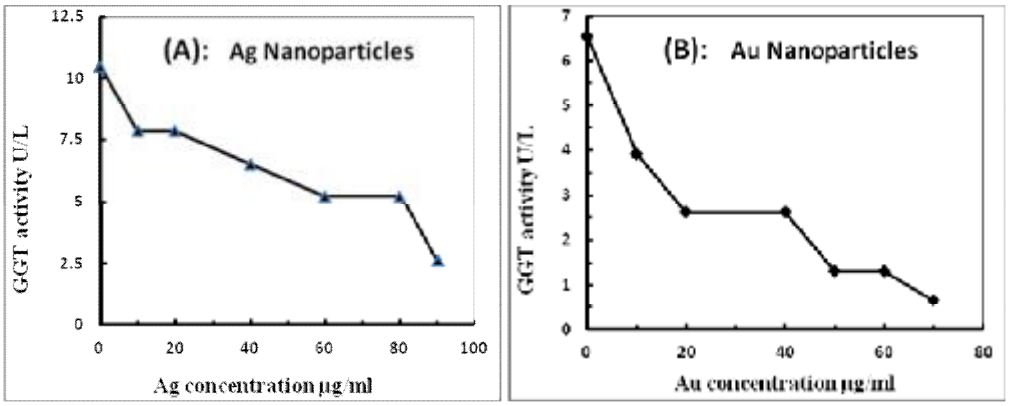


Figure (3):- γ-GT enzyme activity as a function of concentration of silver nanoparticales (A) and gold nanoparticles (B), respectively.

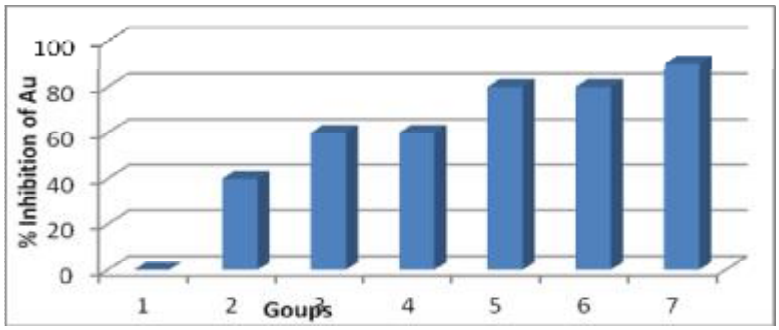


Figure (4):-% Inhibition of γ-GT enzymeand Au particales concentrations.

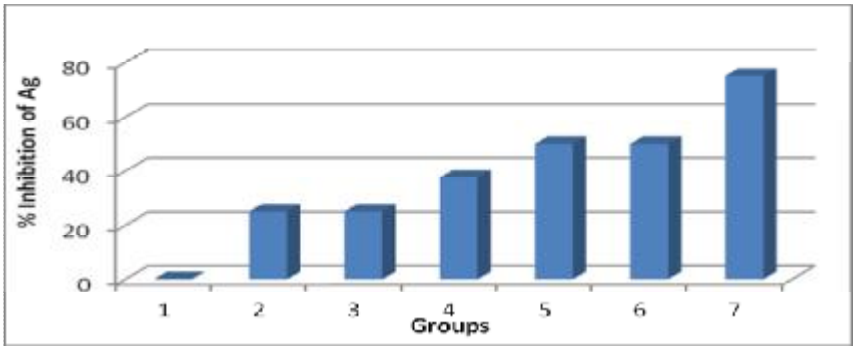


Figure (5):-% Inhibition of γ-GT enzyme and Ag nanoparticles concentrations.

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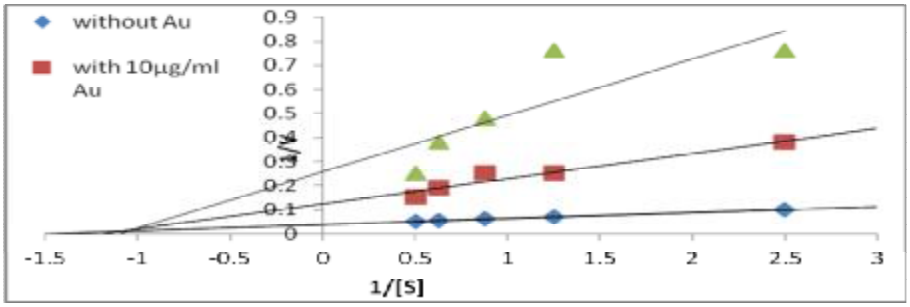


Figure (6):-Lineweaver-Burk plots for Au nanoparticales effects on γ-GT.

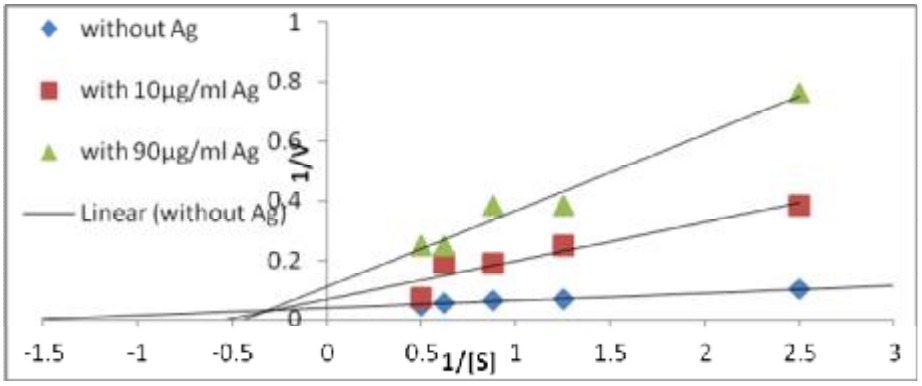


Figure (7):-Lineweaver-Burk plots for Ag nanoparticales effects on γ-GT.

Table (1):-The kinetic properties of γ-GT with Au and Ag nanoparticles.

1	concentration µg/ml	K_{map} (µg/ml)	V_{map} U/L	Ki (µg/ml)	Type of inhibition
Au	10	0.77	0.33	7.14	Mixed
	70	200	0.72	2.17	
Ag	10	2.0	0.92	16.6	Mixed
	90	2.5	9.09	8.33	

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