

Inhibition Effect of Noble Metals Nanoparticles on Acid Phosphate Activity in Sera of healthy subject

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ABSTRACT:

Noble metals nanoparticles have been prepared by laser ablation in water. The structural and optical properties of the Ag and Au nanoparticles have been investigated using (UV-Vis) spectrophotometer and TEM. The produced nanoparticles show small and sharp plasmon peaks around 400nm and 500 nm for silver and gold respectively. The nanoparticles have spherical shape with average size of about 30 nm. The effect of gold and silver NPs was studied on the activity of acid phosphatase (ACP) in blood sera of healthy subjects. The result correlated with the observation that gold and silver nanoparticles had inhibition effect on serum acid phosphatase activity, and this effect increased with increasing the concentration of the nanoparticles.

Keywords: (Au, Ag) nanoparticles, Acid phosphates, Serum.

دراسة تثبيط جسيمات المعادن النانوية النبيلة على انزيم ACP في المصل لعينات سليمة

الخلاصة

حضرة الجسيمات النانوية النبيلة بطريقة بواسطة القشط بالليزر في الماء. تم دراسة الخصائص البصرية والتركيبية للجسيمات المحضرة بواسطة المطياف والمجهر الالكتروني النافذ. الجسيمات النانوية تظهر حزمة بلازمونية حادة حول ال 400 و 520 نانومتر للفضة والذهب على التوالي وكذلك تمتلك حجم نانوي كروي بحدود 30 نانومتر. تم دراسة تأثير الذهب والفضة النانوية على انزيم ال ASP في مصل الدم لعينات صحية. اظهرت نتائج الارتباط ان جسيمات الفضة والذهب تعمل على تثبيط فعالية الانزيم وهذا التثبيط يزداد بزيادة تركيز الجسيمات النانوية.

INTRODUCTION:

Noble metal nanoparticles such as Ag and Au NPs have been a source of great interest due to their novel electrical, optical, physical, chemical and magnetic properties [1,2]. They were very attractive for biophysical, biochemical, and biotechnological applications due to their unusual physical properties, especially due to their sharp plasmon absorption peak at the visible region. Also, Ag and Au NPs prepared by PLAL process were stable for a period of months. Additionally, Gold and silver nanoparticles exhibit surface enhanced Raman scattering SERS in the visible wavelength range, where they may cause a tremendous increase in various optical cross-sections. The resonance frequencies strongly depend on particle shape and size as well as on the optical properties of the material [3].

In the recent past nonomaterials emerged and took their place in lots of field, such as electronics, optics, cosmetics, food....etc. Nano materials were also used in medicine and diagnostics. This dramatic increase of industrial production and use of nanomaterials has led to investigate their effects. Indeed the Nano toxicology is a field well established. Studies of the effects of nanoparticles (NPs) from different industry branches on cells and path ways are emerging, and most of the biological effects of NPs seem due to their interactions with proteins[4-8]. Enzymes are important group of biomolecules synthesized by the living cells. They are catalysts of biological systems (hence are called as biocatalyst), colloidal, thermo labile and protein in nature [9].

Acid phosphatase enzymes as alkaline phosphatase (ALP) and is a hyolrolase that catalyzes the same type of reactions. The major difference between ACP and ALP is the PH of the reaction. ACP functions at an optimal pH of approximately 5.0. Activity of acid phosphatase (ACP) is found in the prostate, bone, liver, spleen, kidney, erythrocytes, and platelets. The prostate is the richest source, with many times the activity found in other tissue. Historically, ACP measurement has been used as an aid in the detection of prostatic carcinoma, particularly metastatic carcinoma of the prostate. Total serum ACP activity raised in Paget's disease of bone, some cases of metastatic bone disease especially with osteosclerotic lesions, Gaucher's disease, and occasionally in thrombocytheamia or polycythaemia.

Until now, a few studies focus on the effect of NPs on enzymes activities, we did not see any study about the effect of gold and silver NPs on ACP activity in serum; therefore, we investigated the effect of these particles on ACP activity in human sera in vitro.

Experimental Nanoparticles Formation

Figure:1 shows the schematic diagram of a simple set up for laser ablation in liquid. Q-switched Nd/YAG laser system type HUAFEI providing pulses of 1064nm wavelength with maximum energy per pulse of 1000 mJ, pulse width of 10 ns, repetition rate of 10 Hz and effective beam diameter of 5 mm, was used for laser ablation. The laser is applied with a lens with 110 mm focal length is used to achieve high laser fluence. Absorbance spectra of NPs solution were measured by UV-VIS double beam spectrophotometers, CECIL C. 7200 (France) and SHIMADZU. All spectra were measured at room-temperature in a quartz cell with 1 cm optical path.

Atomic absorption spectroscopy AAS measurement was carried out for the prepared samples using AAS spectrometer model GBS 933, Australia. Structure and nanosize measurement of nanoparticles samples were identified by the transmission electron microscope TEM type CM10 pw6020, Philips-Germany (electronic microscope centre-collage of medicine/ Al-Nahrien University). The test samples were prepared by placing a drop of suspension of interest on a copper mesh coated with an amorphous carbon film. The drop was dried with an infrared lamp (Philips, 100 W) until all the solvent had evaporated. This process was repeated three to four times.

Enzyme activity assay:

End point method developed by Fishman and optimized by Richterich .In an acid medium, acid phosphatase (EC: 3.1.2)in human serum hydrolyses paranitrophenyl phosphate to paranitrophenol and phosphate. The reaction is stopped in alkaline environment. The intensity of the colored complex which absorbance is proportional to the acid phosphatase activity is measured at 405 nm [10]. A kit from Biolaboreagents(France) have used for measuring the enzyme activity.

Samples of Sera:

The subject of this study was randomly selected from staff of basic science Department, college of Dentistry, University of Baghdad.Blood samples were obtained by rein puncture and allow clotting at room temperature, then the samples were separated by centrifugation at 1500xg for 15 minutes, the sera were removed from the clots and placed immediately in plastic tubes.

Effect of gold and silver nanoparticles on acid phosphatase activity:

From astock (15µg/ml) concentration of silver NPs and a stock (20µg/ml) concentration of gold NPs , the following concentrations (0.94,1.9,4.7,5.7)µg/ml of silver NPs and (1.3,2.5,6.3,10.0)µg/ml of gold NPs were prepared as a final concentration on the total reaction mixture by diluting with deionized water. The inhibition percentage was calculated by comparing the activity with and without the gold or silver NPs and under the same conditions according to the following equation:

$$\text{Inhibition} = 100 - 100 \times (\text{Activity in the presence of inhibitor} / \text{Activity without the inhibitor})\%$$

A constant concentration of Au NPs (7.6 µg/ml) and Ag NPs (5.7µg/ml) were used with different substrates concentrations (0.9, 1.7,2.1,2.6,2.9)mmole/L as a final concentrations in the reaction mixture. The enzyme activities were determined with and without the NPs using the lineweaver-Burk equation: [10]

$$1/v = (k_m/V_{max})1/[s] + 1/V_{max}$$

where

v is measured velocity of reaction (enzyme activity),

V_{max} is maximum velocity(maximum enzyme activity),

[s] is substrate concentration,

and k_m is Michaelis-Menten constant of enzyme for specific substrate and plotting 1/v against 1/[s].

Values of apparent V_{max} , apparent k_m and type of inhibition were evaluated. The simple types of inhibition may be classified by examination of the kinetic effect on the k_m and V_{max} .

Results and Discussion:

Figure (2) shows the colloidal nanoparticles produced by laser ablation of pure metal plate of gold and silver immersed in pure water exposed by 100 laser pulses, with laser fluence ($F= 40 \text{ J/cm}^2$, the laser spot size is 1.27 mm), at laser wavelength of 1064. We observed a visible coloration of the solution after several pulses during the experiment. The color of solutions is faint pink for gold nanoparticles and yellow color for silver nanoparticles. The color of metal nanoparticle is resulted from the coherent oscillation of the conduction band electrons for metallic nanoparticles can be induced by the interacting electromagnetic field, which is named as surface plasmon extinction [11].

Fig. 3 (A and B) shows the UV-VIS absorption spectra that indicated the characteristic absorbance feature of silver and gold nanoparticles, respectively. This was carried out by pulsed laser ablation of a metal plate in water. A focused Nd-YAG laser operated at 1 Hz with a wavelength of 1064 nm was vertically irradiated onto a metal plate placed in the aqueous solution. The laser energy of 600 mJ was employed to ablate a target.

The products formed in the ambient liquid were transparent just after ablation, and then changed to contaminated ones after more application of NPs. Fig. 3-A shows absorption spectra of gold nanoparticles, the surface plasmon related peak could be clearly distinguished. This peak was around 530 nm, which was consistent with the presence of small Au nanoparticles in the solution particle size (3-30) nm [12]. Fig. 3-B shows UV-VIS absorption spectra of Ag NPs. All the spectra exhibit a characteristic peak around 400 nm, indicating the formation of Ag nanocolloids [13].

Figure 4(A and B) shows TEM pictures and size distributions of gold and silver nanoparticles, produced by laser ablation of metal plates immersed in pure water; the laser wavelength is 1064 nm. The nanoparticles thus produced were calculated to have the average diameters of 30 nm at the laser energies 600 mJ. The average particles sizes increase and the size distribution broadens with an increase of applied laser energy. The origin of the surface morphology of the irregularly shaped particles in case of high energy can be explained by absorption by defects and thermally induced pressure pulses which cause cracking [14]. This fragmentation mechanism explained the variation in size distribution. Therefore the population of particles smaller than 10 nm increased markedly in solution when laser energy decrease, compared to higher laser energy. However the density of the ablated species can be changed by adjusting the laser energy.

The biochemical tests revealed that NPs of Au and Ag caused inhibition effect on acid phosphatase activity, the relationships between NPs of Au and Ag concentrations versus the activity of enzyme are shown in figures 5 and 7. These results observed that any increase in nanoparticles of Au and Ag concentration caused increasing in percentage of inhibition of enzymes. The greater inhibition of Au NPs on enzyme activity was 5% at concentration (5.7) $\mu\text{g/ml}$ and Ag nanoparticles was (5.8)% at concentration (10) $\mu\text{g/ml}$, as shown in figures. Competitive, noncompetitive and un competitive 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. 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. Varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzyme.

Table (1) and fig.(7) showed that the kinetic parameters k_{mapp} , v_{maxapp} and type of enzyme inhibition using lineweaver-Burk plot for Au on serum acid phosphatase. Fig.(8) showed type of enzyme inhibition using line weaver-Burk plot for Ag NPs on serum acid phosphatase. The v_{max} and k_m without Au nanoparticles were 0.7U/L, 1.25mmole/L respectively. A liquid 7.6 μ g/ml of Au NPs was mixed inhibition for enzyme activity and 5.7 μ g/ml of Ag NPs was uncompetitive inhibition for acid phosphatase activity.

Heavy metals are toxic and react with proteins, therefore they bind protein molecules, and heavy metals strongly interact with thiol groups of vital enzyme and inactivate them [15]. In addition, it is believed that Ag and Au bind to functional groups of proteins, resulting in protein deactivation and denaturation[16, 17].

We hypothesized that nanoparticles of Au and Ag interact with functional groups of acid phosphatase, resulting in protein inactivation, so nanoparticles of Au and Ag inhibited the enzyme activity. The present work is the first study that demonstrates the effect of gold and silver nanoparticles colloids on the activity of acid phosphatase. Recent researches demonstrated that gold nanoparticles colloids can be used in diagnosis and treatment of some kinds of cancer [18-20].

Gold nanoparticles had attracted a continuous interest due to their unusual properties in DNA hybridization [21-23], and biocatalysts [24]. Moreover, the effectual role of gold nanoparticles as an anti-oxidative agent, by inhibiting the formation of reactive oxygen species (ROS), scavenging free radicals; thus increasing the anti-oxidant defense enzymes and creating a sustained control over hyperglycemic conditions which consequently evoke the potential of Au nanoparticles as an economic therapeutic remedy in diabetic treatments and its complications [25].

In recent year, incorporation of nano-silver into medical products has been of great interest. Properties of nano-structured silver can be controlled and tailored in a predictable manner and impart them with biological properties and functionalities that can bring new and unique capabilities to a variety of medical applications ranging from implant technology and drug delivery, to diagnostics and imaging[26]. Several mechanisms have been postulated for the antimicrobial property of Ag-nanoparticles [27-29].

Conclusion

Only a few studies are conducted on the crucial metabolism enzyme and enzyme dysfunctions, which are related to various pathologies [20,31]. Therefore, it was useful to know what is the effect of gold and silver NPS on activity of acid phosphatase in serum. The present work is the first study that demonstrates the effect of gold and silver nanoparticles colloids on the activity of acid phosphatase. Results showed that Au and Ag NPs had inhibition effect on the enzyme activity, and this effect increased with increasing the concentration of the nanoparticles. The nanoparticles induced protein modifications are promising fields for future research. Proper understanding of such phenomenon is further emphasized by the fact that these materials are utilized for diagnostic and therapeutic purposes.

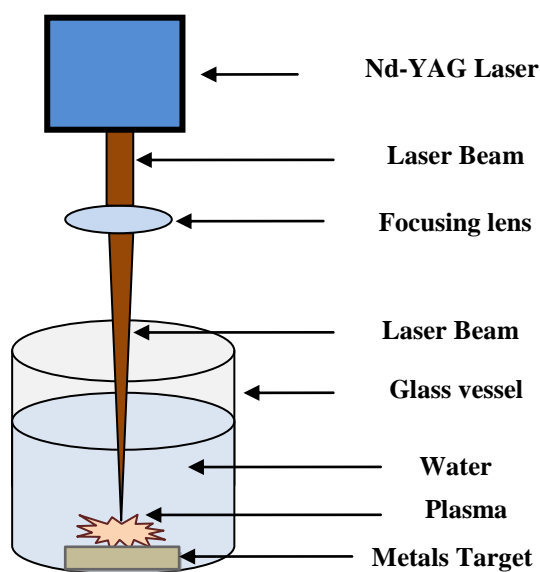


Figure (1): schematic diagram of the set-up of nanoparticles synthesis by laser ablation technique

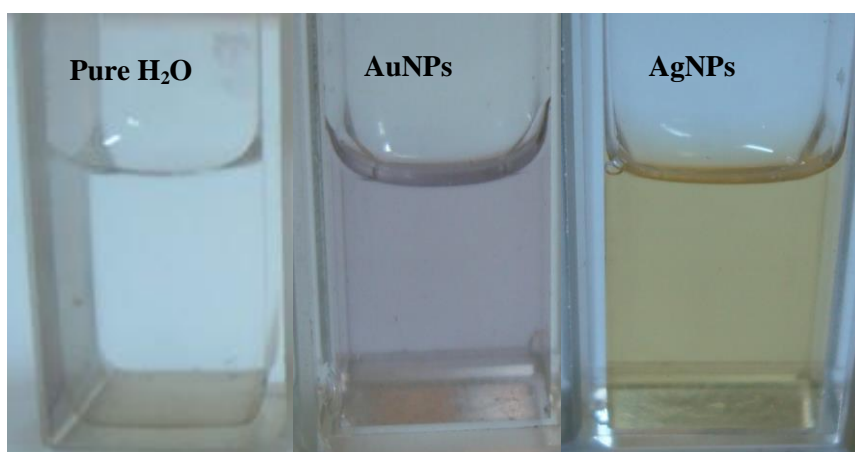


Figure (2): A Colloidal of gold and silver nanoparticles solutions

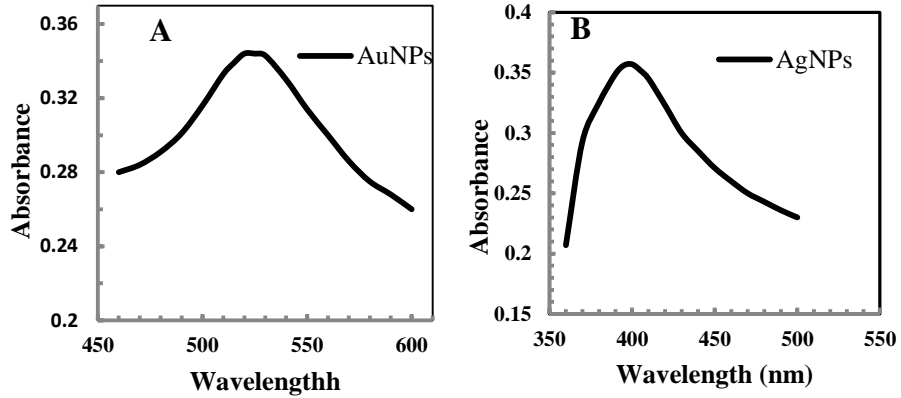


Figure (3): Absorbance spectra of the gold (A), and silver (B) nanoparticles, obtained by laser ablation of metal plates immersed in pure water. The laser shots are 90 pulses at laser energy of 600 mJ and $\lambda=1064$

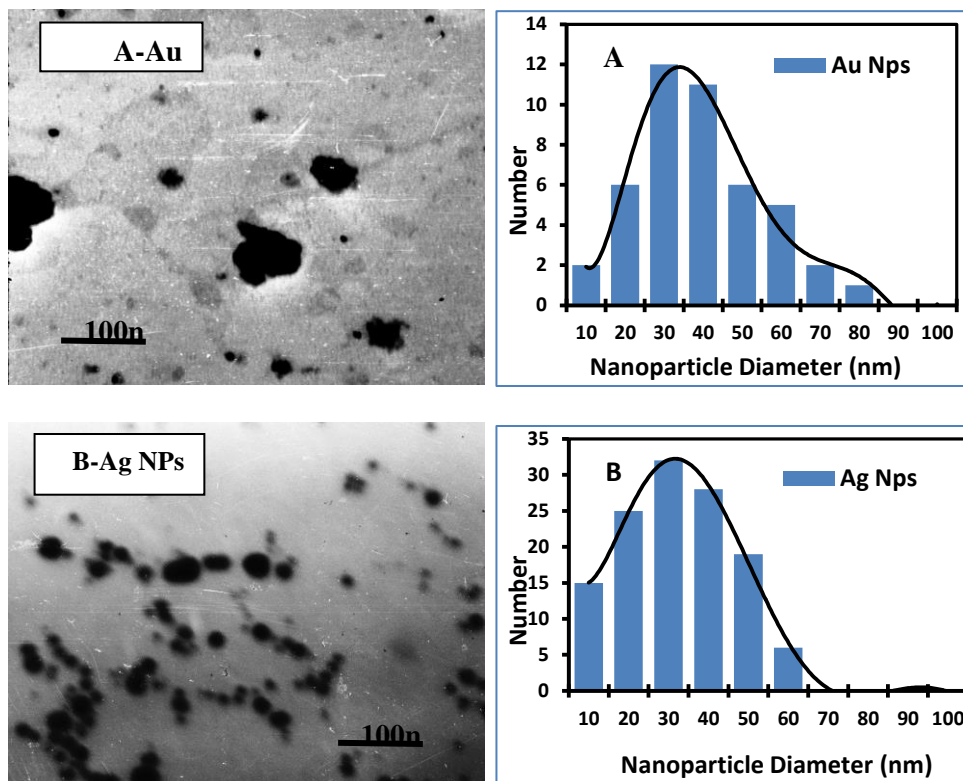


Figure (4): TEM images and size distribution of gold (A) and silver(B) nanoparticles produced by 1064-nm laser ablation ($E=600$ mJ/pulse) of metals plate immersed in 1ml of pure water.

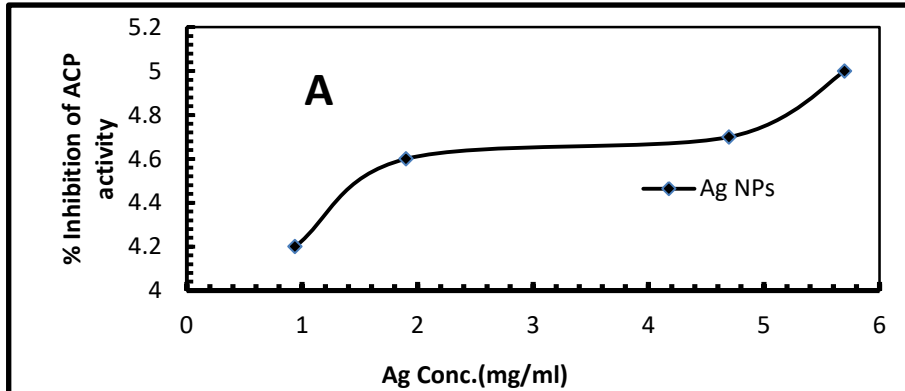


Figure (5) %Inhibition of ACP activity with Ag nanoparticles concentration.

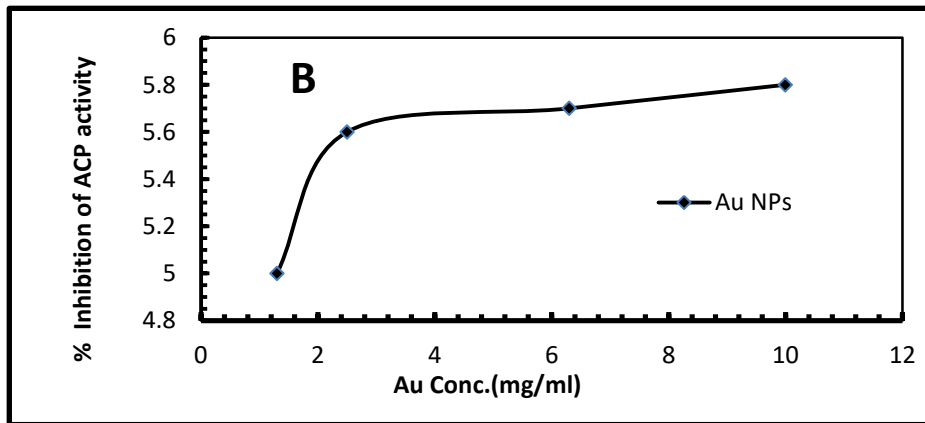


Figure (6) %Inhibition of ACP activity with Au nanoparticles concentration.

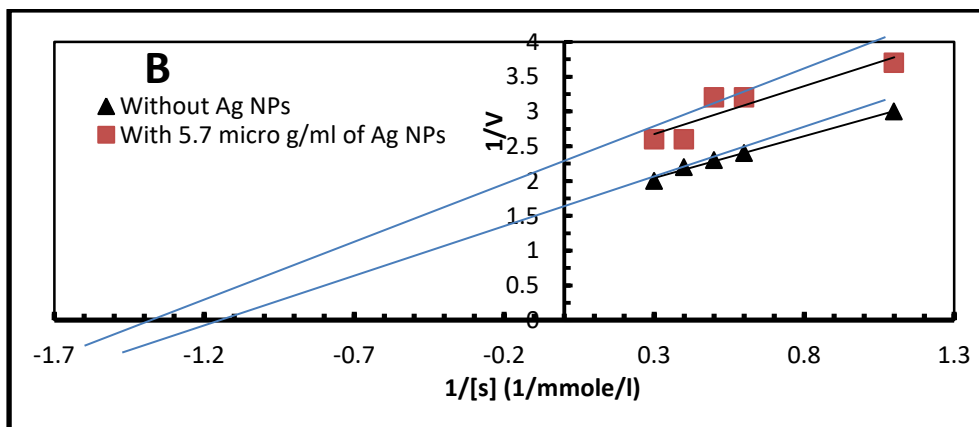


Figure (7) Lineweaver-Burk plot for Ag nanoparticles effect on ACP activity in sera of healthy subjects.

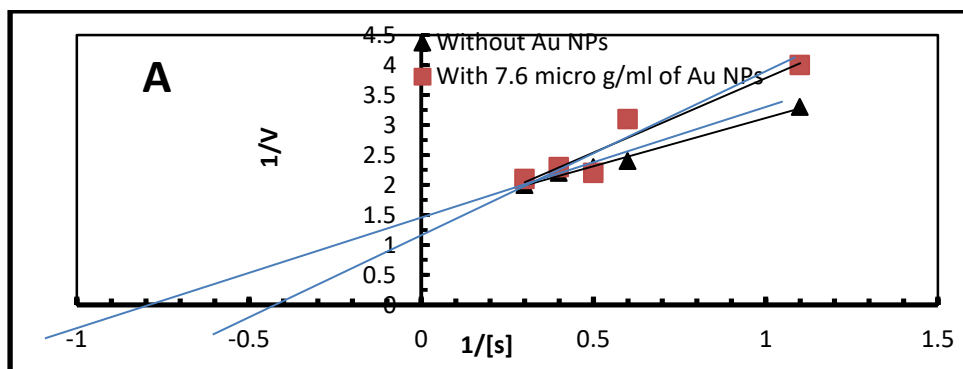


Figure (8) Line weaver-Burk plot for Au nanoparticles effect on ACP activity in sera of healthy subjects

Table (1): The kinetic properties of ACP with Au and Ag NPs.

V_{max} (U/L)	K_m (mmole/L)	Nanoparticles	k_{mapp} (mmole/L)	V_{maxapp} (U/L)	Inhibition Type
0.7	1.25	Au	1.85	0.77	Mixed
		Ag	0.9	0.5	uncompetitive

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