Nanoparticles Impression on Creatine Kinase Activity

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Abstract

Nanoparticles (NPs) are purchased from Sigma-Aldrich (730785). Sodium citrate as stabilizer comprises 10 nm particle size (TEM), dispersion (NPs), stabilizer Silver, and 0.02 mg/mL in aqueous buffer relative molecular mass (107.87). The concentration number (3.6×1012) packaging 25mL in a glass bottle. 10 nm diameter golden nanoparticles OD 1, stable 0.1 mM suspension is an aqueous salt solution that contains common salt, dihydrogen phosphate, and dilute phosphate-buffered saline (PBS). It also contains potassium dihydrogen phosphate and potassium chloride in certain formulations. While the pH rises, the role of the buffer is to regulate it. NPs, equipped with (Sigma-Aldrich752584) relative molecular mass (196.97). Structural and optical nanoparticles are studied. Findings show a nanoparticle size of approximately 10 nanometers. The silver and gold sources' impact on healthy people's creatine kinase (CK) activity within the blood was in vitro-studied. The results indicated that the nanoparticles of silver and gold had a dissimilar impact on serum CK activity. The gold particles tonic impacts serum CK activity and increases the activity of enzymes as the concentration of nanoparticles increases because it acts as an enzyme activator. At the same time, silver nanoscale has an inhibitory effect thereon.

Key Words: Nanoparticles (NPs), CK Activity, Blood of Healthy People.

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Introduction

Nanoparticles are accurate and tiny in sizes ranging from 1 to 100 nanometers, and have distinct characteristics and transport methods. Nanoparticles have been studied in many advanced technologies as potential components in medical chemistry in terms of diagnosis, treatment identification, and design of biologically active compounds, including toxicity control and the use of metal to reach a specific target of tissues or organs and cells (Al-Hadedee *et al*, 2020). Some nanoparticles can be used in a group of successful nanoscale such as gold and silver due to its plasmodium properties and relative stability for therapeutic and diagnostic agents to understand diseases (Azharuddin *et al*, 2019). Thus, nanoparticles provide the ability to design new promising field for medicine applications.

Creatine kinase Ec (2.7.3.2) enzyme catalyzes reverse phosphorylation of ADP to ATP or creatine conversion into phosphocreatine and this conversion supplies the instantaneous energy required by the cell. This enzyme is that the main key to the transfer and building of energy, present within the cytoplasm likewise as in mitochondria within the tissues requiring consumption high energy and elevated in serum indicates several medical conditions (Moghadam *et al*, 2016), involved with physical structure, there is an essential role Creatine kinase plays, such as efficiently buffering the system of cellular adenosine triphosphate (ATP) levels.

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It does this in high-energy consuming tissues like heart and brain muscles, where it metabolizes them. The enzyme catalyzes the phosphoryl group's reversible transfer to adenosine diphosphate (ADP) from phosphocreatine, regenerating ATP (Wallimann *et al* (1998). Generally, when excitement occurs, there is a resultant 10-fold rise of cellular turnover. During these rapid changes, the creatine/phosphocreatine/CK system becomes crucial for buffering the energy system to elude significant fluxes of cellular ATP/ADP levels in the relevant tissues. Ostensibly, a reduction of CK activity could lead to impaired energy homeostasis, causing necrobiosis (Pilla *et al* 2003).

Methods

The activity of the Creatine enzymes was measured using the linear kit. The formulation of this test followed the IFCC standardized procedure (Clin, 2002).

The reaction between phosphocreatine (CP) and '-diphosphate (ADP) is catalyzed by CK, forming adenosine 5' triphosphate (ATP) and creatine. In the presence of hexokinase (HK), the phosphorylates glucose to glucose-6-phosphate (G6P) occurs. Another oxidation process occurs in G6P conversion to Gluconate-6P in the presence of lowered nicotinamide-adenine dinucleotide phosphate (NADP). That occurs when an oxidation process happens by glucose-6-phosphate dehydrogenase (G6P-D. That happens by kinetically monitoring the process at 340 nm checking how the increased speed occurs in absorbance caused by the reduction of NADP to NADPH relative to the sample CK activity. While this takes place, it is essential to test 1,2 any present N-acetyl cysteine (NAC) that permits the enzyme's activation at an optimal level. Excluded hemolyzed specimens as plasmas comprising heparin, EDTA, fluoride or citrate. Lipemia, drugs and substances may interfere since they'll produce unpredictable reaction rates. The working rule depends on the rise in absorbance at wavelength nm340 because of formation of NADPH by creatine kinase consistent with the subsequent reaction:

Creatin phosphate + ATP ---------- CK (AMP, NAC) ---------> creatine + ATP
ATP + Glucose ------- HK ----------> ADP + G6P
G6p +NADP+H2O - ----------G6DH---> NADPH +H + Gluconate-6P

Solutions Used: NAC/ Glucose/ Buffer. Glucose 20 mmol/L, NADP, 2.5 mmol/L, HK ≥ 4 KU/L, NAC 20 mmol/L, Imidazol buffer 100 mmol/L pH 6.7, magnesium acetate10 mmol/L, EDTA 2 mmolR2; AMP 5 mmol/L, ADP2 mmol/L, Substrate/Coenzymes. CP 30 mmol/L, di(adenosine-5´) pentaphosphate 10 mol/L, G6PDH ≥1.5 KU/L. Preparing the working solution, mix 1 ml of R2 + 4 ml of R1, and this solution becomes steady for a month, faraway from light. 1 ml of labor solution was taken at 37 C degrees lml of the sample, 20µl was added mixed well and left for 3 minutes then Absorbance read out Initial reading at 340 nm. The reading was repeated after 1,2,3 minutes' absorbance rate change / min. A constant concentration of Au NPs (0.1mM) were used with different substrates Concentrations (0, 0.0125,0.025,0.05,0.1) mmole/L as a last solution concentration in the reaction mixture. The Ag NPs (0.02mg/ml) sample were added to dilution buffer into each eppendorf tubes labeled with (0, 0.0025, 0.005, 0.01, 0.02) mg/ml, which are represented as (2.5; 5;10; 20 ppm). The Line Weaver-Burk equation helped determine enzyme happenings in the presence and absence of the NPs. Superficial Vmax, superficial Km, and reserve kind were tested (Burtis *et al* 2012).

Calculations: U / L Creatine kinase activity = change in absorbance / min x8095

Statistical Analysis

The analysis of variance (ANOVA) one-way analysis of variance was performed and means were compared. A value of $p < 0.05$ was determined significant using Fisher's exact test differences.

Results

The dispersion silver and gold nanoparticles (NPs) have been purchased from (Sigma-Aldrich) as illustrated in figure (1).

Figure 1. Shows the dispersion silver nanoparticles (NPs) have been purchased from (Sigma-Aldrich 730785), and gold Au(NPs), from (Sigma-Aldrich752584)

The optical and structural properties of the Au and Ag nanoparticles have been studied. A simple absorption spectrometer can help record optical absorption spectra to show typical spectra. Seemingly, the silver optimum shows at about 396 nm and the one for gold at approximately 530 nm. they represent the surface plasmon resonance(spr) peaks mentioned above.

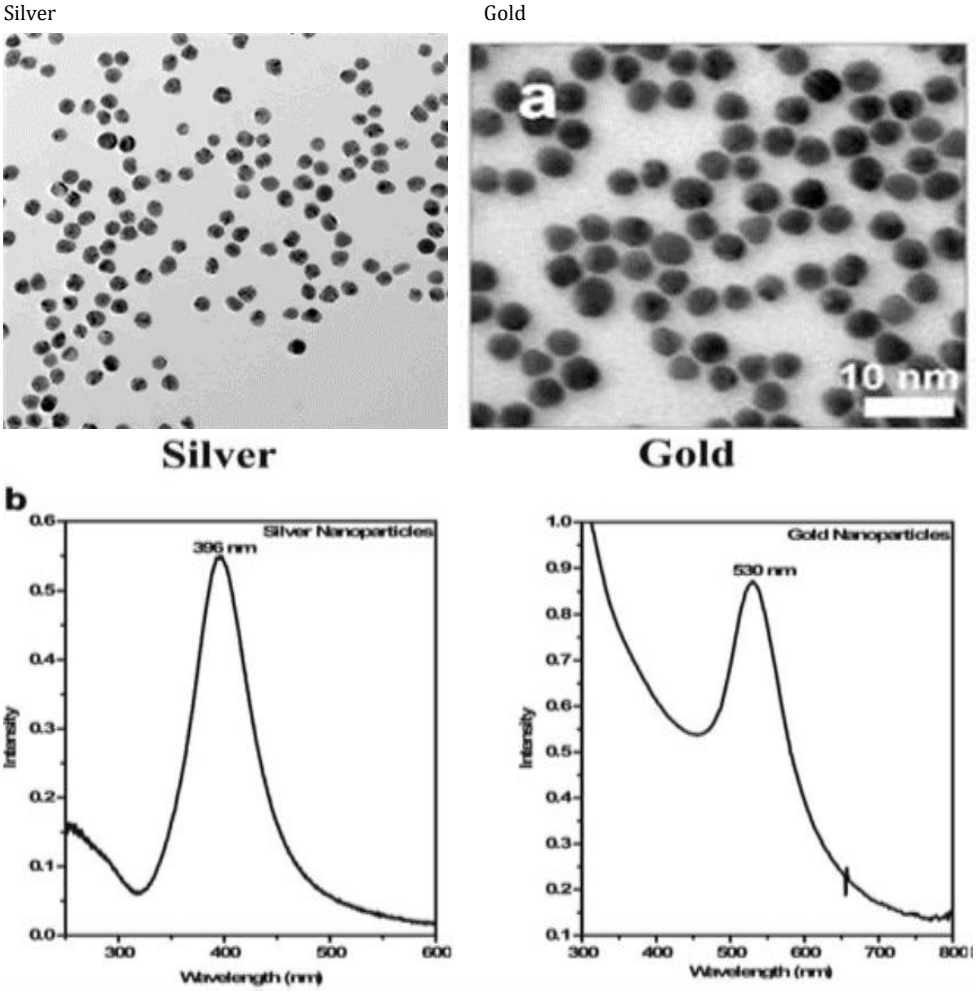

Figure 2. Gold nanoparticles (10 nm diameter, OD1, stabilized suspension in 0.1 mM PBS abs. max 530-400), Silver nanoparticles (10nm, 0.02mg/ml in 2mM sodium citrate, abs. max. 390-400)

The results showed the Ag and Au NPS had various impacts on CK, as illustrated in Figures 3 and 4 below. The figures show the correlation between concentrations of Au, Ag and NPS against the enzyme activity. The findings revealed that a rise in nanoparticles of Ag had a negative impact on CK

enzyme which leads increase inhibition percentage. The highest Ag NPS inhibition on enzyme activity was recorded at 80.1%, with a concentration of 0.02 mg/ml Moreover, an increase in Au NPS activation effect caused a resultant positive impact on enzyme activation. The peak Au NPS activation on CK was recorded at 95.5% with a 0.2mM concentration level.

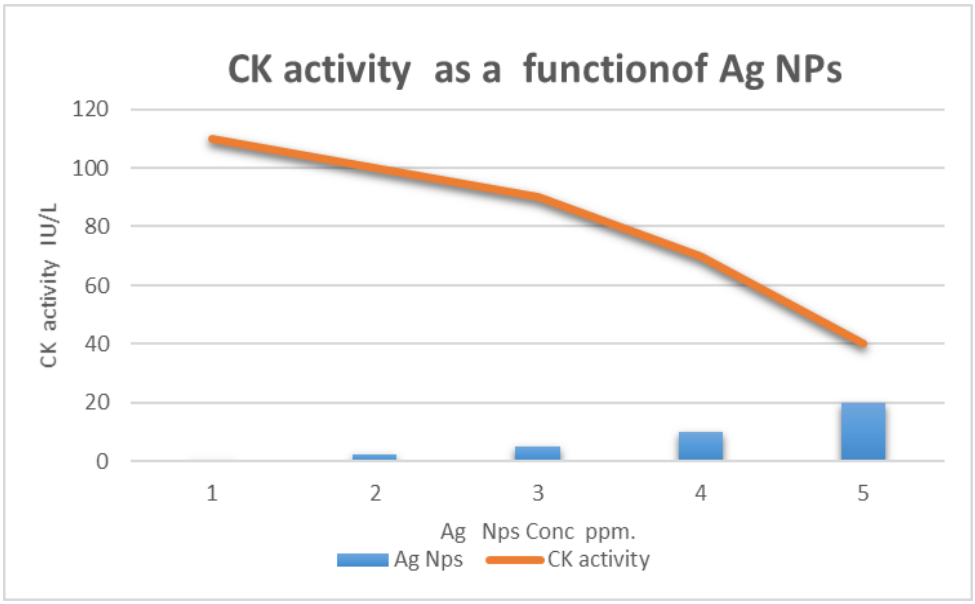

Figure 3. Ag NPs concentration impact on CK activity

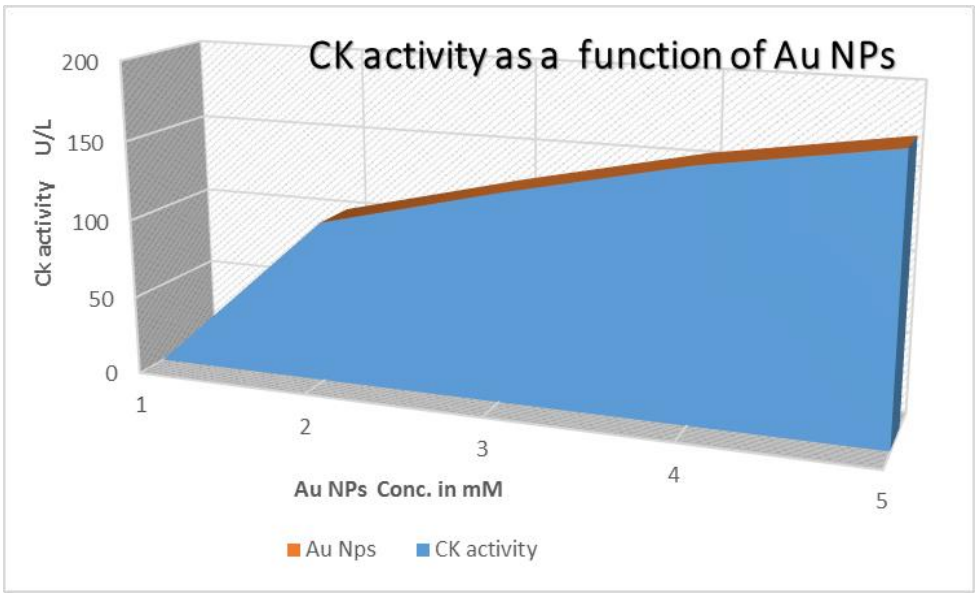

Figure 4. Au NPs concentration impact on CK activity

Showed the correlation between enzyme activity against NPS of Au solution Table (1) leads to an increase in serum CK activity while the effects of silver nanoparticles decrease the activity of enzyme as reported in the table.

Table 1. The evaluations of kinetic biochemical showed NPs of Au and Ag impacted CK

	Before adding NPs	**After adding gold**	
Creatine Kinase IU/L	73± 3.4 IU/L	108±15.74 IU/L	T-test =12, P<0.05 significant
		After adding silver	
		57.± 4.21 IU/L	T-test =9, P<0.05 significant

The evaluations of kinetic biochemical showed that NPs of Au and Ag impacted CK activity in various ways see figure (5) and (6). That used kinetic parameters Km app, Vmax app, and kind of enzyme inhibition applying Line-Weaver-Burk formula for Ag NPs on CK. The Vmax and Km in the absence of Ag nanoparticles recorded (73.45) U/L and (0.12) mmole/L, respectively. A (0.02) mg/ml of Ag NPs concentration was inadequate inhibition for enzyme activation. The Kmapp and Vmaxapp recorded (0.05) mmole/L and (57.04) U/L, respectively.

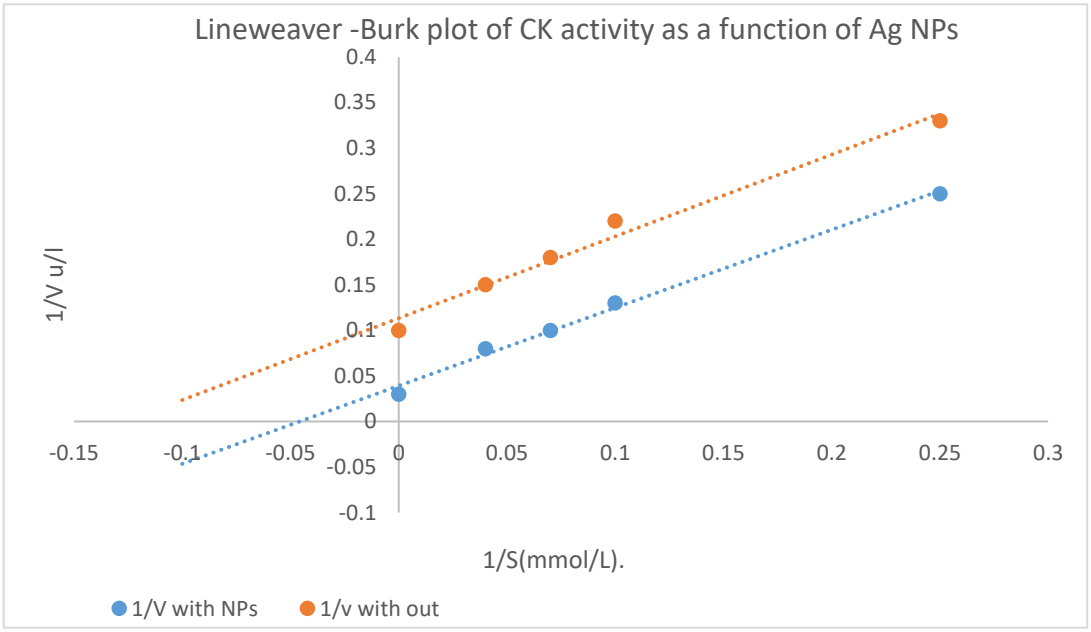

Figure 5. The Km and Vmax with and without Ag nanoparticles

The kinetics study of the CK enzyme after the usage of gold nanoparticles indicates a competitive binding on the active site of the enzyme and is complemented by a change in km value, but Vmax remains constant as shown by the Line-Weaver-Burk plot figure (6).

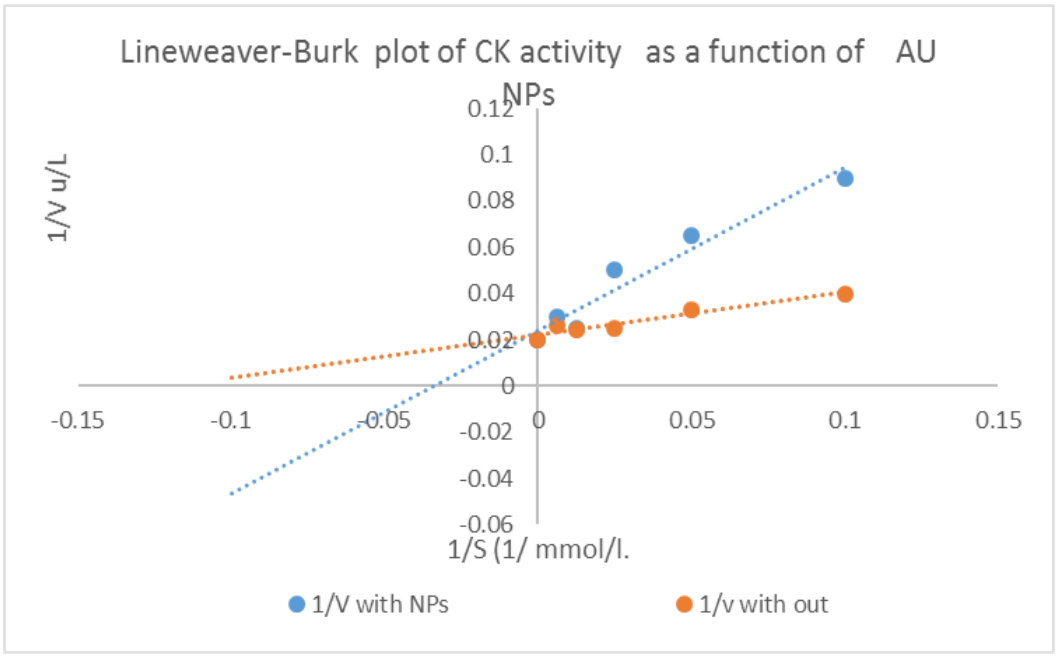

Figure 6. The Km and Vmax with and without Au nanoparticles

Discussion

The creatine kinase (CK) plays an important role in hemostasis the cellular energy mainly in case of transient states such as exercise and energy needed in certain muscles under fluctuating condition, so this situation requires appropriate control of CK. NPs holds as a potential agent for therapeutic and diagnostic service of disease (Goswami, *et al* 2018). The results reveal varying concentrations of AgNPs cause uncompetitive inhibition on the CK serum activation due to directly bound to thiol groups present in the protein structure induce unfolding and change the geometry of the active site. The structure of myofibrillar CK proposed installation that Cys283 was selected as essential for the catalysis action as the schematic representation in figure (7) (Mejsnar, *et al 2002).*

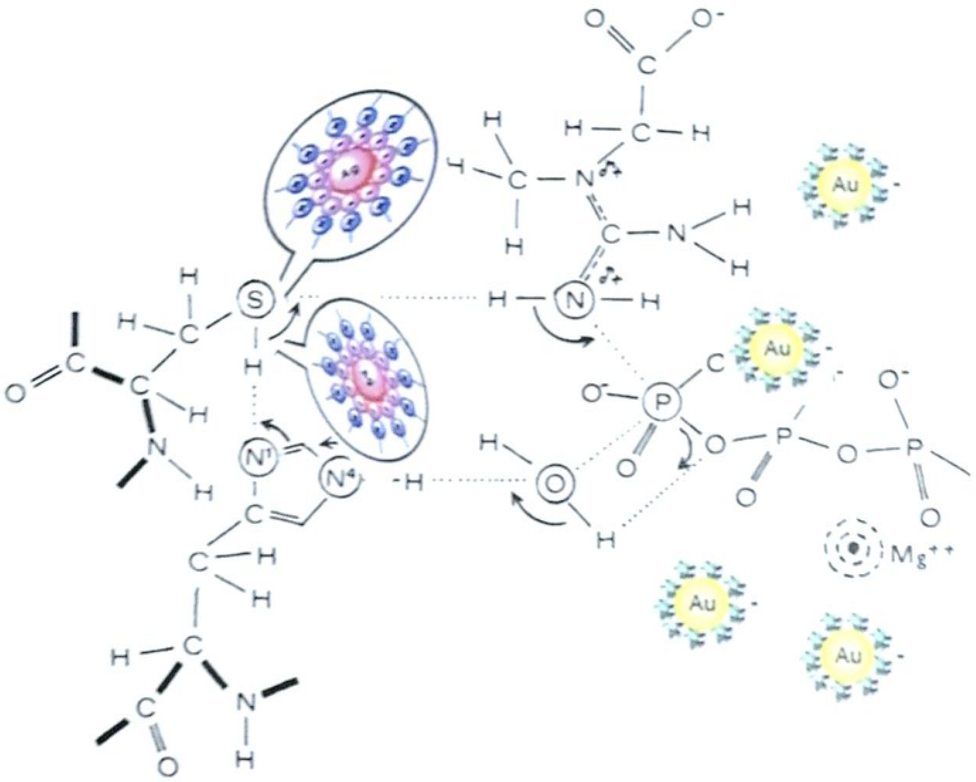

Figure 7.

Figure (7) schematic representation involve interaction between AgNPs and thiols groups decreased the activity by denaturation of the active site. While Au NPs was competitive inhibition for CK activity (Mejsnar, *et al 2002*). There is evidence that protein deactivation and denaturation is a result of Ag binding to functional groups of proteins (Lubick, *et al* 2008). Our results revealed that the effects of gold nanoparticles had a competitive inhibition on CK serum activity, that impact soared with rising AuNPs concentration, as illustrated in figure (8) (Chen, *et al* (2017).

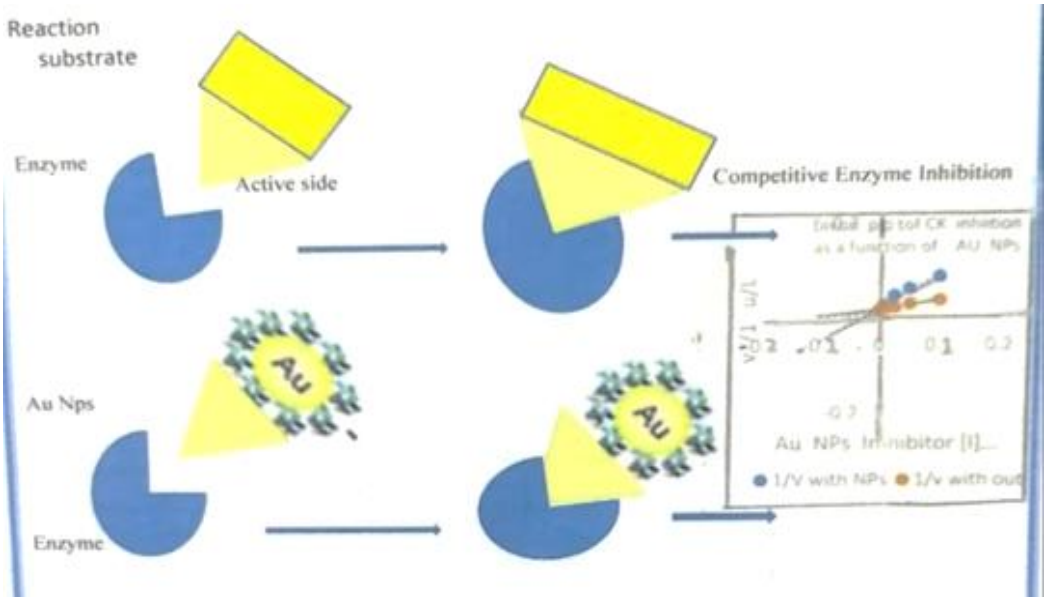

Figure 8.

Those findings correlated with other findings that combined and synergistic effects resulted from high local peptide density around the nanoparticle. The outcome is an increased rate and unique catalytic features compared to the peptide, which is unconjugated (Watanabe, *et al* 2018). Other researchers summarize the contributions and advances around the area of catalytically nanoparticle coupling or in the active non-peptide. The origin of the distinguishing features is possible uses. A future study on the reversible control of combined states of plasmo NPs via exogenous stimuli is wished. There are various proved and strongly rooted tactics for regulating catalytic activity by interacting with a necessary corresponding ligand/cofactor naturally appearing here as justified designed peptides nanoparticle conjugates (Mikolajczak, *et al* 2017). Besides, three interesting developments are presented here for using active modifiers in synthetic enzyme systems to enhance the existing nanoparticles' catalytic progress. A case in point other study where they described the lysozyme-stabilized gold nanocomposites' catalytic activity at neutral pH could be made possible using graphene oxide as a modulator. That is possible because graphene oxide has significant potential for usability in biological functions. That paper has revealed that by incorporating mutations into synthetic enzymes, it is possible to provide simple yet more impactful methods for improving their whole catalytic performance or perceiving catalytic reactions that would not have been practical before. It is probable that distinguished nanoparticle features will lead to an increased need for further research, opening more doors for new studies in this field (Mikolajczak *et al* 2020).

Conclusion

The results indicated that the nanoparticles of silver and gold had a dissimilar impact on serum CK activity. The gold particles tonic impacts serum CK activity and increases the activity of enzymes as the concentration of nanoparticles increases because it acts as an enzyme activator. At the same time, silver nanoscale has an inhibitory effect thereon.

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Conflict of Interest

None.

Funding Source

None.

References

AL-Hadedee LT, Sadoon THA, Chechan RA. Biocompatibility of Nanoparticles and some of it's Applications. *Biochemical and Cellular Archives* 2020; 20(1): 131-136.

Azharuddin M, Zhu GH, Das D, Ozgur E, Uzun L, Turner AP, Patra HK. A repertoire of biomedical applications of

noble metal nanoparticles. *Chemical Communications* 2019; 55(49): 6964-6996.

Moghadam-Kia S, Oddis CV, Aggarwal R. Approach to asymptomatic creatine kinase elevation. *Cleveland Clinic journal of medicine* 2016; 83(1): 37-42.

Wallimann T, Dolder M, Schlattner U, Eder M, Hornemann T, Kraft T, Stolz M. Creatine kinase: an enzyme with a central role in cellular energy metabolism. *Magnetic Resonance Materials in Physics, Biology and Medicine* 1998; 6(2-3): 116-119.

Pilla C, De Oliveira Cardozo RF, Dutra-Filho CS, Wyse ATS, Wajner M, Wannmacher CMD. Effect of leucine administration on creatine kinase activity in rat brain. *Metabolic brain disease* 2003; 18(1): 17-25.

Burtis A, Ashwood R, Bruns E. (eds): Tietz Textbook of Clinical Chemistry and Molecular Diagnosis (5th edition) Elsevier, St. Louis, USA 2012.

Goswami P, Mittal D, Kumar P. Nanotheranostic: Nanomedicine and future development. *Journal of Biomedical and Pharmaceutical Research* 2018; 7(4), 56- 59.

Mejsnar JA, Sopko B, Gregor M. Myofibrillar creatine kinase activity inferred from a 3D model. *Physiological research* 2002; 51(1): 35-42.

Lubick N. Nano silver toxicity: ions, nanoparticles--or both? *Environmental Science & Technology* 2008; 42(23): 8617.

Chen Y, Xianyu Y, Jiang X. Surface Modification of Gold nanoparticles with small molecules for biochemical analysis. *Accounts of Chemical Research* 2017; 37: 238-250.

Watanabe K, Kuroda K, Nagao D. External-stimuli-assisted control over assemblies of plasmonic metals. *Materials* 2018; 11(5): 794.

Mikolajczak J, Heier L, Schade B, Koksch B. Catalytic Activity of Peptide-Nanoparticle Conjugates Regulated by a Conformational Change. *Biomacromolecules* 2017; 18(11): 3557-3562.

Mikolajczak DJ, Berger AA, Koksch B. Catalytically active peptide–gold nanoparticle conjugates: prospecting for artificial enzymes. *Angewandte Chemie International Edition* 2020; 59(23): 8776-8785.

Mohammed AA, Alshabander BM, Nasir EM. The role of water absorption on thermal conductivity and mechanical properties for (Recycling hdpe-coal ash) composite. *NeuroQuantology* 2020; 18(4): 11-19.