International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Volume 3, Issue 7 - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-7-12

Determination of Vmax and Km of ALP, AST and Peroxidase in Saliva of Chronic Periodontitis Patients with and without ZnO NPs: A Kinetic Study

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Abstract

Background: enzyme activities can be utilized as good indicators for diagnostic purposes. Thus, determination of kinetic parameters of the target enzymes facilitates the interpretation of the effect that may be occurred on activities of the studied enzymes. The aim of this study is to determine of Vmax and Km of ALP, AST and peroxidase in presence and absence of ZnONPs in saliva of chronic periodontitis patients.
Materials and Methods: saliva samples of 60 patients, aged (30-60) years, with chronic periodontitis were collected as a patients group. Control group included 20 samples of saliva were obtained from 20 healthy subjects, aged (30-60). Nanoparticles of ZnO were used in this study in a diameter less than 80 nm. 4-Nitrophenyl disodium orthophosphate, L – aspartic acid and hydrogen peroxide were used as substrates of ALP, AST and peroxidase , respectively.
Results: Km and Vmax values of ALP in absence and presence of ZnO NPs were found to be [21.28mmol/L, 2.414 U/L], [24.39 mmol/L, 2.145 U/L], respectively. While the values of Km and Vmax for AST in absence and presence of NPs were recorded to be[2.288 mmol/L, 6.051U/L], [2.203mmol/L, 6.466 U/L], correspondingly. The Vmax and Km values of salivary peroxidase in absence and presence of ZnO NPs were calculated to be [120) U/L, and (0.012) mmol /L], [120) U/L, 0.024 mmol/L], in that order.
Conclusion: the results of this study confirmed the inhibited effect of ZnO NPs on both salivary ALP and peroxidase activities. In contrast, the activity of salivary AST was increased in presence of ZnO NPs. The effects of ZnO NPs on these enzyme activities may be attributed to activity of this NPs type together with conformational changes that can be occurred on the protein structure after interaction with NPs.

Keywords: Kinetic study, salivary AST, ALP and Peroxidase, Chronic periodontitis, ZnO NPs.

Introduction

More recently, in our previous studies, we reported the effect of ZnO NPs on salivary ALP, Peroxidase and AST activities [1-3]. The effect of nanoparticles of many metals or metal oxide were estimated toward different biological systems, in recent study, the effect of Au, Ag, TiO_2 nanoparticles on activities of acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were investigated [4-6]. Studies were reported that of ZnO NPs possess an excellent biological activity against certain pathogenic

Bactria [7-9]. Sanif E, at al [10] were referred to the kinetic studies and the significance alteration of the enzymatic activity that induced by NPs in a size dependent-manner [10]. Kinetic studies were applied in nanoparticle drugs; the study was suggested for future studies with NP formulations of poorly water-soluble drugs [11].

The aim of this study is to determine of the kinetic parameters (Km, Vmax) of salivary ALP, AST and peroxidase of chronic periodontitis in presence and absence of ZnO NPs to knowledge the effect nature of this type of NPs on the activities of the studied enzymes.

Materials and Methods

Nanoparticles

Zinc oxide nanoparticles have been obtained from Nanjing, china. This product supplies as ZnO nano powder. UV-VIS spectrum of NPs stock solution was recorded by UV- VIS spectrophotometer. Structure and nano size measurement of ZnO NPs powder were identified by the Scanning Electron Microscope SEM (Electronic Microscope Centre- College of applied Science, University of Technology, Iraq).

Collection of saliva samples

Un-stimulated whole saliva was collected. A sample was collected after an individual was asked to rinse his mouth thoroughly with water to insure the removal of any possible debris or contaminating materials and waiting for 1-3 min for water clearance.

The samples were collected at least 1 h after the last meal. Saliva was collected between 9-11 a.m. Each one of the groups' subjects was asked to spit saliva into the polyethylene tubes until 5 ml was collected. Samples containing blood were discarded. Then the container was labelled with the number of the subject and kept in the cooling box.

Then the collected saliva was separated by centrifuge at 4000 rpm for 10 minutes, the clear supernatant saliva divided into 3 parts by micropipette into eppendorf tubes and store at -20˚C (freeze) until biochemical analysis .

Kinetic studies

ALP kinetics:

The enzyme activity was carried out under optimum reaction conditions by using the enzyme substrate; 4-Nitrophenyl disodium orthophosphate in different concentrations (20, 30, 40, 50, 60, 80, and 100) mmol/l. The relationship between each substrate concentration and the enzyme activity was plotted in order to indicate the type of enzyme kinetic. Then, the values of Km and Vmax for the binding of enzyme ALP to the substrate were determined using the Lineweaver- Burk plot.

The same experiment was repeated by adding (18.25µg/ml) of ZnO NPs concentration in order to determine the effect of ZnO NPs on enzyme kinetic parameters .The relationship between substrate concentration and the enzyme activity was plotted in order to determine the values of Km apparent and Vmax apparent for the ALP activity by using Lineweaver- Burk plot [13].

AST kinetics:

L – aspartic acid as substrate of AST activity was used under optimum reaction conditions. Different concentrations (40, 60, 100, 140, 160, 170, and 180) mmol/l of the substrate was prepared. The relationship between substrate concentrations and the enzyme activities was plotted .Then, the values of Km and Vmax for the binding of enzyme AST to the substrate were determined using the Lineweaver- Burk plot.

The same experiment was repeated by adding (0.33µg/ml) of ZnO NPs concentration. Relationship between substrate concentration and the enzyme activity was plotted in order to indicate the values of Km apparent and Vmax apparent for the AST activity by using Lineweaver- Burk plot [13].

Peroxidasekinetics:

Peroxidase enzymatic reaction was carried out by using hydrogen peroxide as substrate under optimum reaction condition using different concentrations (0.02, 0.04, 0.06, 0.08, 0.17, 0.2, 0.25, 0.3, and 0.4) mmol/l. Then, relationship between each substrate concentration and the enzyme activity was plotted in order to indicate the type of enzyme kinetic. Km and Vmax values for the binding of enzyme peroxidase to the substrate were determined using the Lineweaver-Burk plot.

Then the same experiment was repeated by adding (2.58µg/ml) of ZnO NPs concentration in order to indicate the effect of ZnO NPs on enzyme kinetic parameters .The relationship between substrate concentration and the enzyme activity was plotted. Km apparent and Vmax apparent for the peroxidase was determined using Lineweaver- Burk plot [13].

Characterization of ZnO NPs

UV- VIS absorption spectra:

Spectra of UV-VIS were indicated the characteristic absorbance feature of Zinc oxide nanoparticles, the maximum absorption peak of ZnO NPs, which suspended in ethanol-water mixture, was showed at 375 nm as shown in figure (1). This absorption peak considers as a hallmark of ZnO NPs at applied nanoparticles size (<80 nm).

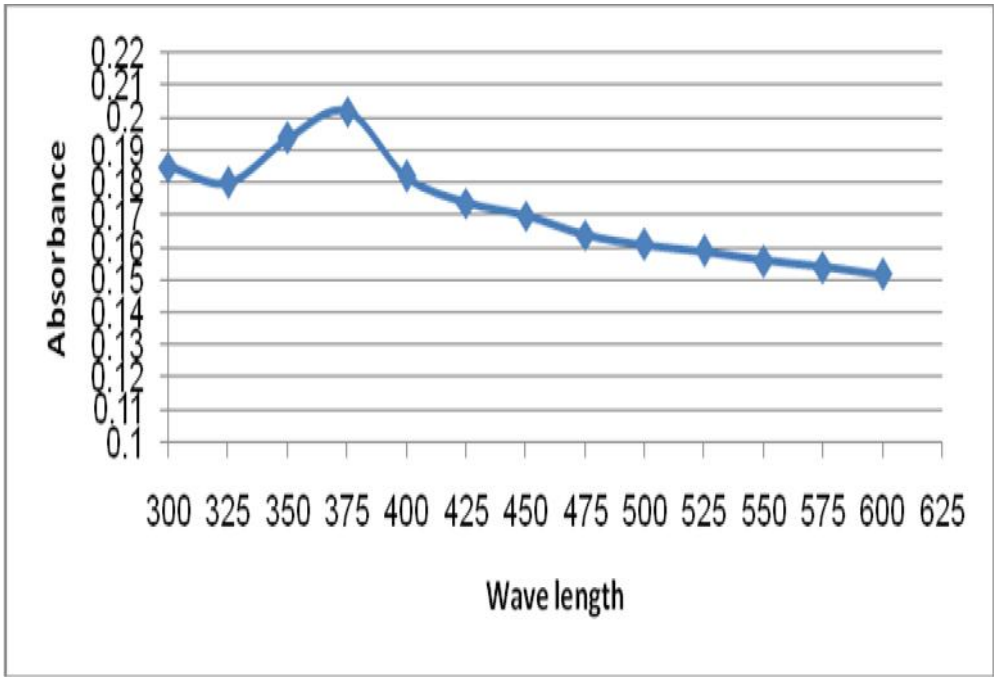

Figure (1): UV – VIS spectrum of the ZnO NPs

Scanning Electron Microscope (SEM):

Figure (2) shows SEM picture and size distribution of ZnO NPs using in this study. The average diameters of the produced NPs were found to be less than 80nm.

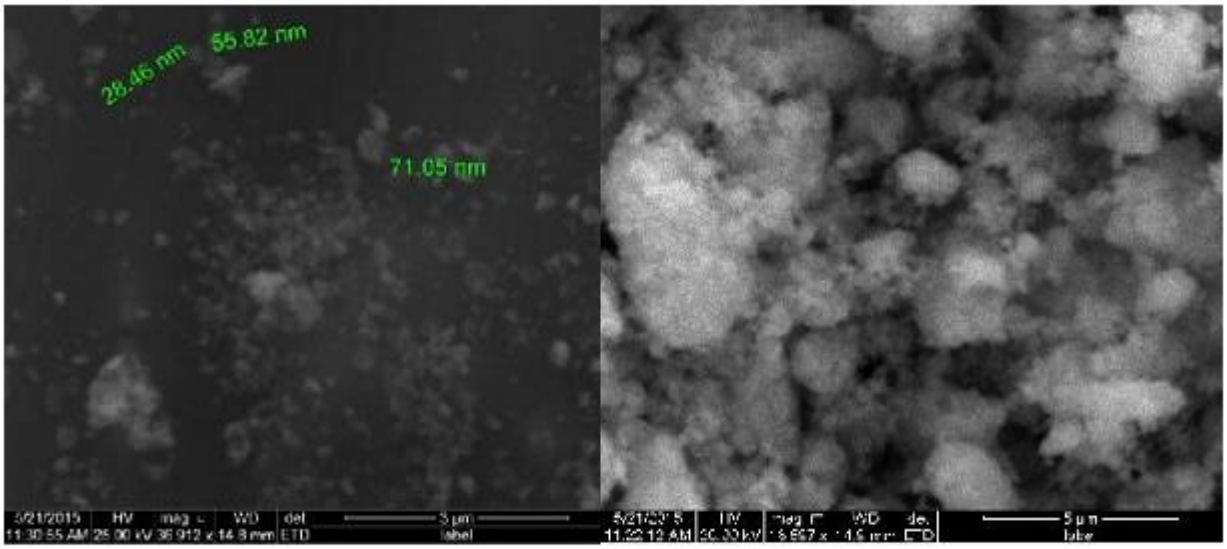

Figure (2): SEM picture and size distributions of ZnO NPs

Results and Discussion

Effect of Substrate Concentration on Salivary ALP activity:

A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate, [S]. However, studying the effects of substrate concentration is complicated by the fact that [S] changes during the course of an in vitro reaction as substrate is converted to product [13].

Figure (3) shows the relationship between the velocity [salivary ALP activity (U/L)] and substrate concentration [(4-Nitrophenyl disodium orthophosphate mmol/L)] in absence and presence of ZnO NPs in chronic periodontitis patients.

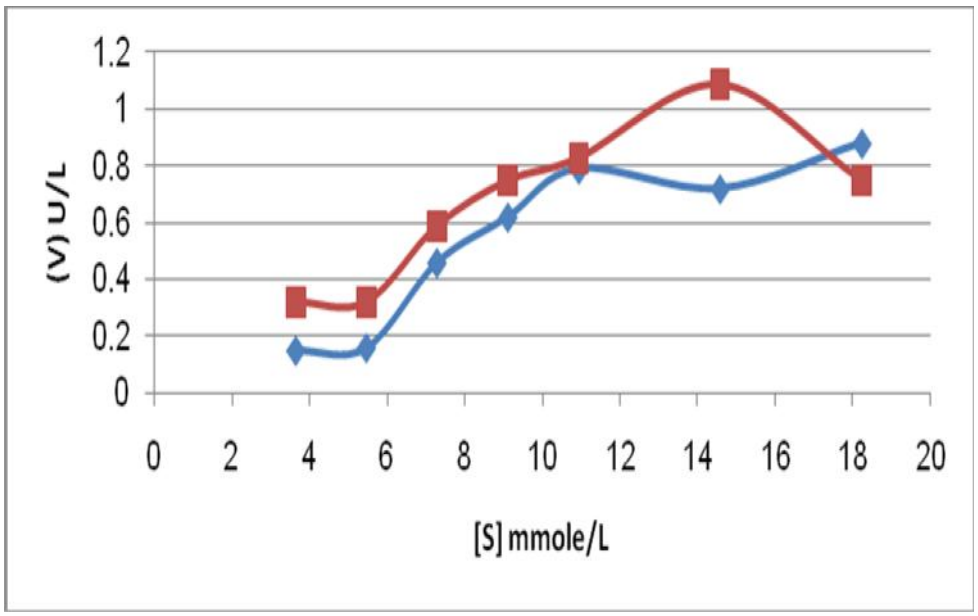

Figure (3): The effect of substrate concentration on ALP activity in saliva of patients in absence and presence ZnO NPs.

Our results showed that there is an inhibition effect on the ALP activity due to presence of ZnO NPs in all concentrations of ALP substrate except the highest one (18.25 mmol/L).

The results in present study are matching with the results in our previous study of the activity of salivary ALP with and without NPs in chronic periodontitis case. Moreover, the kinetic results were support the results that regarding with the effect of NPs on salivary ALP activity in patients with chronic periodontitis [1].

The kinetic parameters of Kmapp., and Vmaxapp., were obtained from Lineweaver – Burk plot. Km and Vmax values in absence and presence of NPs were found to be [21.28mmol/L, 2.414 U/L], [24.39 mmol/L , 2.145 U/L] respectively as shown in table (1).

Table (1): V_{max} and K_m values of reaction catalyzed by ALP with absence and presence of ZnO NPs.

Kinetic parameters	Vmax(U/L)	Km (mmole/L)	Type of inhibition
Absence ZnO NPs	2.414	21.28	Mixed
Presence ZnO NPs	2.145	24.39	

Our results in ALP kinetic study are in agreement with many other studies, which were studied the kinetic of ALP with and without NPs, such as Ag and Au NPs, and showed the inhibition effects on activities of ALP, ACP and LDH enzymes due to the effects of these NPs[4,6].

The results have been demonstrated that ZnO NPs is a mixed inhibitor for salivary ALP activity. Mixed inhibitor also binds at a site distinct from the substrate, but it binds to either E or ES of an enzyme [13]

Effect of Substrate Concentration on Salivary AST activity:

The kinetic study of AST was conducted using L- aspartic acid as a substrate to determine the effect of substrate concentration on reaction velocity in absence and presence of ZnO NPs. The relationship between the velocity [salivary AST activity (U/L)] and substrate concentration [L – aspartic acid (mmol/L)] in absence and presence ZnO NPs in chronic periodontitis patients was plotted as shown in figure (4).

The results of our study showed there is an activation effect on the AST activity due to the presence of ZnO NPs in all concentrations of AST substrate. Thus, the kinetic results of AST are consistent with salivary AST activity which was obtained in previous study [3].

The kinetic parameters of Kmapp., and Vmaxapp., were obtained from Lineweaver – Burk plot. Km and Vmax values in absence and presence of NPs were found to be [2.288 mmol/L, 6.051U/L], [2.203mmol/L, 6.466 U/L] respectively as shown in table (2).

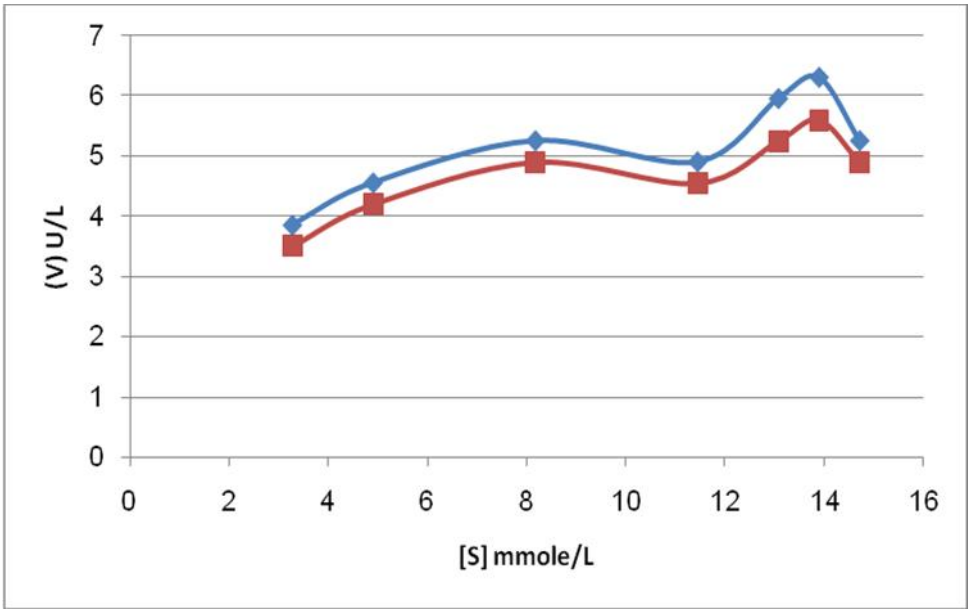

Figure (4): The effect of substrate concentrations on AST activity in salivary of patients in absence and presence ZnO NPs.

Table (2): Vmax and Km values of reaction catalyzed by AST with absence and presence of ZnO NPs.

Kinetic parameters	Vmax(U/L)	Km (mmole/L)
In absence ZnO NPs	6.051	2.288
In presence ZnO NPs	6.466	2.203

Results of the present study are consistent with more recent study which showed that ZnO NPs effect on AST activity is a dose-dependent manner [14]. In contrast, Abbas et al [15] were reported that both gold and silver nanoparticles are inhibiting the activity of AST and ALT. In another recent study, it was found that Au NPs activated the activity of LDH [6]. Similar results was obtained of gold and silver nanoparticles on choline esterase (ChE) and monoamino oxidase (MAO) enzymes in the serum, which showed an activated effects on their activities[16].

Effect of Substrate Concentration on Salivary Peroxidase activity:

To determine the effect of substrate concentration on reaction velocity which catalysed by salivary peroxidase in absence and presence of ZnO NPs. The kinetic study of salivary peroxidase was conducted

using hydrogen peroxide as substrate. The results of this study showed the inhibition effect on salivary peroxidase activity in presence of ZnO NPs in all the substrate concentrations as shown in figure (5).

The results in present study are matching with the results in previous study of the activity of salivary peroxidase with and without NPs in chronic periodontitis case. Furthermore, the kinetic results were support the results that regarding with the effect of NPs on salivary peroxidase activity in patients with chronic periodontitis [2].

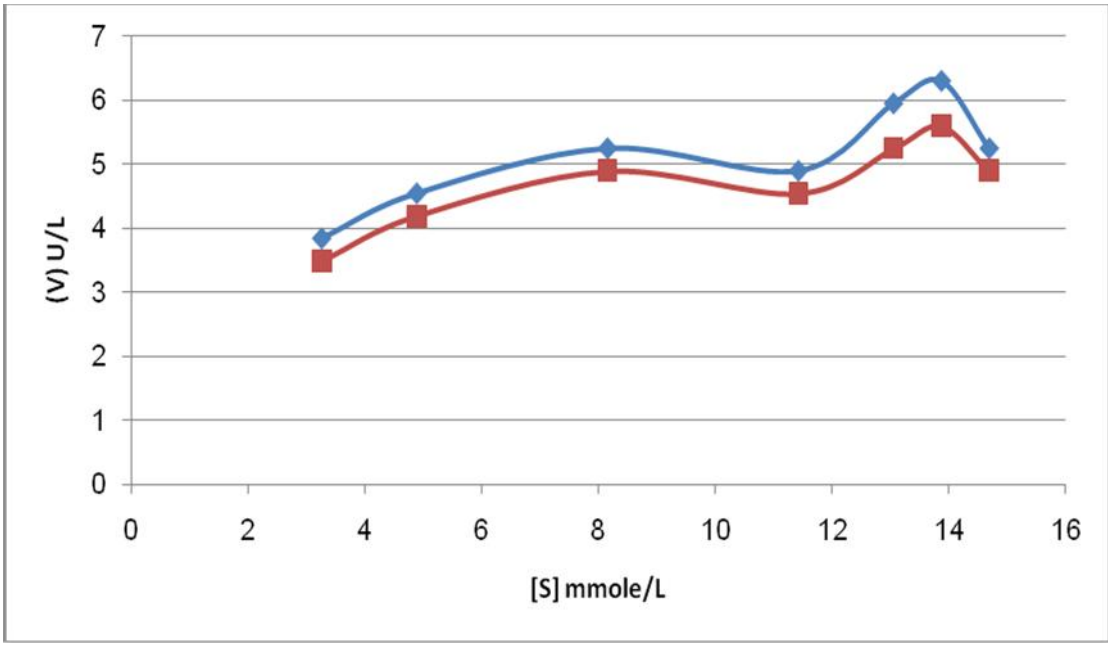

Figure (5): The effect of substrate concentration on Peroxidase activity in salivary of patients in presence and absences ZnO NPs.

Table (3) showed the kinetic parameter Kmapp, Vmaxapp and type of enzyme inhibition using Lineweaver – Burk equation for ZnO NPs on salivary ALP activity. The values of Vmax and Km in absence and presents ZnO NPs were found to be [120 U/L, and 0.012mmol /L], [120 U/L, 0.024mmol/L] respectively.

Table (3): shows Vmax and Km values of reaction catalyzed by peroxidase in absence and presence of ZnO NPs.

Kinetic parameters	Vmax(U/L)	Km (mmole/L)	Type of inhibition
Absence ZnO NPs	120	0.012	Competitive
Presence ZnO NPs	120	0.024	

Results of the present study are consistent with more recent study which showed that NPs inhibited the total salivary peroxidase (TSP) activity [17]. Kinetic analysis of the initial rates of peroxidase extracted from palm tree vs. H2O2 and reducing substrate concentration were seen to be consistent with a substrate inhibited Ping – Pong Bi-Bi reaction mechanism [18].Exposure to Ag NPs leads to the inhibition of selenoprotein synthsis and inhibition of Thioredoxin Reductase in oxidative stress, increases endoplasmic reticulum stress, and reduced cell proliferation during exposure to Ag [19].Our results show that ZnO NPs was competitive inhibitor for salivary peroxidase activity. Competitive inhibitor competes with the substrate for the active site of an enzyme [13].

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How to cite this article:

Kadhim K. Ghudhaib, Leka´a M. Ibrahim, Eaman A. Al-Rubaee, Zainab A. Salman. (2016). Determination of Vmax and Km of ALP, AST and Peroxidase in Saliva of Chronic Periodontitis Patients with and without ZnO NPs: A Kinetic Study. Int. J. Adv. Res. Biol. Sci. 3(7): 85-91.

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