

The Effect of Ferric Oxide Nanoparticles on Peroxidase and Catalase Enzyme Activity in Healthy Human Serum

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Article Info

Received
19/03/2018

Accepted
14/05/2018

Published
15/08/2019

Abstract

In this work, ferric oxide nanoparticles were prepared by sol-gel method from oleic acid .the size of nanoparticles was studied by Scanning electron microscopy (SEM) and Thermal gravimetric analysis (TGA). The effect of ferric oxide nanoparticles on catalase and peroxidase enzyme activity in healthy human serum was studied. The results showed that ferric nanoparticles acted as competitive inhibitor with peroxidase and un competitive inhibitor with catalase enzyme.

Keywords: Ferric oxide nanoparticles, Peroxidase, Catalase.

الخلاصة

تم في هذا البحث تحضير الدقائق النانوية لأكسيد الحديد باستخدام طريقه محلول الهلام من حامض الاوليك .تم دراسته حجم الدقائق النانوية بواسطة مسح المجهر الالكتروني وتحليل الجاذبيه الحراريه .تم دراسة تأثير الدقائق النانوية لأكسيد الحديد على فعاليه انزيم الكاتلاز والبيروكسيداز . اظهرت النتائج ان للدقائق النانويه المحضره تأثير تثبيطي تنافسي على انزيم البيروكسيداز ولاتنافسي على الكاتلاز.

Introduction

Iron nanoparticles have attracted interest in the fields of nanoscience and nanotechnology because of their unique characteristics, such as chemical stability, durability and economic abundance [1]. The most application of ferric nanoparticles are: drug for specific carrier, gene carrier in gene therapy, in MRI studies, as nano adjuvant for vaccine and antibody production, and used in cancer treatment that is based on semiconductor ability to produce free radicals [2].

There are several methods for preparation of iron nanoparticles, from ascorbic acid [3], amino acid(lysine) [4], hemoglobin and myoglobin [5], reducing sugar (glucose) [6], tannic acid [7], biosynthesis from bacteria (acinetobacter species) [8], fungi [9], algae [10], and from fruits and plants extract[11, 12]. Catalase (E.C.1.11.1.6) is tetrameric protein with four identical subunits, each subunit contain hundreds amino acid residues, one

head group, iron called protoporphyrin and molecule of NADPH [13].

Catalase has catalytic action on hydrogen peroxide, high concentration of this enzyme in mammals is found in erythrocytes, kidney, liver, and saliva [14].

Catalase decreases in patients with diabetes mellitus, breast cancer, lymphoma, head and neck cancer, and urological cancer [15].

Catalase increase in hepatitis, sever liver cell damage, cardiac circulating failure and fatty liver [16].

Peroxidase (E.C.1.11.1.7) is the enzyme that catalase the biochemical reactions of peroxide in presence of electron donor substances. Salivary, mammary and mucosal glands can secrete peroxidase enzyme. Peroxidase found in high concentration in liver, spleen, salivary glands, leukocytes, intestines, and stomach. In the body, a peroxidase function is a defense against harmful effect of free radicals, so it has an important role in defending against diseases [17].

Materials and Methodologies

The study considers the preparation of (α - Fe_2O_3) using Oleic acid by sol-gel method [18]. Fe_2O_3 nanoparticles were prepared by sol-gel method. (1.62g) FeCl_3 was dissolved in (100cm^3) distilled water with stirring for (30min.). Gelatin (Oleic acid) (6.44ml) was dissolved in few amount of absolute ethanol and 100 ml distilled water and then stirred for (30min.) and the mixture was heated at 60°C for one hour at pH 8 by adding drops of (30 %) sodium hydroxide. The color of solution red-brown and the solution turns to gel followed by drying in oven at 80°C for four hours. The obtained compound was calcined at different temperatures for two hours using muffle furnace.

Determination of serum catalase enzyme activity by using manual method [19]

Principle: catalase react with peroxide (substrate), the remaining peroxide react with ammonium molybdate to form a coloring complex

Reagents	Test	Blank 1	Blank 2	Blank 3
Serum	0.2ml	----	---	---
Substrate(hydrogen peroxide)	1ml	1ml	1ml	---
D.W	20 μl	20 μl	20 μl	20 μl
Buffer (sodium phosphate) Ph7.4	----	---	---	1ml
Ammonium molybdate	1ml	1ml	1ml	1ml
Buffer(sodium phosphate)Ph7.4	----	---	0.2ml	0.2ml
Serum	----	0.2ml	---	---

Determination of serum peroxidase enzyme activity [20]

Serum peroxidase activity assay was achieved by using colorimetric method, In test tube 1.4ml of phenol solution was added, then 1.5ml of peroxide (substrate 0.0017mmol/L) was added with incubation 3min., then 100 μl of serum and 20 μL D.W. were added.

Serum catalase and peroxidase activities were determined by using colorimetric method. Different concentration of ferric nanoparticles (0.02, 0.01, 0.005, 0.002, 0.001 $\mu\text{g}/\text{mL}$) were prepared from stock solution 0.1 $\mu\text{g}/\text{mL}$.

Serum catalase and peroxidase were detected by using above method; the same steps were conducted with replace the volume of D.W. by nanoparticles volume to evaluate the effect of nanoparticles on enzyme activity.

Results and Discussion

Characterization of Fe_2O_3 Nanoparticles

Scanning electron microscopy (SEM)

Scanning electron microscopy gives the information about the morphological and topographical characteristics of the solid surfaces that is necessary in understanding the behavior of the surfaces. The SEM works by focusing a high-energy electron beam generated from a field emission gun on to the sample using electromagnetic apertures and lenses. A raster scan patterns then performed with the electron beam by scanning coils. The Electro emission is due to absorption of the electron beam (primary electrons) in order to image the surface topography of the sample. The electron photomicrography of α - Fe_2O_3 nanoparticles obtained from sol-gel derived gelatin media calcined at 700°C for 2hr. are illustrated in Figure 1. The particles are dispersed and hexagonal single crystals shape with particle size (54nm). These results are good agreement with those reported in literatures [21-23].

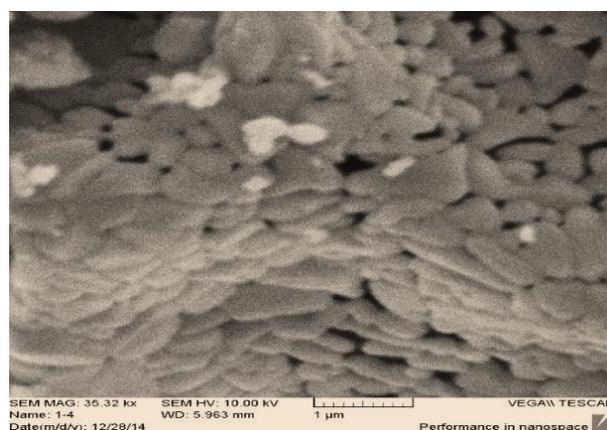


Figure 1: SEM images of α - Fe_2O_3 nanoparticles derived from oleic acid; after calcinations at 700°C through sol – gel method.

Thermal gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) is an analytical technique for measuring changes in the mass of a material that occur in response to

programmed temperature changes [24]. The measured weight loss curve gives information about changes in sample composition. The thermal analysis was carried out from 25 – 700°C. TGA of the prepared compound was performed by heating rate at 10°C/min. figure 2 show that the initial weight loss for the water departure is 18.98% while 52.74% weight loss in the temperature range 230 – 650°C corresponds to the decomposition of the organic constituents.

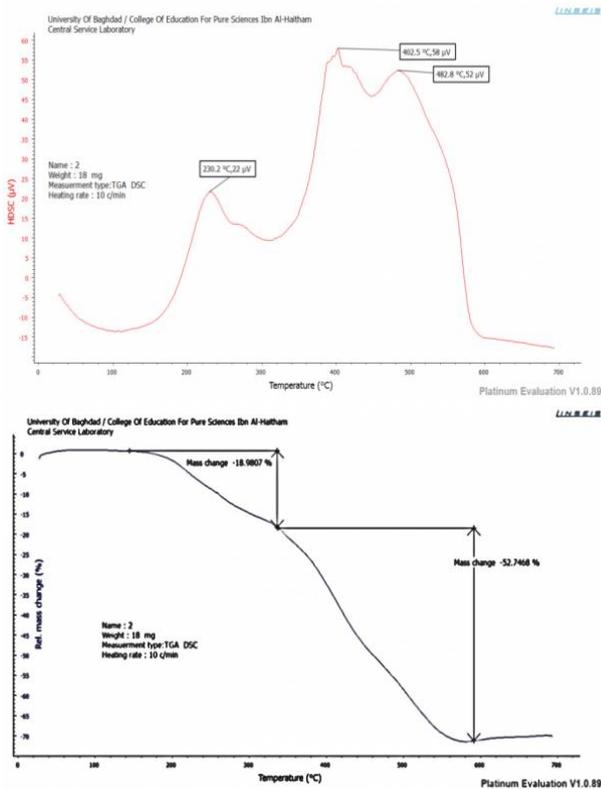


Figure 2: TGA pattern of complex synthesized by sol-gel technique before calcinations.

1. Biochemical analysis

The biochemical tests show that ferric nanoparticles showed inhibitory effect on catalase and peroxidase enzyme, the relation between enzyme activity and nanoparticles concentration was shown in Table 1 and 2.

The greater percentage of inhibition of ferric nanoparticles on peroxidase enzyme was 86.54% at 0.02µg/mL as shown in tTable 1, and the greater percentage of inhibition of ferric nanoparticles on catalase enzyme was

81.94% at 0.02µg/mL as shown in Table 1, 2 and Figure 4, 5.

Kinetic parameters were studied using lineweaver burk plot as shown in Table 3 and Figure 3.

The forces that may be involved in the protein –NPs interactions are hydrophobic interaction, van der waals forces, lone pair electron, polarization, and hydrogen bond. The type of interaction depends on protein and NPs, the kind of interaction could be very helpful in nanomedicine to eliminate certain type certain type of nanoparticles based on their relative Nano descriptors [25].

So, ferric nanoparticles caused inhibition of catalase and peroxidase because it was interact with functional group in active site of enzyme and causing enzyme inhibition and protein denaturation.

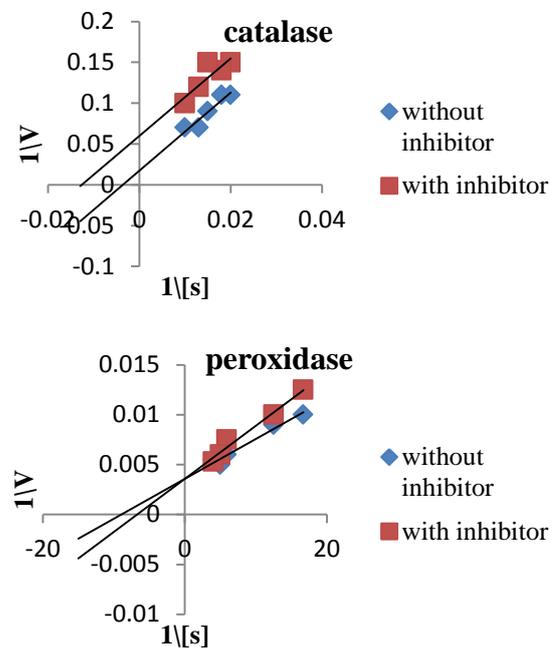


Figure 3: Lineweaver burk plot for ferric oxide nanoparticles with peroxidase and catalase enzyme.

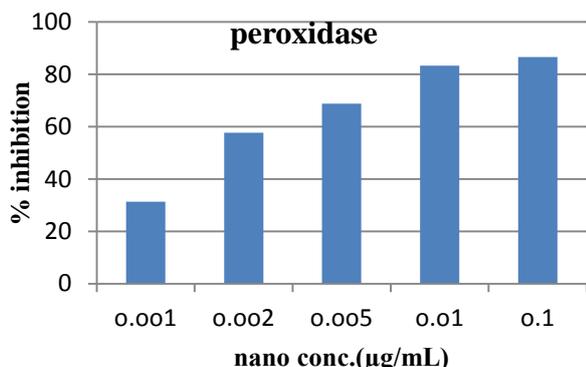


Figure 4: The relation between ferric oxide nanoparticles and % inhibition for peroxidase.

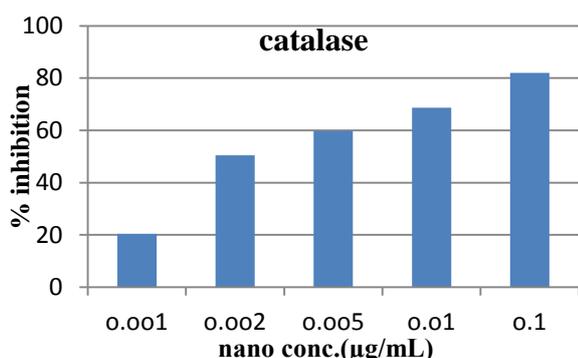


Figure 5: The relation between ferric oxide nanoparticles and % inhibition for catalase.

Table 1: The effect of Fe₂O₃ nanoparticles on peroxidase activity.

NANO CONC. (µg/ml)	Peroxidase activity (U/L)	% inhibition
Nil	165.15	
0.001	113.73	31.37
0.002	69.92	57.66
0.005	51.67	68.71
0.01	27.55	83.31
0.02	22.23	86.54

Table 2: The effect of Fe₂O₃ nanoparticles on catalase.

NANO CONC. (µg/ml)	Catalase Activity (U/L)	% inhibition
Nil	41.6	
0.001	33.14	20.33
0.002	20.59	50.50
0.005	16.73	59.78
0.01	13.02	68.70
0.02	7.51	81.94

Table 3: Kinetic parameters of peroxidase and catalase with Fe₂O₃ nanoparticles.

Enzymes	V _{map} (U/L) ⁻¹	K _{map} (mmol/L) ⁻¹	Inhibition type
peroxidase	22.2	0.16	Competitive
catalase	16.7	80.3	UN-Competitive

Conclusion

We conclude that there was competitive inhibition for ferric oxide nanoparticles with peroxidase enzyme activity and un-competitive inhibition with catalase enzyme activity.

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