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Effect of Hematite Nanoparticles Synthesized by Sol –Gel Method on Activity of Liver Enzymes

Asmaa J. Al-lamei[1], Enas, F. Shtewy[2]

[1]Chemistry Department, College of Science, Al-Mustansyriah University, Iraq

[2]Ministry of Health the National Center of Drugs Control and Research

Abstract: ***This paper describes the synthesis of hematite nanoparticles by sol-gel routeusing carboxylic acid as gelatin media at different temperatures. At 700°C the α-Fe_2O_3nanoparticlesare formed with particle size 54nm.The nanoparticles are characterized by XRD, SEM and AFM.The effect of nanoparticles on activity of liver enzyme (GPT and GOT) is also studied by using different concentration of NPs.It is found that the activity of GPT and GOT enzymes increases with decreases in nano particles concentrations and decreases in inhibition percentage. The greater activation of nano was demonstrated at concentration (10^{-1} M).***

Keywords: Hematitenanoparticles, sol-gel method, liver enzymes

1. Introduction

Iron oxide nanoparticles have been of great interest, not only because of their special fundamental properties, but also due to their multivalent oxidation states, abundant polymorphism and mutual polymorphous changes in crystal phase such as hematite (α-Fe_2O_3) and maghemite (γ-Fe_2O_3).Hematite (α-Fe_2O_3), the thermodynamically stable crystallographic phase of iron oxide, is a very attractive material because of its wide applications in various fields. For instance, high density magnetic recording media, gas sensors, catalysts, pigment and clinical uses. Its nontoxicity, low cost, and relatively good stability are definitely very attractive features for these applications[1].In the recent few years, many studies on synthesis of iron oxide nanoparticles have focused on controlling the shape and size of nanoparticle via various synthesis methods. Some studies on synthesis of magnetic Fe_2O_3 nanoparticles have been reported in recent decade, and most of them focused on the synthesis of γ- Fe_2O_3, Fe_3O_4 nanoparticles and α- Fe_2O_3nano spheres [2, 3, 4].The principal iron oxide nanoparticles used in catalysis of industrial reactions are magnetite and hematite. Both are semiconductors and can catalyze oxidation/reduction reactions. They can also be used in: biotechnology research, for catalytic reaction, for environment application and high performance liquid pigment.Enzymes are giant macromolecules which catalyse biochemical reactionsThe difference being that enzymes possess catalytic activity. The part of the enzyme tertiary structure which is responsible for the catalytic activity is called the 'active site' of the enzyme The active site is usually a hydrophilic cleft or cavity containing an array of amino acid side chains which bind the substrate and carry out the enzymatic reaction. A liver enzymes are a protein that helps to speed up a chemical reaction in the liver Aspartate aminotransferase (GOT) and alanineaminotransferase (GPT) are enzymes found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys.GPT having the function of transferring amino group from alpha–amino acid (alanine) to alpha– keto acids (α–ketoglutrate), therefore; named transaminase[5, 6]. The transamination reaction is an important in intermediary metabolism due to synthesis and degradation of amino acids. The keto acids formed by the reaction are altimatly oxidized by the tricarboxylic acid cycle to provide a source of energy [7].

2. Material and Methods

Preparation of (α–Fe_2O_3) using Oleic acid:
Iron oxide nanoparticles are synthesized by sol- gel route using ferric chloride as iron source from SDFCL (97%) analytical grad and olic acid as a gelatin agent. In a typical synthesis (1.62 g, 9.8 m mole) $FeCl_3$ was dissolved in (100 $cm^{3)}$ D.W with stirring for (30 min.). Gelatin (Oleic acid) (6.44 ml) was dissolved in a mount of absolute ethanol and 100 ml D.W and then stirred for (30 min.) and the mixture was heated at 60ºC for one hour at pH 8 by adding drops of (30%) ammonium hydroxide. As in the past, The obtained α-Fe_2O_3 nanoparticles red – brown color.

Characterizations : The identification phase , particle size and crystallinestructure analysis are determined by XRD using shimadzu –6000 model witha Cu radiation (λ= 1.54 A^{O}) , voltage 40Kv and current 30 mA with speed 5^{O} /min. The Atomic force microscopy (AFM)CAPM type AA3000 is used toinvestigate the particle size and morphology of the derived nanoparticles.

Procedure the effect of nanoparticles on GPT &GOT enzyme activity

1) Preparation different concentration of nanoparticles ($1x10^{-1}$, $1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$, $1x10^{-5}$ M) in deionized water.
2) Preparation of working reagent: mix 8 ml of reagent (R1) with 2 ml of reagent (R2) the working reagent is stable for 30 days at 2-8 ºC.
3) Six test tubes were used to put in each one mix (1000 µl) from working Reagent (GPT enzyme) with (100 µl) nanoparticles and (100 µl) serum and incubate at 37 ºCfor one minute. Then the change in absorbance per minute (Δ O.D / min) was measured at λ = 340 nm.
4) A control solution was prepared by mixing (1000 µl) GPT enzyme with (100 µl) serum and deionized water (100 µl) and incubate at 37ºC for 1minute.

Then the change in absorbance per minute. (Δ O.D/ min.) was measured during 3 minute. The inhibitor percentage was calculated by comparing the activity with and without the nano and under the same conditions. According to the equation[8]:

$$\% \text{ Inhibition} = 100 - \frac{\text{the activity in the presence of nano}}{\text{the activity in the absence of nano (control)}} \times 100$$

The enzyme GPT &GOT activity was measured in human serum by using this equation: GPT activity (U / L) = Δ O.D / min. x 1745

3. Result and Discussion

AFM analysis

Figure (1) and table (1) shows the AFM imagesand the corresponding size distributionsof the α–Fe_2O_3 nanoparticles. It is clearfrom Figure that the average diameter ofα–Fe_2O_3nanoparticles is 54nmwhich are observed over the entire surface, as shown in the inset.The3–dimensional (3D) AFM images of material nanoparticle in which the irregular and randomly distributed α–Fe_2O_3 nanoparticles and can be seen with a maximum value of (2.9 nm) exhibit morphology with a root–mean square (RMS) roughness of (0.494nm) for carboxylic acid. A number of earlier studies have investigated the surface structure ofhematite dispersions characterized by a variability of morphology and particlesize from AFM and TEM techniques [9, 10, 11].

Table 1: Effect of Calcination temperature on the average particle size of α–Fe_2O_3 nanoparticles using carboxylic acid bysol-gel method

Temp. (º C)	400	500	600	700	800	900
Particlesize (nm)Oleic acid	102.97	82.02	76.68	54.67	88.02	99.56

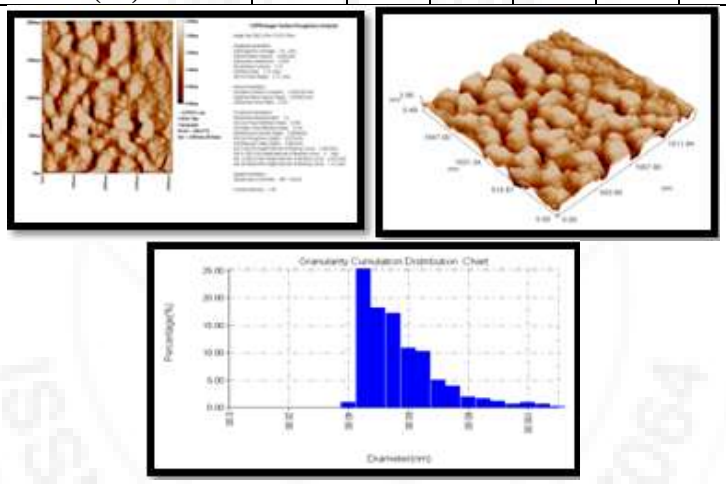

Figure 1: AFM images for nanoparticles synthesized from carboxylic acid (Oleic acid) at 700 ºC calcination through sol-gel

Scanning electron microscopy (SEM):

Scanning electron microscopy gives the information about themorphological and topographical of the solid surfaces that is necessary in understanding the behavior of the surfaces[12].Fig. (2) the SEM image of α–Fe_2O_3 nanoparticles derived from Oleic acid as gelatin media. The particles are dispersed and hexagonal single crystals shape with particle size (54 nm). Details of the characterization of the study synthetic hematite samples are presented elsewhere Colombo and et al. Andreu and co-workers[13, 14].

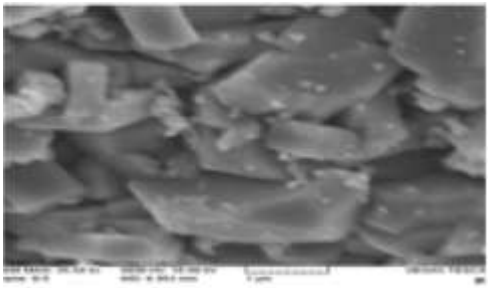

Figure 2: SEM image of α–Fe_2O_3 nanoparticles derived from oleic acid; after calcination at 700 ºC through sol – gel method

X-Ray diffraction (XRD) analysis

The X-Ray diffraction patterns for hematite nanoparticles (calcined at 700ºCfor2 hr.) prepared by sol –gel method using gelatin as a media was illustrated in figure (3).The complete transformation of γ– Fe_2O_3 to α-Fe_2O_3 can be seen at 700ºC.These results are contradictory to those obtained by Sahoo and et al. where they had reported the complete transformation at 880º C[15].In the present synthetic method, 700ºCis the temperature at which the α-Fe_2O_3particles with size (54nm)were obtained.It can be seen the particles size decreases with temperature increases from 400 to700ºCthis might be explained by the phase transformation from gamma–Fe_2O_3 to α–Fe_2O_3 in this range of temperatureThe XRD peaks in whole angle range of 2 θ from 10º -70 º with Cu radiation (voltage 40 Kv and current 30 mA) λ = 1.540 Aº with speed5 º / min.It can be see from figure (3) nine characteristic peaks were observed for α –Fe_2O_3 nanoparticles (2 θ = 24.2 º , 33.2º , 35.6º , 41.1º , 49.5º , 54.1º , 57.4º , 62.4º and 64.0º).The diffraction peak of the synthesized α–$Fe_2O_3$are in good agreement with those reported in literatures[16, 17, 18].

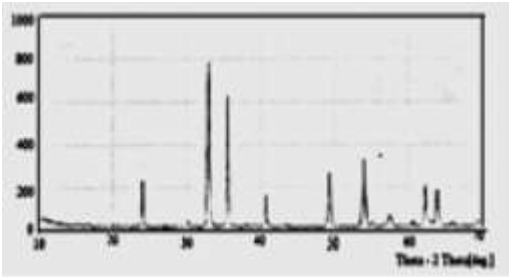

Figure 3: XRD pattern of α–Fe_2O_3 nanoparticles obtained from oleic acid after calcination 700 ºC

The effect of α–Fe_2O_3 nanoparticles on GPT and GOT enzyme Activity

GPT and GOT levels are a valuable aid primarily in the diagnosis of liver disease it can be used in combination with other enzymes to monitor the course of various liver disorders.The effect of nano (α-Fe_2O_3) of serum GPT and GOT enzymes activity was investigated in this study, the biochemical tests revealed that nanoparticles (hematite) caused inhibitory effects on GPT and GOT enzymes. The relationship between nanoparticles concentration versus the activity of enzymes as shown in table (2)and table (3)for GPT and GOT respectively. These results observed that any increase in nano concentrations caused decreases in activation of enzymes and increases in inhibition percentage. Figure (4)and figure (5) for GPT and GOT enzymes respectively.The greater inhibition of nano was demonstrated at concentration (10^{-1}M).The enzymes plays an important role in amino acid metabolism and in the urea tri- carboxylic acid cycles[19]. We suggested that nano molecule changes the active sides of amino acids on GPT and GOT enzymes due to decreasing affinity of active sides of enzymes or the change in the stereo structure of the enzymes in the presence of nano caused to inhibite the enzymes.The results of our study are in agreement with previous studies of same enzyme[20].

Table 2: Shows the effect of different concentration of nano on activity and inhibitior percentage of GPT enzyme.

[conc.ofnano] M	Enzyme activity (U/L)	Inhibition%
10^{-1}	5.235	62.5
10^{-2}	8.725	37.5
10^{-3}	10.47	25
10^{-4}	10.47	25
10^{-5}	12.564	10
control	13.96	-------

Table 3: Shows the effect of different concentration of nano on activity and inhibitior percentage of GOT enzyme.

[conc.ofnano] M	Enzyme activity (U /L)	Inhibition%
10^{-1}	1.745	85.71
10^{-2}	3.49	71.42
10^{-3}	4.362	64.28
10^{-4}	5.235	57.14
10^{-5}	8.725	21.43
control	12.215	-------

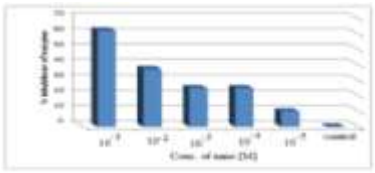

Figure 4: % Inhibition of GPT enzyme with different concentration of nanoparticles

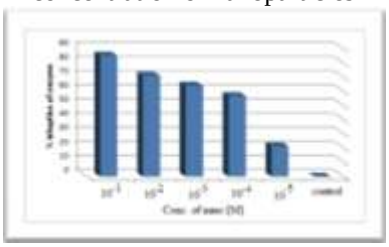

Figure 5: % Inhibition of GOT enzyme with different concentration of nanoparticles

[21]Y.M.Jeon and et al.were examined the liver tissue damage induced by nano sized–TiO_2 in mouse the biochemical parameters of liver namely GOT, GPT and Alkaline phosphatase enhanced approximately 18% , 35% and 69 % by exposure to nano sized–TiO_2respectively.The GPT activity is greater more than GOT activity due to GPT sensitive to nanoparticles more than GOT enzyme.

4. Conclusion

Iron oxide particles in the size range of 54 nm have been synthesized by sol-gel method with different annealing temperature. The XRD results show that at the lower annealing temperature nano particle comprise both the γ-Fe_2O_3 and α-Fe_2O_3 phase as the temperature got increased up to 700ºC the maghemite phase seemed to be converted to the hematite phase in all acid modified at700º C.Nanoparticales (hematite) caused inhibitory effects on ALT (GPT) and AST (GOT)enzymes.The greater inhibition of nano was demonstrated at concentration (10^{-1}M).Inhibitor percentage of GPT enzyme exhibits (62.5%) lower than GOT enzyme (85.71%).

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