

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

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Abstract: This paper describes the synthesis of hematite nanoparticles by sol-gel route using carboxylic acid as gelatin media at different temperatures. At 700°C the α -Fe₂O₃ nanoparticles are 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: Hematite nanoparticles, 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 -Fe₂O₃) and maghemite (γ -Fe₂O₃). Hematite (α -Fe₂O₃), 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 Fe₂O₃ nanoparticles have been reported in recent decade, and most of them focused on the synthesis of γ -Fe₂O₃, Fe₃O₄ nanoparticles and α -Fe₂O₃ nano 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 catalyze biochemical reactions. The 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 alanine aminotransferase (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 -ketoglutarate), 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 ultimately oxidized by the tricarboxylic acid cycle to provide a source of energy^[7].

2. Material and Methods

Preparation of (α -Fe₂O₃) using Oleic acid:

Iron oxide nanoparticles are synthesized by sol-gel route using ferric chloride as iron source from SDFCL (97%) analytical grade and oleic acid as a gelatin agent. In a typical synthesis (1.62 g, 9.8 μ mole) FeCl₃ was dissolved in (100 cm³) 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 60°C for one hour at pH 8 by adding drops of (30%) ammonium hydroxide. As in the past, The obtained α -Fe₂O₃ nanoparticles red – brown color.

Characterizations : The identification phase, particle size and crystalline structure analysis are determined by XRD using shimadzu –6000 model with Cu radiation ($\lambda = 1.54 \text{ \AA}$), voltage 40Kv and current 30 mA with speed 5°/min. The Atomic force microscopy (AFM) CAPM type AA3000 is used to investigate 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 °C.
- 3) Six test tubes were used to put in each one mix (1000 μ l) from working Reagent (GPT enzyme) with (100 μ l) nanoparticles and (100 μ l) serum and incubate at 37 °C for one minute. Then the change in absorbance per minute (Δ O.D / min) was measured at $\lambda = 340 \text{ nm}$.
- 4) A control solution was prepared by mixing (1000 μ l) GPT enzyme with (100 μ l) serum and deionized water (100 μ l) and incubate at 37°C for 1 minute.

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 images and the corresponding size distributions of the α -Fe₂O₃ nanoparticles. It is clear from Figure that the average diameter of α -Fe₂O₃ nanoparticles is 54nm which are observed over the entire surface, as shown in the inset. The 3-dimensional (3D) AFM images of material nanoparticle in which the irregular and randomly distributed α -Fe₂O₃ 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 of hematite dispersions characterized by a variability of morphology and particle size from AFM and TEM techniques [9, 10, 11].

Table 1: Effect of Calcination temperature on the average particle size of α -Fe₂O₃ nanoparticles using carboxylic acid by sol-gel method

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

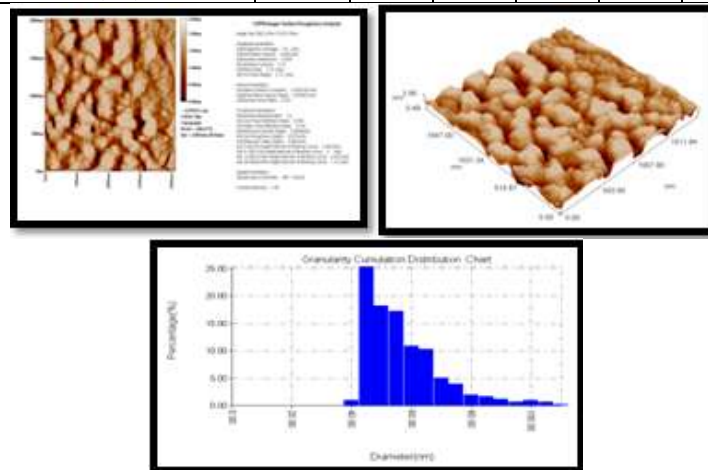


Figure 1: AFM images for nanoparticles synthesized from carboxylic acid (Oleic acid) at 700 °C calcination through sol-gel

Scanning electron microscopy (SEM):

Scanning electron microscopy gives the information about the morphological and topographical of the solid surfaces that is necessary in understanding the behavior of the surfaces^[12]. Fig. (2) the SEM image of α -Fe₂O₃ 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].

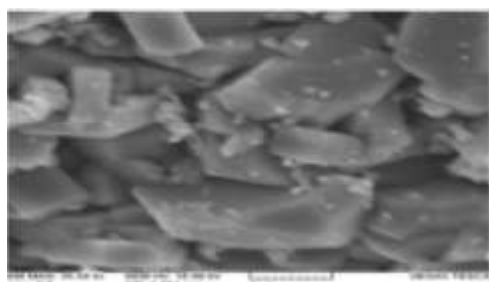


Figure 2: SEM image of α -Fe₂O₃ nanoparticles derived from oleic acid; after calcination at 700 °C through sol-gel method

X-Ray diffraction (XRD) analysis

The X-Ray diffraction patterns for hematite nanoparticles (calcined at 700°C for 2 hr.) prepared by sol-gel method using gelatin as a media was illustrated in figure (3). The complete transformation of γ -Fe₂O₃ to α -Fe₂O₃ can be seen at 700°C. These results are contradictory to those obtained by Sahoo and et al. where they had reported the complete transformation at 880° C^[15]. In the present synthetic method, 700°C is the temperature at which the α -Fe₂O₃ particles with size (54nm) were obtained. It can be seen the particles size decreases with temperature increases from 400 to 700°C this might be explained by the phase transformation from gamma-Fe₂O₃ to α -Fe₂O₃ in this range of temperature. The XRD peaks in whole angle range of 2 θ from 10° -70 ° with Cu radiation (voltage 40 Kv and current 30 mA) λ = 1.540 Å with speed 5 ° / min. It can be seen from figure (3) nine characteristic peaks were observed for α -Fe₂O₃ nanoparticles (2 θ = 24.2 ° , 33.2° , 35.6° , 41.1° , 49.5° , 54.1° , 57.4° , 62.4° and 64.0°). The diffraction peak of the synthesized α -Fe₂O₃ are in good agreement with those reported in literatures^[16, 17, 18].

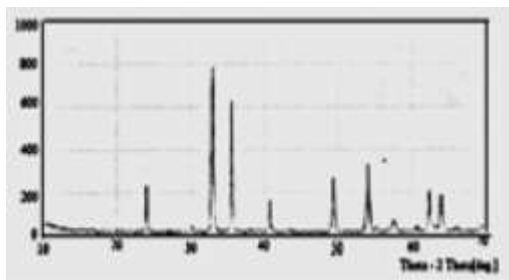


Figure 3: XRD pattern of α -Fe₂O₃ nanoparticles obtained from oleic acid after calcination 700 °C

The effect of α -Fe₂O₃ 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 -Fe₂O₃) 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 (10⁻¹M). The enzymes play 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 inhibit 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 inhibitor 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 inhibitor 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	-----

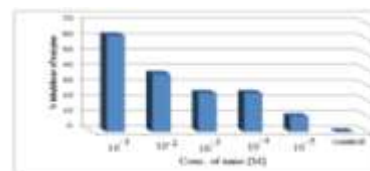


Figure 4: % Inhibition of GPT enzyme with different concentration of nanoparticles

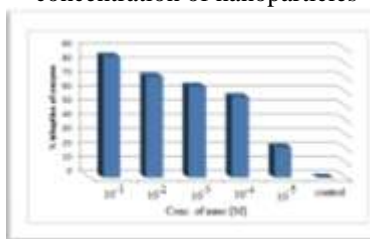


Figure 5: % Inhibition of GOT enzyme with different concentration of nanoparticles

^[21]Y.M.Jeon and et al. were examined the liver tissue damage induced by nano sized-TiO₂ in mouse the biochemical parameters of liver namely GOT, GPT and Alkaline phosphatase enhanced approximately 18% , 35% and 69 % by exposure to nano sized-TiO₂ respectively. 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 -Fe₂O₃ and α -Fe₂O₃ phase as the temperature got increased up to 700°C the maghemite phase seemed to be converted to the hematite phase in all acid modified at 700° C. Nanoparticles (hematite) caused inhibitory effects on ALT (GPT) and AST (GOT) enzymes. The greater inhibition of nano was demonstrated at concentration (10⁻¹M). Inhibitor percentage of GPT enzyme exhibits (62.5%) lower than GOT enzyme (85.71%).

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