New colorimetric method to determine catalase mimic activity

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Abstract

A new colorimetric method was used to determine catalase mimic activities of manganese dioxide (MnO\textsubscript{2}), iron oxide (Fe\textsubscript{2}O\textsubscript{3}) nanoparticles were prepared by a hydrothermal method (autoclave), and its composite. The MnO\textsubscript{2} nanoparticles were annealed at different temperatures (250–700 °C), while MnO\textsubscript{2}:Fe\textsubscript{2}O\textsubscript{3} composite in the mole ratio of 3:1 annealed at 400 °C. The structures and surface morphology were characterized by FT-IR measurements, x-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Atomic Force Microscope (AFM). This new method succeeds to determine catalase mimic activity, and found the activity of the composite was lower than its activity of MnO\textsubscript{2} alone, in the same annealing temperature.

1. Introduction

Catalase (CAT) is a common enzyme (metalloprotein) that presents in all oxygen metabolizing cells. This enzyme exists in a soluble state in erythrocyte, and human erythrocytes are normally rich in catalase, so the catalase activity of blood is practically all due to the erythrocyte [1]. Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is produced naturally in the aerobic organisms and human body. It is a strong oxidizing (one of reactive oxygen species) and bleaching agent and can be converted to highly reactive hydroxyl radicals, which are extremely toxic and can cause damage to dopaminergic neurons. Increased lipid peroxidation, elevated iron levels, expanded creation of reactive oxygen species (ROS) and diminished degrees of weakened glutathione have also been identified in the substantial nigra of patients suffering from Parkinson’s disease [2]. Catalase stops the accumulation of hydrogen peroxide and defends cellular organelles and tissues from destruction by peroxide, which is constantly shaped by many metabolic reactions. Catalase causes H\textsubscript{2}O\textsubscript{2} into H\textsubscript{2}O and O\textsubscript{2} and defends organisms from free radicals. It also has manufacturing uses to avoid certain pollutants in food and as a disinfectant for interaction lenses and a cleansing agent in some other yields.

The catalase action can be estimated by deciding the lessening of H\textsubscript{2}O\textsubscript{2} absorption (at λ = 240 nm) [3, 4]. The complications combined with this method, caused by using great levels of the substrate about (5–50 mM) to acquire adequate absorbance [5]. Furthermore, the excessive levels of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) lead to the realization of small bubbles in the solution which cause an error in the measurements [6]. CAT activity can be measured by other approaches such as the titration method, which is the sample does not allow in the spectrophotometer method when the precipitation or pigmentation was formed [1, 7]. Goth [8] used other colorimetric methods for CAT by measuring spectrophotometrically the unreacted H\textsubscript{2}O\textsubscript{2} by reacting a compound with the ammonium molybdate. Another method used by Sinha and Hadwan [9, 10] which are the hydrogen breakdown of H\textsubscript{2}O\textsubscript{2} measured (spectrophotometrically) by the reaction of dichromate and acetic acid as a reagent to formation complex. The titration method considered as a method for measurement of catalase activity, this method used when precipitation or pigmentation was formed, these do not allow in the UV-spectrophotometric method [5]. Manganese oxides (MnO\textsubscript{2}) for many years, with various crystal is giving a lot of attention, since their different chemical and physical properties, which are used in the different applications, such as catalysts, biosensors, water treatment, molecular sieves, and supercapacitors [11]. MnO\textsubscript{2} is n-type
semiconductor oxide [12]. Iron oxide (Fe₂O₃) prepared by different methods such as oxidation precipitation, co-precipitation, Sol-Gel, and thermal decomposition. The physical and chemical properties are affected by the type method used for preparation of nanoparticles, for example uniformity, crystallinity, and size [13, 14]. Fe₂O₃ has different crystal structure for example wustite, hematite, maghemite, and magnetite which summarize as FeO, α-Fe₂O₃, β-Fe₂O₄ and γ-Fe₂O₄ respectively [15–17]. Fe₂O₃ nanoparticles have the advantage of low cost and environmental harmless, MnO₂ and Fe₂O₃, two very low-cost metal oxides and MnO₂:Fe₂O₃ are widely used in many applications such as supercapacitor [18]. MnO₂ and Fe₂O₃ nanoparticles were prepared by a hydrothermal method, the hydrothermal method considered as a powerful for synthesizing numerous forms of MnO₂ because of the it is a simple method, cheap technique, can be control temperature, pH, choice of precursors, and reaction time [19].

The present work is a new improved method that uses a colorimetric method for determination of hydrogen peroxide (H₂O₂) in acidic solution contain potassium permanganate.

2. Experimental method

2.1. Chemicals and equipment

The reagents used in this research were of analytical grade purity. Potassium permanganate (KMnO₄), purity 99.9%; sulfuric acid (H₂SO₄), purity 95%; ethanol (EtOH), grade, purity 97%; iron chloride anhydrate (FeCl₃), purity 99.8%, hydrochloric acid (HCl), purity 99.9%, and urea (NH₂CONH₂) 99.9% from BDH company. Hydrogen peroxide (H₂O₂), purity 50%; Merck company. Fourier Transform Infra-Red spectroscopy (FTIR) (FTIR-8400S Shimadzu) in the wavelength range 400–4000 cm⁻¹ and x-ray Diffraction (XRD) (6000 Shimadzu) using CuKα, λ = 0.15406 nm radiations. Atomic force microscopy (AFM) were measured using (SPM-AA 3000), and UV/Visible spectrophotometric.

2.2. Preparation of MnO₂ nanoparticles

The reaction was performed by the hydrothermal method in a Teflon-lined (100 ml) stainless steel autoclave. The synthesis in briefly, 4.115 g of KMnO₄ into deionized water (70 ml) was added with stirring about 20 min. The solution should be filtered to remove any contaminates, after that added about 3.4 ml of HCl (concentrated) to the above solution (filtrated) with vigorous stirring to get the precursor solution. After that the solution transferred into an autoclave, sealed and treated with hydrothermally at 200 °C for 9 h. After complete the reaction, taken out the autoclave and cooled to room temperature (naturally). The resulting precipitates (brown-black) were filtered by centrifugation and washed by deionized water several times, to remove other products, washed with ethanol, and finally the as-prepared dried at 100 °C in air for 120 min. The reaction between KMnO₄ and HCl took place as the following steps:

\[
2\text{KMnO}_4 + 6\text{HCl} + \text{H}_2\text{O} \xrightarrow{\text{Autoclave}} 2\text{Mn(OH)}_2 + 2\text{Cl}_2 + 2\text{KCl} + 1/2\text{O}_2 \tag{1}
\]

\[
2\text{Mn(OH)}_2 \xrightarrow{\text{Heat}(100 ^\circ \text{C})} 2\text{MnO}_2 + 4\text{H}_2\text{O} \tag{2}
\]

The final above reactions can be write by the following equation:

\[
2\text{KMnO}_4 + 6\text{HCl} + \text{H}_2\text{O} \xrightarrow{1.\text{Autoclave} \ 2.\text{Heat}(90 ^\circ \text{C})} 2\text{MnO}_2 + 2\text{Cl}_2 + 2\text{KCl} + 1/2\text{H}_2\text{O} \tag{3}
\]

The MnO₂ precipitate (brown-black) annealed at (250 °C, 400 °C, 550 °C and 700 °C) temperatures for 120 min.

2.3. Preparations of iron oxide (Fe₂O₃) nanoparticles

Iron oxide nanoparticles were prepared by the hydrothermal method using an autoclave, by dissolved 6 g of FeCl₃ (anhydrous), in 20 ml of distilled water. The solution mixed in a beaker about 10 min at room temperature to ensure dissolve all the salt. In another beaker, 3.33 g of NH₂CONH₂ were dissolved in 10 ml distilled water with stirring for 10 min at room temperature, until the solution became colorless. The first solution pours on the urea solution and stirred by using a magnetic stirrer for 20 min at room temperature. The mixed solutions were placed into an autoclave, then placed in the oven and heated at 200 °C for 12 h. After he tend of time, the autoclave cooled at normal temperature slowly. The precipitate form in reddish-brown color was washed by water (several times) after that collected by centrifugation, then the precipitate washed several times with ethanol. Finally dried the precipitate in an oven at 100 °C for 120 min. The dark red color precipitate was obtained when annealed at 400 °C.

The reaction between FeCl₃ and NH₂CONH₂ write by the following equations:

\[
3\text{NH}_2\text{CONH}_2 + 9\text{H}_2\text{O} \rightarrow 6\text{NH}_4\text{OH} + 3\text{CO}_2 \tag{4}
\]
The final equation can be assumed as:

$$\begin{align*}
2\text{FeCl}_3 + 6\text{NH}_4\text{OH} & \rightarrow 2\text{Fe(OH)}_3 + 6\text{NH}_4\text{Cl} \\
2\text{Fe(OH)}_3 & \xrightarrow{\text{Heat}} \text{Fe}_2\text{O}_3 + 3\text{H}_2\text{O}
\end{align*}$$

(5)

(6)

The final equation can be assumed as:

$$\begin{align*}
2\text{FeCl}_3 + 3\text{NH}_2\text{CONH}_2 + 6\text{H}_2\text{O} & \xrightarrow{\text{Heat}} \text{Fe}_2\text{O}_3 + 6\text{NH}_4\text{Cl} + 3\text{CO}_2 \\
\end{align*}$$

(7)

### 2.4. Preparation of MnO$_2$:Fe$_2$O$_3$ Composite

This composite was prepared by mixing (3:1) mole ratio of MnO$_2$:Fe$_2$O$_3$ respectively, starting from the equivalent amount of these metal hydroxides. In a beaker containing distilled water the metal oxides were dispersed and mixed for 3 h by using a magnetic stirrer. Then the mixture was placed in the autoclave and located in the oven at temperature 200 °C for 6 h. After end of the time the dark powder is wished several times with distilled water and annealing at temperature 400 °C for 120 min.

### 2.5. Catalytic activity procedure

The first time, the KMnO$_4$ concentration was measured by titration with sodium oxalate solution (known concentration). The second time, the unknown concentration of H$_2$O$_2$ was measured by titration with the KMnO$_4$ (known concentration). The standard curve contained the following concentration of KMnO$_4$ (0, 1, 2, 3, 4 and 5) $\times 10^{-5}$ M, was prepared to calculate the different color concentrations absorbed by KMnO$_4$ at the length wave 525 nm (as shown in figure 1).

The mimic activity, was measured using the reaction with the solution of MnO$_2$ nanoparticle (2 mM), and solution of H$_2$O$_2$ (750 $\mu$M), as shown in the reaction [10]:

$$2\text{H}_2\text{O}_2 \xrightarrow{\text{MnO}_2} \text{O}_2 + 2\text{H}_2\text{O}$$

(8)

After five minutes, the solution of potassium permanganate (300 $\mu$M), acidity by some drops of sulfuric acid (H$_2$SO$_4$) was added. The purple color of KMnO$_4$ solution, will be reacting with the H$_2$O$_2$ (the excess residual), and converted to colorless produce (MnSO$_4$), as explain s in the following equation:

$$3\text{H}_2\text{SO}_4 + 2\text{KMnO}_4 + 5\text{H}_2\text{O}_2 \rightarrow 2\text{MnSO}_4 + \text{K}_2\text{SO}_4 + 5\text{O}_2 + 8\text{H}_2\text{O}$$

(9)

The concentration of H$_2$O$_2$ is directly comparative to the KMnO$_4$ concentration. The decreasing which happen in the KMnO$_4$ color (concentration), was determined calorimetrically at $\lambda = 525$ nm using the equation get from figure 1. The following steps in table 1, explain the work of mimics activity.

### 3. Result and discussion

#### 3.1. The XRD analysis of MnO$_2$ and MnO$_2$:Fe$_2$O$_3$ composite nanopowders

The XRD pattern of MnO$_2$ nanoparticles is explained in figure 2. All the measured peaks are indicated to the tetragonal $\alpha$-MnO$_2$ (tetragonal phase), which agrees with the JCPDS Card No. 44–0141, with the lattice constants equal to ($a = b = 9.78475$ Å, and $c = 2.86302$ Å) while the angles ($\alpha = \beta = \gamma = 90^\circ$). As in figure 2(a) the diffraction peaks that are (211), (310), and (110) at $2\theta = (37.5720^\circ)$, (28.8056$^\circ$), and (18.1551$^\circ$) respectively, of MnO$_2$ at annealing temperature 250 °C, refer to the structure of tetragonal and belonged to alpha phase. When MnO$_2$ powder is annealed at 400 °C, (211), (310) and (101) planes appeared at ($2\theta = 37.7886^\circ$, 28.9918$^\circ$ and 18.3566$^\circ$) which are related to the alpha phase as shown in figure 2(b). After annealing at 350 °C the XRD of MnO$_2$ results were similar to 400 °C as shown in figure 2(c), the peaks located at ($2\theta = 37.7943^\circ$, 29.0091$^\circ$ and 18.3465$^\circ$) are related to the (211), (310) and (101) of the $\alpha$-MnO$_2$. All peaks are agreement with the
The intensity of diffraction peak (211) increased when annealing temperatures increase from 250 to 550 °C. At 400 °C and 550 °C the sample showed orientation of regular grains shaped, that suggesting to the pure phase of \(\alpha\)-MnO\(_2\) nanoparticle was increased, these results are identical to the results of the XRD spectra of the reference \[20\].

The new phase as cubic Mn\(_2\)O\(_3\) was identified at annealing temperature 700 °C (JCPDS Card No.41−1442) as in figure 2(d). The average crystallite size (D) for the peak alone was estimated using the Debye-Scherer formula:

\[
D = \frac{K\lambda}{\beta\cos \theta}
\]

Table 1. The steps describe the procedure used to calculate catalase mimic activity.

| Reagents              | Test | Control |
|-----------------------|------|---------|
| Solution of metal oxide | 0.5 ml | 0.5 ml |
| Distilled water       | 1 ml  | 2 ml    |
| Hydrogen peroxide     | 1 ml  | —       |
| Mix variously for 5 min, then, add: |       |         |
| Acidic solution of KMnO\(_4\) | 0.5 ml | 0.5 ml |
| Total volume          | 3 ml  | 3 ml    |

same card (JCPDS Card No.44−0141).
Table 2. The results obtained of the XRD for MnO$_2$ at different temperatures (from 250 °C to 700 °C), while Fe$_2$O$_3$ and MnO$_2$:Fe$_2$O$_3$ at 400 °C for 120 min.

| Annealing temperature for 120 min | 2θ (deg) | hkl | FWHM (deg) | Grain size (Å) | d (Å) | Lattice constant a (Å) | Lattice constant c (Å) |
|-----------------------------------|----------|-----|------------|----------------|-------|------------------------|------------------------|
| MnO$_2$ 250 °C                    | 37.572   | 211 | 0.552      | 151.928        | 2.391 | 9.793                  | 2.855                   |
| MnO$_2$ 400 °C                    | 37.805   | 310 | 0.506      | 162.018        | 3.096 |                        |                        |
| MnO$_2$ 550 °C                    | 18.155   | 101 | 0.597      | 134.781        | 4.882 |                        |                        |
| MnO$_2$ 700 °C                    | 37.888   | 211 | 0.545      | 153.894        | 2.378 | 9.731                  | 2.840                   |
| MnO$_2$:Fe$_2$O$_3$ at 400 °C     | 28.991   | 310 | 0.636      | 128.982        | 3.077 |                        |                        |
|                                  | 18.356   | 101 | 0.724      | 111.086        | 4.829 |                        |                        |
| Fe$_2$O$_3$ at 400 °C             | 37.794   | 211 | 0.541      | 155.119        | 2.378 | 9.728                  | 2.840                   |
|                                  | 29.001   | 310 | 0.645      | 127.226        | 3.076 |                        |                        |
|                                  | 18.346   | 101 | 0.694      | 115.879        | 4.831 |                        |                        |
| MnO$_2$:Fe$_2$O$_3$ at 400 °C     | 37.715   | 211 | 0.621      | 135.230        | 2.383 | 9.767                  | 2.843                   |
|                                  | 28.883   | 310 | 0.699      | 117.420        | 3.088 |                        |                        |
|                                  | 18.245   | 101 | 0.672      | 119.633        | 4.858 |                        |                        |

Where D is the crystallite size, k is the constant (0.9), β is the full width at half maximum (FWHM) intensity of the diffraction peak, λ is the wavelength of the x-ray radiation, and θ is the diffraction angle [21].

Table 2 shows the diffraction patterns of x-ray nanoparticles product at annealing temperatures (250, 400, 550 and 700 °C) for 120 min. The hydroxide phase (Mn(OH)$_4$) decrease when increase annealing temperature from 250 °C to 400 °C and convert from hydroxide (Mn(OH)$_2$) to only oxide phase (MnO$_2$), whereas the increasing temperature to 550 °C cause formed mixture phases from MnO$_2$ and Mn$_2$O$_3$, the increased in both of the lattice constant and the diffraction peaks (intensity), are agreement with the results of the reference [22]. The lattice constant is decreases when annealing temperature becomes 700 °C, due to the formation of one phase called Mn$_3$O$_4$ [23].

The result of the XRD of Fe$_2$O$_3$ and MnO$_2$:Fe$_2$O$_3$ composite powders at (3:1) mole ratio annealed at 400 °C (figure 2(e)). The diffraction peaks for the planes (012), (104), (110) and (113) at (2θ = 24.1, 32. 9, 35.6 and 40.8) according to (JCPDS Card No: 44-1087).

The XRD patterns for MnO$_2$:Fe$_2$O$_3$ composites powders are shown (figure 2(f)) giving the planes (200) and (110) at (2θ = 16.9097 and 14.0818) respectively. These belonged to the tetragonal structure ($\alpha$-phase) for MnO$_2$. The strength reflection planes (104) and (110) at (2θ = 33.3728° and 35.8370°) belonged to the hexagonal structure for Fe$_2$O$_3$ ($\alpha$-hematite phase). The greater intensity planes (200) for (2θ = 16.9097°) belongs to tetragonal structure of MnO$_2$ alpha phase.

3.2. Atomic force microscope (AFM) for MnO$_2$, Fe$_2$O$_3$, and MnO$_2$:Fe$_2$O$_3$ composite nanopowders

Figures 3(a) to (d) show atomic force microscope (AFM) images 3D and 2D of MnO$_2$ nanopowders annealing at several temperatures (250–700 °C), and the chart of granularity accumulation distribution. It is clear that at an average diameter of prepared nanoparticles and its composite are change with different annealing temperatures, as in table 3. The surface morphologies of the MnO$_2$ are different. We found the average diameter of the nanoparticles were (66.27–81.65 nm). According to the results of AFM the average diameter increasing by increase annealing temperature this result maybe relate to improvements in the crystalline of the nanoparticle and the agglomeration of small grains form larger grains [24]. But when the annealing temperature is 700 °C the average diameter decreases due to the formation of a new phase (Mn$_3$O$_4$) and these results correspond to XRD analysis.

Figures 3(e) and (f) shows a model two dimensional (2D) and three dimensional (3D) AFM image of Fe$_2$O$_3$ and MnO$_2$:Fe$_2$O$_3$ composite nanoparticles annealed at 400 °C. The average diameter of Fe$_2$O$_3$ nanoparticle was 61.53 nm, and decreases to 58.23 nm when composite MnO$_2$:Fe$_2$O$_3$ in mole ratio (3:1), this maybe relate to the interaction between nanoparticles of these oxides, as explained in the table 3.
3.3. Surface morphology by (FE-SEM)

3.3.1. Surface morphology by (FE-SEM) for MnO₂

Figure 4 shows the FE-SEM images of α-MnO₂ nanostructures prepared by hydrothermal method and annealed at temperatures 400 °C, for 120 min, at two magnifications (1 μm and 500 nm). The top-view FE-SEM image presented in figure 4 shows that the sample is composed of nanorods and nanorods bundle with average diameters about 65 nm.

3.3.2. The morphology surface for MnO₂:Fe₂O₃ by (FE-SEM)

The high magnification FE-SEM images of the Fe₂O₃ powder and MnO₂-Fe₂O₃ composite powders prepared by hydrothermal method with annealing temperature 400 °C for 120 min are shown in figures 4(b) and (c)) with magnifications 500 nm, Nanoparticles were observed as individual clusters with few conglomerates over the surface. The expected shapes are hexagonal micro pyramid which belongs to α-Fe₂O₃ was a match the XRD patterns results. The FE-SEM images of MnO₂-Fe₂O₃ shows that there are nanorods and the bundle of nanorods with different diameters and lengths, the faces of nanorods have quadrilateral shapes which belonged to tetragonal crystalline structure for α-MnO₂ nano-powder. The figure also shows that there are aggregations of granules appear around the nanorods which belonged to hexagonal crystalline structure for α-Fe₂O₃.

3.4. Fourier transform infrared spectrum (FTIR) study

FT-IR spectroscopy was carried out in order to ascertain the purity and nature of manganese dioxide nanostructures synthesized by the hydrothermal method. FTIR spectroscopy was carried out in order to ascertain the purity and nature of manganese dioxide nanostructures synthesized by the hydrothermal method. FTIR spectroscopy was carried out in order to ascertain the purity and nature of manganese dioxide nanostructures synthesized by the hydrothermal method. FTIR spectroscopy was carried out in order to ascertain the purity and nature of manganese dioxide nanostructures synthesized by the hydrothermal method.

Figures 5(a)–(e) represent the FTIR spectra of Mn(OH)₄ (as-prepared figure 5(a)), and powders annealing at (250–700°C). There is broad peaks around 3400 cm⁻¹ and 1650 cm⁻¹, which are distinguishing of surface-adsorbed hydroxyl groups in water (vibration of the stretching and bending hydroxyl (O–H) group, respectively). These peaks are reduced and became smaller with increasing annealed temperature (250–700 °C), corresponding to decrease the amount of water in samples and the Mn-O stretching became broad and more significant. The broadband absorption in the wavelength at 3420 and 1620 cm⁻¹ are assigned both the stretching and bending of H–O–H [25]. The bands at 522 cm⁻¹ correspond to the Mn–O bond. The broadband at 3320 cm⁻¹ and 1620 cm⁻¹ (figure 5(b)) relate to both the stretching and bending of residual hydroxyl groups respectively. The absorption peak at the wavelength 704 cm⁻¹ signified the surface bonding of −OH groups of Mn–OH for MnO₂ nanostructures. An absorption band is observed at 534 cm⁻¹ and 461 cm⁻¹ relates to the characteristic stretching of O–Mn–O, which confirmed the existence of the MnO₂ in the sample [26].

Figures 5(c), (d) and (e) show a decrease in both bands near to 3300 cm⁻¹ and 1660 cm⁻¹ which relate to vibration and bending of absorbed water. Meanwhile, the absorbance at 1007 cm⁻¹, from 715 to 662 cm⁻¹ and
Table 3. The Average diameter obtained by AFM of MnO₂ at different temperatures (from 250 °C to 700 °C), while Fe₂O₃ and MnO₂:Fe₂O₃ at 400 °C for 120 min.

| Sample annealing for 120 min | Average diameter (nm) |
|-----------------------------|-----------------------|
| MnO₂ (at 250 °C)            | 69.62                 |
| MnO₂ (at 400 °C)            | 72.36                 |
| MnO₂ (at 550 °C)            | 81.65                 |
| MnO₂ (at 700 °C)            | 66.27                 |
| Fe₂O₃ (at 400 °C)           | 61.53                 |
| MnO₂:Fe₂O₃ (at 400 °C)      | 58.23                 |
from 520 to 460 cm\(^{-1}\) are corresponding to stretching of Mn–O–Mn, Mn–O and O–Mn–O respectively [25].
The FTIR spectrum of the iron oxides annealing at 400 °C for 120 min, the peak at 3361 and 1640 cm\(^{-1}\) correspond to the water of hydration. The peaks at 547 and 474 cm\(^{-1}\) correspond to the metal–oxygen (Fe–O) vibration modes [27], as in figure 5(f).

FTIR spectra of the prepared MnO\(_2\):Fe\(_2\)O\(_3\) through the hydrothermal method and annealed at 400 °C is presented in figure 5(g). The bands at 3458 cm\(^{-1}\) and 1651 cm\(^{-1}\) can be assigned to the stretching and bending vibration of free water molecules in the sample. The peaks at 566 cm\(^{-1}\) and 474 cm\(^{-1}\) may be due to stretching of both O–Mn–O and Fe–O vibrations bond of hematite in the MnO\(_2\)/Fe\(_2\)O\(_3\) nanocomposite of rhombohedral structure [28, 29].

3.5. Catalase mimic activity of MnO\(_2\), Fe\(_2\)O\(_3\) and MnO\(_2\):Fe\(_2\)O\(_3\) composite

We used a new and simple colorimetric method to determine catalase mimic activity of MnO\(_2\), Fe\(_2\)O\(_3\) nanoparticles and MnO\(_2\):Fe\(_2\)O\(_3\) composite. This method is simple, cheap, fast and easy in the application. In order to estimate the catalase mimic activity (rate of reaction) of MnO\(_2\) nanoparticles as-prepared and when the annealing of the samples at different temperatures (250 °C–700 °C) for 120 min, we used the following equation:

\[
\text{Rate of catalase activity} \quad (K) = (2.303/t) \times (\log(C_o/C))
\]

Where: t mean the reaction time (in seconds); C\(_o\) and C are the entire concentration of H\(_2\)O\(_2\) in the test tube before and after reaction respectively. We found that there is increase in rate of reactions (K) with increase annealing temperatures and become maximum at annealing temperature is 400 °C (K = 2.59 \times 10^{-2} \text{ Ssc}^{-1}) as shown in figure 6 and Table 4, this may be related to conversion of manganese compound from metal hydroxide (Mn(OH))\(_4\) to metal oxide (MnO\(_2\)), after that the rate of reactions decreases with increasing the temperatures from 550 °C to 700 °C. This may be due to a decrease in the surface area of particles when increase annealing and accumulate particles.

At the same annealing temperature (400 °C) the catalase mimic activity of Fe\(_2\)O\(_3\) (1.44 \times 10^{-2} \text{ s}^{-1}) is lower than MnO\(_2\) (2.59 \times 10^{-2} \text{ s}^{-1}). Therefore, we suggested to use the mole ratio (3:1), (of the composite MnO\(_2\):Fe\(_2\)O\(_3\)) respectively, while its composite with MnO\(_2\) shows a decrease in activity compared with MnO\(_2\) only, and an increase in activity when compared with Fe\(_2\)O\(_3\) alone. These changes in activities maybe relate to the difference in ionic potential (charge/radius) between these ions (for Fe\(^{3+}\) = 4.138 and for Mn\(^{4+}\) = 5.970). This differences in ionic potential make the strong one (Mn\(^{4+}\)) effect on oxygen atoms of Fe\(_2\)O\(_3\), therefore, the bond strength between Mn\(^{4+}\) ion and oxygen relate to Fe\(_2\)O\(_3\) will increase and cause a decrease in bond strength (elongation of bond length) between Mn–O relate to MnO\(_2\) molecules compared with MnO\(_2\) alone. These results
Figure 5. FTIR transmittance spectrum of MnO₂ annealing at (a) 100 °C, (b) 250 °C, (c) 400 °C, (d) 550 °C, (e) 750 °C, (f) Fe₂O₃ at 400 °C and (g) MnO₂·Fe₂O₃ at 400 °C for 120 min.

Figure 6. The curve explains the rate of reaction (K) of prepared compounds MnO₂ annealing at (1) 100 °C, (2) 250 °C, (3) 400 °C, (4) 550 °C, (5) 750 °C, (7) Fe₂O₃ at 400 °C and (8) MnO₂·Fe₂O₃ at 400 °C for 120 min.
agree that found from XRD which found the increase in the bond length of Mn–O from 9.7316 Å to 10.478 Å. This result maybe make a decrease in catalase activity of MnO2:Fe2O3 composite compared with MnO2 alone, in figure 6.

4. Conclusion

In summary, we successfully used a new and simple colorimetric method to determine catalase mimic activity of MnO2, Fe2O3 nanoparticles and its composite (MnO2: Fe2O3), against low concentration (750 μM) of H2O2 solution as a substrate. The MnO2 and Fe2O3 nanoparticles were successfully prepared by the hydrothermal method (autoclave), then MnO2 annealed at different temperatures (250 °C–700 °C). By using our new colorimetric method the results show the annealing temperature at 400 °C of MnO2 nanoparticles, had the greatest catalase mimic activity (2.59 × 10⁻² s⁻¹) among all other annealing temperatures. Therefore this annealing temperature (400 °C), was used to calculate the catalase mimic activity of Fe2O3 and MnO2:Fe2O3 composite. The results show these are decreasing in the catalase mimic activities of both Fe2O3 nanoparticles and the composite (MnO2: Fe2O3) which were (1.44 × 10⁻² s⁻¹, 1.78 × 10⁻² s⁻¹) respectively, compared with MnO2 alone. The catalase mimic activity it is a special specification for each material, but the decrease in the activity of composite may be due to the interference between them may be effected on the activity.

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ORCID iDs

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Table 4. The rate of reaction (K) of prepared compounds MnO2 annealing at different temperatures, Fe2O3 and MnO2:Fe2O3 at 400 °C for 120 min.

| Annealing temperature | Rate of reaction (K × 10⁻²(s⁻¹)) |
|-----------------------|----------------------------------|
| MnO2 (at 100 °C)      | 1.69                             |
| MnO2 (at 250 °C)      | 2.10                             |
| MnO2 (at 400 °C)      | 2.59                             |
| MnO2 (at 550 °C)      | 1.97                             |
| MnO2 (at 700 °C)      | 1.49                             |
| MnO2 (at 400 °C)      | 1.44                             |
| MnO2:Fe2O3 (at 400 °C)| 1.78                             |
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