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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_2), iron oxide (Fe_2O_3) nanoparticles were prepared by a hydrothermal method (autoclave), and its composite. The MnO_2 nanoparticles were annealed at different temperatures (250–700 °C), while MnO_2: Fe_2O_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_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_2O_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 pauses H_2O_2 into H_2O and O_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_2O_2 absorption (at $\lambda = 240$ 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_2O_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_2O_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_2O_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_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_2 is n-type

semiconductor oxide [12]. Iron oxide (Fe_2O_3) 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]. Fe_2O_3 has different crystal structure for example wustite, hematite, maghemite, and magnetite which summarize as FeO, α-Fe_2O_3, β-Fe_3O_4 and γ-Fe_3O_4 respectively [15–17]. Fe_2O_3 nanoparticles have the advantage of low cost and environmental harmlessness, MnO_2 and Fe_2O_3, two very low-cost metal oxides and MnO_2:Fe_2O_3 are widely used in many applications such as supercapacitor [18]. MnO_2 and Fe_2O_3 nanoparticles were prepared by a hydrothermal method, the hydrothermal method considered as a powerful for synthesizing numerous forms of MnO_2 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 (H_2O_2) 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 ($KMnO_4$), purity 99.9%; sulfuric acid (H_2SO_4), purity 95%, ethanol (EtOH), grade, purity 97%; iron chloride anhydrate ($FeCl_3$), purity 99.8%, hydrochloric acid (HCl), purity 99.9%, and urea (NH_2CONH_2) 99.8% from BDH company. Hydrogen peroxide (H_2O_2), 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, $\lambda = 0.15406$ nm radiations. Atomic force microscopy (AFM) were measured using (SPM-AA 3000), and UV/Visible spectrophotometric.

2.2. Preparation of MnO_2 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 $KMnO_4$ 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 $KMnO_4$ and HCl took place as the following steps:

$$2KMnO_4 + 6HCl + H_2O \xrightarrow{\text{Autoclave}} 2Mn(OH)_4 + 2Cl_2 + 2KCl + 1/2O_2 \quad (1)$$

$$2Mn(OH)_4 \xrightarrow{\text{Heat(100 °C)}} 2MnO_2 + 4H_2O \quad (2)$$

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

$$2KMnO_4 + 6HCl + H_2O \xrightarrow[\text{2.Heat(90 °C)}]{\text{1.Autoclave}} 2MnO_2 + 2Cl_2 + 2KCl + 1/2H_2O \quad (3)$$

The MnO_2 precipitate (brown-black) annealed at (250 °C, 400 °C, 550 °C and 700 °C) temperatures for 120 min

2.3. Preparations of iron oxide (Fe_2O_3) nanoparticles

Iron oxide nanoparticles were prepared by the hydrothermal method using an autoclave, by dissolved 6 g of $FeCl_3$ (anhydrous), in 20 ml of distilled water. The solution mixed in a beaker about 10 min at room temperature to ensure dissolute all the salt. In another beaker, 3.33 g of NH_2CONH_2 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 $FeCl_3$ and NH_2CONH_2 write by the following equations:

$$3NH_2CONH_2 + 9H_2O \rightarrow 6NH_4OH + 3CO_2 \quad (4)$$

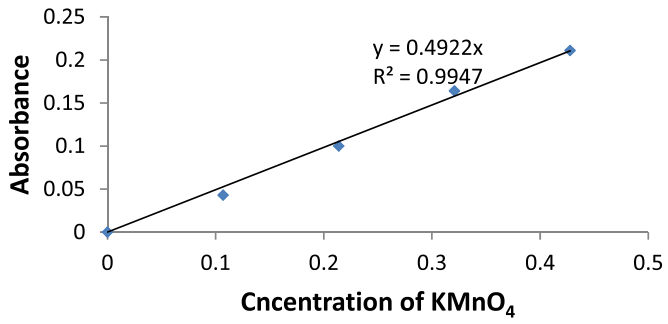

Figure 1. Standard curve of the $KMnO_4$ solution measured at 525 nm.

$$2FeCl_3 + 6NH_4OH \rightarrow 2Fe(OH)_3 + 6NH_4Cl \quad (5)$$

$$2Fe(OH) \xrightarrow{Heat} Fe_2O_3 + 3H_2O \quad (6)$$

The final equation can be assumed as:

$$2FeCl_3 + 3NH_2CONH_2 + 6H_2O \xrightarrow{Heat} Fe_2O_3 + 6NH_4Cl + 3CO_2 \quad (7)$$

2.4. Preparation of MnO_2.Fe_2O_3 Composite

This composite was prepared by mixing (3:1) mole ratio of MnO_2.Fe_2O_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 time 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_2O_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_2O_2 (750 μM), as shown in the reaction [10]:

$$2H_2O_2 \xrightarrow{MnO_2} O_2 + 2H_2O \quad (8)$$

After five minutes, the solution of potassium permanganate (300 μM), acidity by some drops of sulfuric acid (H_2SO_4) was added. The purple color of $KMnO_4$ solution, will be reacting with the H_2O_2 (the excess residual), and converted to colorless produce ($MnSO_4$), as explain s in the following equation:

$$3H_2SO_4 + 2KMnO_4 + 5H_2O_2 \rightarrow 2MnSO_4 + K_2SO_4 + 5O_2 + 8H_2O \quad (9)$$

The concentration of H_2O_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_2O_3 composite nanopowders

The XRD pattern of MnO_2 nanoparticles is explained in figure 2. All the measured peaks are indicated to the tetragonal α-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 ($\alpha = \beta = \gamma = 90°$). As in figure 2(a) the diffraction peaks that are (211), (310), and (110) at 2θ = (37.5720°), (28.8056°), and (18.1551°) 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 (2θ = 37.7886°, 28.9918° and 18.3566°) which are related to the alpha phase as shown in figure 2(b). After annealing at 550 °C the XRD of MnO_2 results were similar to 400 °C as shown in figure 2(c), the peaks located at (2θ = 37.7943°, 29.0091° and 18.3465°) are related to the (211), (310) and (101) of the α-MnO_2. All peaks are agreement with the

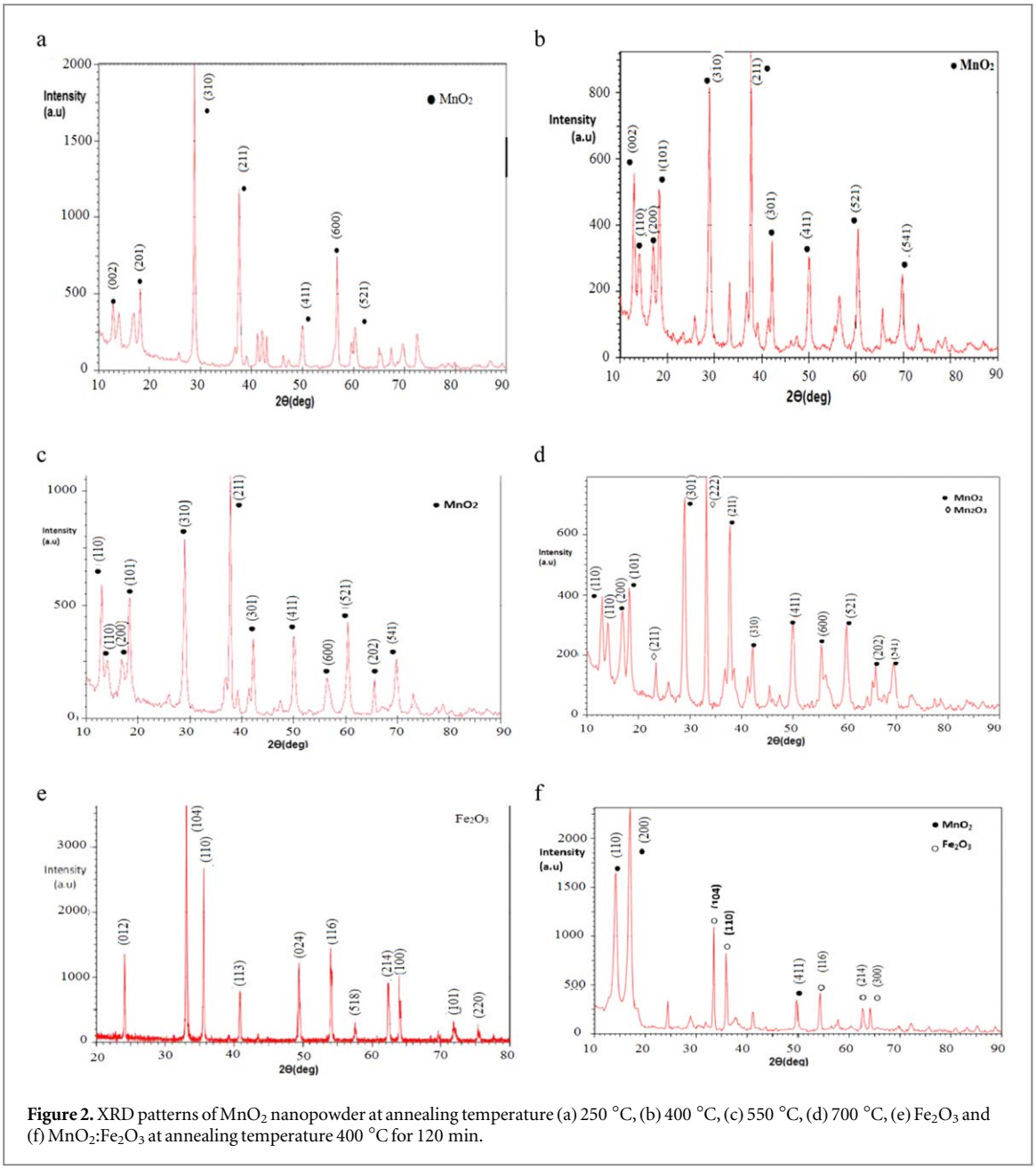

Figure 2. XRD patterns of MnO_2 nanopowder at annealing temperature (a) 250 °C, (b) 400 °C, (c) 550 °C, (d) 700 °C, (e) Fe_2O_3 and (f) MnO_2:Fe_2O_3 at annealing temperature 400 °C for 120 min.

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 KMnO4	0.5 ml	0.5 ml
Total volume	3 ml	3 ml

same card (JCPDS Card No.44-0141). 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 α-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_2O_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:

Table 2. The results obtained of the XRD for MnO_2 at different temperatures (from 250 °C to 700 °C), while Fe_2O_3 and MnO_2: Fe_2O_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
	28.805	310	0.506	162.018	3.096		
	18.155	101	0.597	134.781	4.882		
MnO_2 400 °C	37.788	211	0.545	153.894	2.378	9.731	2.840
	28.991	310	0.636	128.982	3.077		
	18.356	101	0.724	111.086	4.829		
MnO_2 550 °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_2 700 °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		
Fe_2O_3 at 400 °C	33.144	104	0.163	507.879	2.700		
	35.625	110	0.158	527.786	2.518		
	54.065	116	0.181	491.657	1.694		
MnO_2:Fe_2O_3 at 400 °C	16.909	200	0.772	103.967	5.239	10.47	2.787
	14.081	110	0.798	100.271	6.284		
	33.372	104	0.515	161.058	2.682		

$$D = K\lambda/\beta \cos\theta \tag{10}$$

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 ($Mn(OH)_4$) to only oxide phase (MnO_2), whereas the increasing temperature to 550 °C cause formed mixture phases from MnO_2 and Mn_2O_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_2O_3 [23].

The result of the XRD of Fe_2O_3 and MnO_2-Fe_2O_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_2O_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 (α-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_2O_3 α-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_2O_3 and MnO_2:Fe_2O_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 nanoparticles 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_2O_3) 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_2O_3 and MnO_2: Fe_2O_3 composite nanoparticles annealed at 400 °C. The average **diameter** of Fe_2O_3 nanoparticle was 61.53 nm, and decreases to 58.23 nm when composite MnO_2: Fe_2O_3 in mole ratio (3:1), this maybe relate to the interaction between nanoparticles of these oxides, as explained in the table 3.

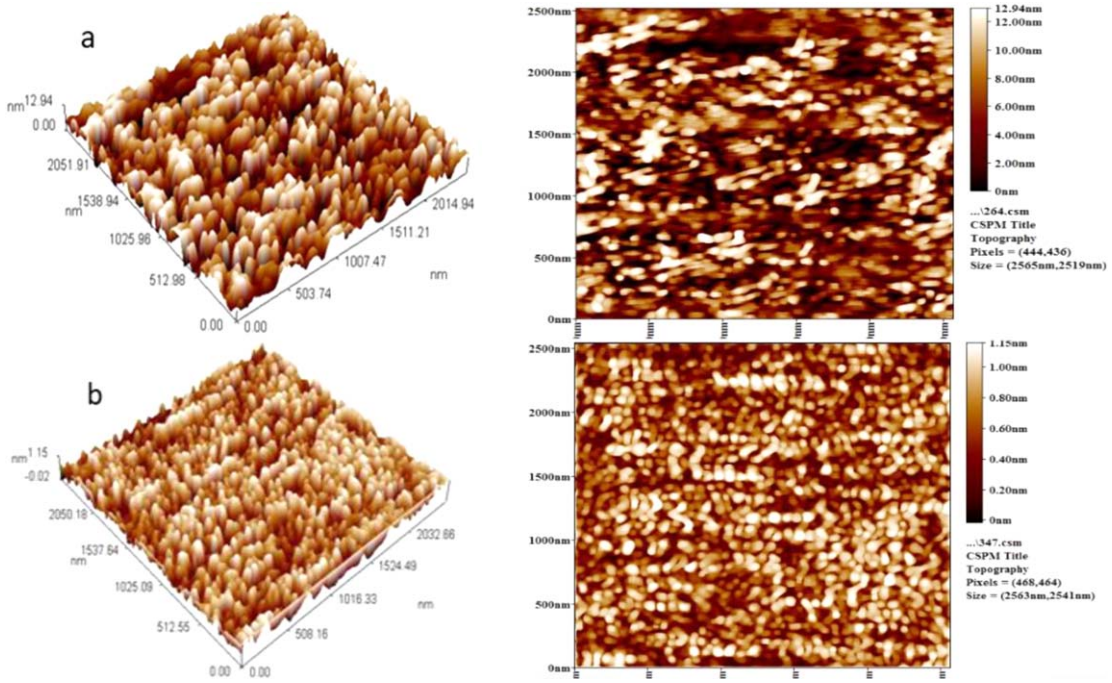

Figure 3. AFM images 3D and 2D of MnO_2 at annealing temperature: (a) 250 °C (b) 400 °C (c) 550 °C, (d) 700 °C , (e) Fe_2O_3 and MnO_2: Fe_2O_3 annealing at 400 °C for 120 min.

3.3. Surface morphology by (FE-SEM)

3.3.1. Surface morphology by (FE-SEM) for MnO_2

Figure 4 shows the FE-SEM images of α-MnO_2 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 MnO_2:Fe_2O_3 by (FE-SEM)

The high magnification FE-SEM images of the Fe_2O_3 powder and MnO_2-Fe_2O_3 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-Fe_2O_3 was a match the XRD patterns results. The FE-SEM images of MnO_2-Fe_2O_3 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-MnO_2 nano-powder. The figure also shows that there are aggregations of granules appear around the nanorods which belonged to hexagonal crystalline structure for α-Fe_2O_3.

3.4. Fourier transform infrared spectrum (FTIR) study

FT-IR spectra 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. 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 MnO_2 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 MnO_2 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

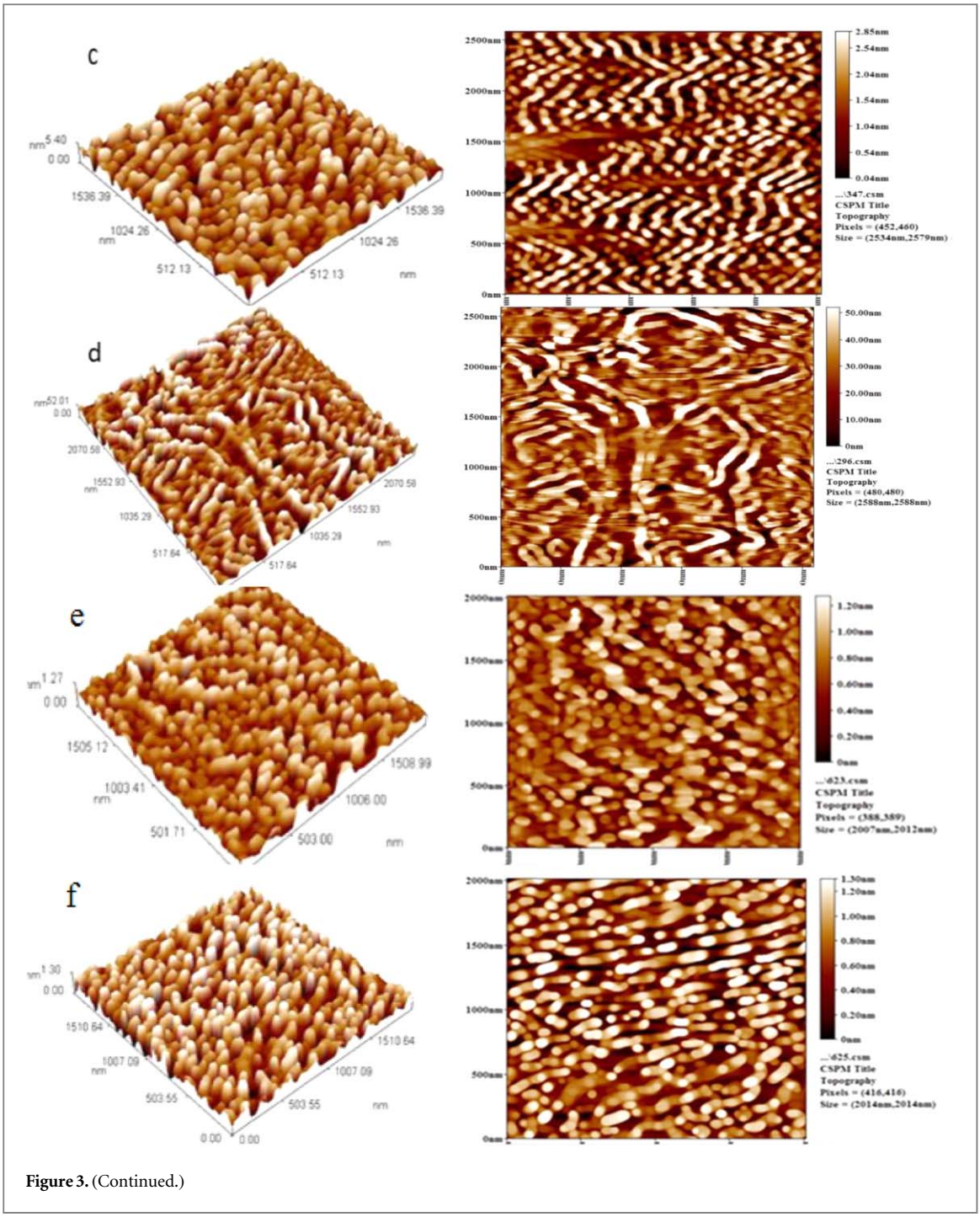

Figure 3. (Continued.)

Table 3. The Average diameter obtained by AFM of MnO_2 at different temperatures (from 250 °C to 700 °C), while Fe_2O_3 and MnO_2: Fe_2O_3 at 400 °C for 120 min.

Sample annealing for 120 min	Average diameter (nm)
MnO_2 (at 250 °C)	69.62
MnO_2 (at 400 °C)	72.36
MnO_2 (at 550 °C)	81.65
MnO_2 (at 700 °C)	66.27
Fe_2O_3 (at 400 °C)	61.53
MnO_2: Fe_2O_3 (at 400 °C)	58.23

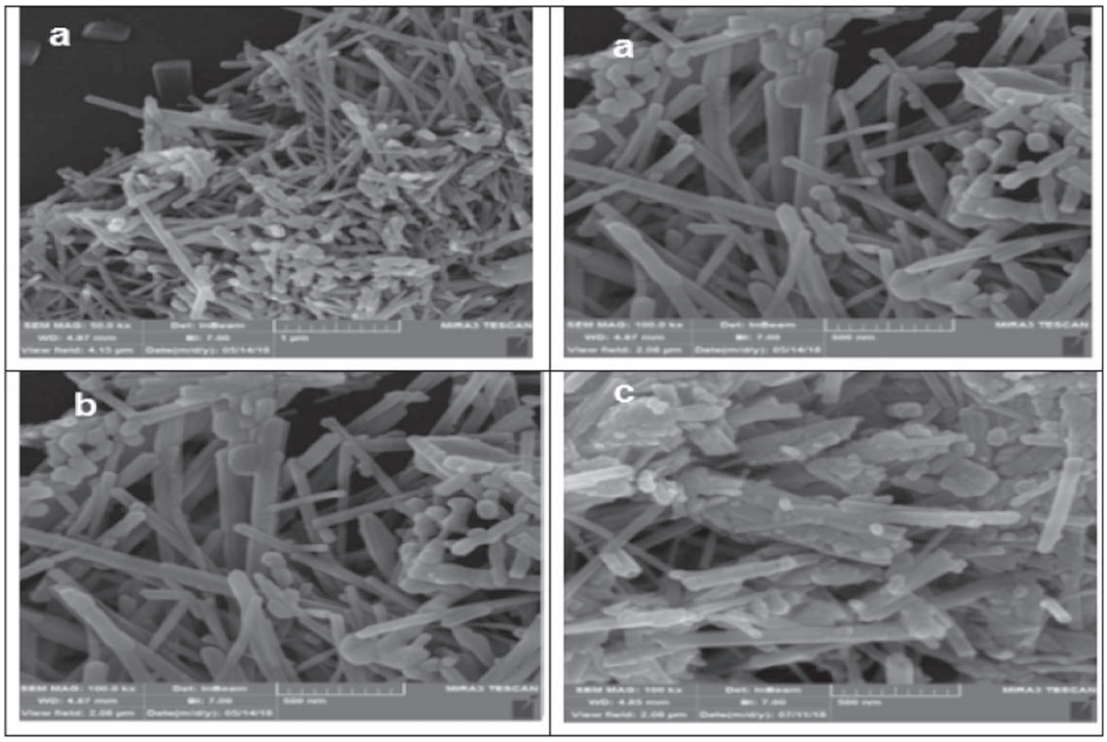

Figure 4. FE-SEM images of MnO_2 nanoparticles (a) at magnification 1 μm and 500 nm, (b) Fe_2O_3 nanoparticles and (c) MnO_2:Fe_2O_3 at annealing temperature 400 °C for 120 min.

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_2O_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_2O_3 nanocomposite of rhombohedral structure [28, 29].

3.5. Catalase mimic activity of MnO_2, Fe_2O_3 and MnO_2:Fe_2O_3 composite

We used a new and simple colorimetric method to determine catalase mimic activity of MnO_2, Fe_2O_3 nanoparticles and MnO_2:Fe_2O_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:

$$\text{Rate of catalase activity } (K) = (2.303/t) \times (\log(C_o/C)) \quad (11)$$

Where: t mean the reaction time (in seconds); C_o and C are the entire concentration of H_2O_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 ($K = 2.59 \times 10^{-2}$ 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_2O_3 (1.44×10^{-2} s^{-1}) is lower than MnO_2 (2.59×10^{-2} s^{-1}). Therefore, we suggested to use the mole ratio (3:1), (of the composite MnO_2: Fe_2O_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_2O_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_2O_3, therefore, the bond strength between Mn^{4+} ion and oxygen relate to Fe_2O_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

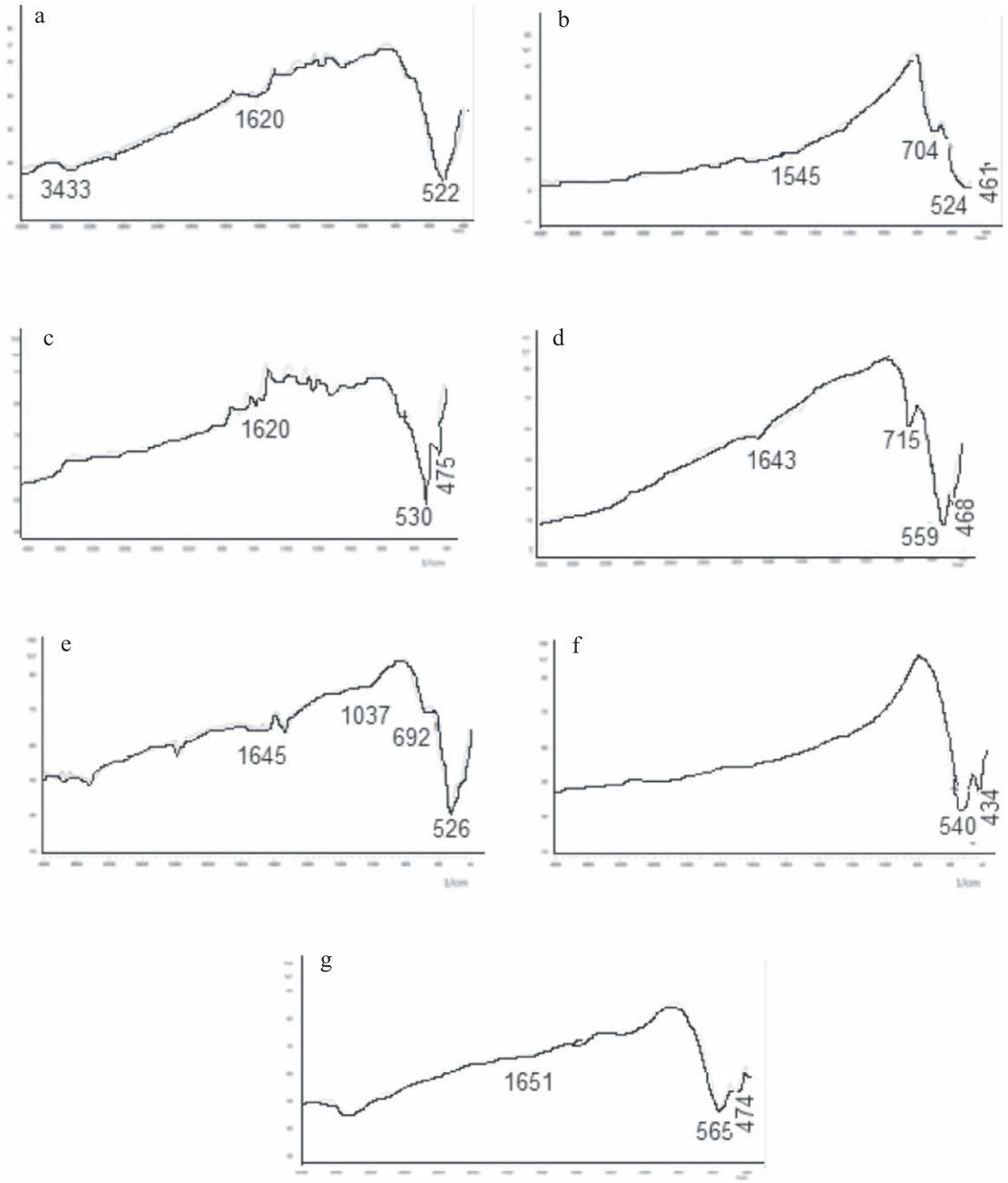

Figure 5. FTIR transmittance spectrum of MnO_2 annealing at (a). 100 °C, (b). 250 °C, (c). 400 °C, (d). 550 °C, (e). 750 °C, (f). Fe_2O_3 at 400 °C and (g). MnO_2: Fe_2O_3 at 400 °C for 120 min.

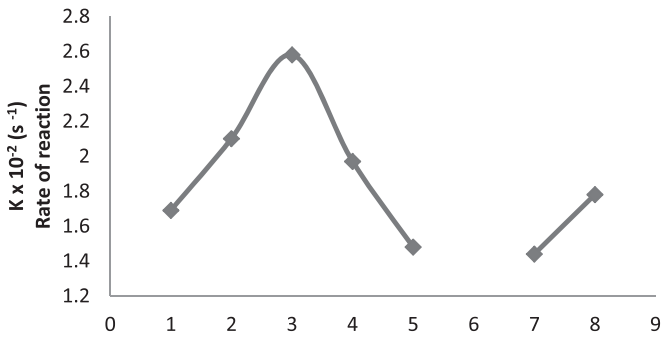

Figure 6. The curve explains the rate of reaction (K) of prepared compounds MnO_2 annealing at (1). 100 °C, (2). 250 °C, (3). 400 °C, (4). 550 °C, (5). 750 °C, (7). Fe_2O_3 at 400 °C and (8). MnO_2: Fe_2O_3 at 400 °C for 120 min.

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 × $10^{-2}(s^{-1})$)
MnO_2 (at 100 °C)	1.69
MnO_2 (at 250 °C)	2.10
MnO_2 (at 400 °C)	2.59
MnO_2 (at 550 °C)	1.97
MnO_2 (at 700 °C)	1.49
MnO_2 (at 400 °C)	1.44
MnO_2: Fe_2O_3 (at 400 °C)	1.78

agree that found from XRD which found the increase in the bond length of Mn-O from 9.7316 A to 10.478 A. This result maybe make a decrease in catalase activity of MnO_2:Fe_2O_3 composite compared with MnO_2 alone, in figure 6.

4. Conclusion

In summary, we successfully used a new and simple colorimetric method to determine catalase mimic activity of MnO_2, Fe_2O_3 nanoparticles and its composite (MnO_2: Fe_2O_3), against low concentration (750 μM) of H_2O_2 solution as a substrate. The MnO_2 and Fe_2O_3 nanoparticles were successfully prepared by the hydrothermal method (autoclave), then MnO_2 annealed at different temperatures (250 °C–700 °C). By using our new colorimetric method the results show the annealing temperature at 400 °C of MnO_2 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 Fe_2O_3 and MnO_2: Fe_2O_3 composite. The results show these are decreasing in the catalase mimic activities of both Fe_2O_3 nanoparticles and the composite (MnO_2: Fe_2O_3) which were (1.44 × 10^{-2} $s^{-1,}$ 1.78 × 10^{-2} s^{-1}) respectively, compared with MnO_2 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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