# Synthesis and Catalase Mimic Activity of $MnO_2$ Nano Powder Prepared by Hydrothermal Process

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**Abstract**

Manganese dioxide ($MnO_2$) nanopowder has been synthesized by hydrothermal method. $MnO_2$ was annealed at different temperatures (250, 400, 550, 700˚C). The crystal structure and surface morphology of these nanostructures were characterized by X-ray diffraction (XRD), Atomic Force Microscope (AFM) and Scanning Electron Microscopy (SEM). The catalase mimic activity (catalytic activity) of $MnO_2$ against hydrogen peroxide ($H_2O_2$) was studied by using a new method and found that 400˚C is the best annealing temperature.

***Keywords:*** Manganese Dioxide ($MnO_2$) Nanopowder, Hydrothermal Method, Catalase Mimics Activity.

## 1. Introduction

For many years, manganese dioxide with diverse crystal morphologies are attracting a lot of attention, because of their outstanding structural flexibility combined with novel physical and chemical properties, which are of interest for the following applications, for example, molecular sieves, supercapacitors, catalysts and biosensors [1]. It is n-type semiconductor material [2]. Manganese dioxide exists in various polymorphic forms including α-, β-, γ- and δ-$MnO_2$ which are different in the arrangement of basic octahedral [$MnO_6$] units [3]. The hydrothermal method is a powerful synthesis approach for synthesizing various forms of manganese oxides because of the choice of precursors that can be used and control of reaction time, pH, and temperature and it is a simple and inexpensive technique [4].

The catalytic (catalase) activity can be measured by determining the decrease of $H_2O_2$ absorption (at 240 nm) [5,6]. The difficulties associated to this method, due to using high levels of substrate approximately (5-50 mM) to get acceptable absorbance [7]. Moreover, the high levels of $H_2O_2$ lead to formation of bubbles in the test cell which cause mistake measurements [8]. Catalase (catalytic) activity can be determined in other methods such as by titrimetric determination of $H_2O_2$ concentration, determination of oxygen production from decomposition of $H_2O_2$ by oxygen electrode [9,10]. There are simple colorimetric methods such as by Goth [11] for catalase, by measuring of hydrogen peroxide (unreacted) spectrophotometrically by a complex reaction with ammonium molybdate. Sinha and Hadwan [12,13] use another simple method, in which the decomposition of hydrogen peroxide determined spectrophotometrically by a complex reaction with dichromate/acetic acid reagent. Another method for catalase activity measurement is the titration method, which is used when high (UV) absorption pigmentation or precipitation of the sample does not allow the use of the spectrophotometric method [8].

Our work is new modified method which use spectrophotometric assay to determination of $H_2O_2$ by potassium permanganate in acidic solution.

## 2. Theoretical Part

In the present work, we have prepared $MnO_2$ nanopowder using $KMnO_4$ and HCl as a precursor. The crystalline size for that peak alone calculated, using the Debye- Scherer formula [14]:

$$D = k\lambda / \beta\cos\theta \quad \ldots\ldots\ldots (1)$$

Where k is the constant (0.9), λ is the wave length of X-ray (1.54 nm), β is the full width half maximum (FWHM) of the peak and θ is the reflection angle.

## 3. Materials used

All reagents were of analytical grade purity and no further purification was done before use. Potassium permanganate ($KMnO_4$), purity 99.9%; and hydrochloric acid (HCl), purity 99.9%, sulphuric acid ($H_2SO_4$), purity 95% from British Drug House (BDH) company. Hydrogen peroxide ($H_2O_2$), purity 50 %; Merck company.

### 3.1 Synthesis of $MnO_2$ Nano powder

The hydrothermal reaction was done in a 100 mL Teflon-lined stainless steel (autoclave) under autogenous pressure. In this synthesis, 4.115 g (47.298 mmol) of $KMnO_4$ was added into 70 mL of deionized water with vigorous stirring, and stirred for about 10 min. at room temperature. The solution filtered, then 3.405 ml concentrated HCl were added to the filtrated solution under stirring to form the precursor solution. Then the solution poured into a 80 Teflon-lined stainless steel autoclave. The autoclave was sealed and placed in an oven at 200 °C for 6 h. and hydrothermally treated at 200 °C for 12 h. After that, the autoclave was allowed to cool to room temperature naturally. The brown black precipitate ($Mn(OH)_4$) was washed with distilled water (4-5 times), and collected by centrifugation, washed with ethanol (2 times) and lastly the washed precipitates were dried at 90˚C for 2 hours in air.

The reaction took place between potassium permanganate and hydrochloric acid as following steps:

1. $2KMnO_4 + 6HCl + H_2O \xrightarrow[\text{Heat (90˚C)}]{\text{Autoclave}} 2Mn(OH)_4 + 2Cl_2 + 2KCl + 1/2O_2$ ….(1)
2. $2Mn(OH)_4 \longrightarrow 2MnO_2 + 4H_2O$ …..(2)

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The overall reaction can be described by equation:

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

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

### 3.2 Catalase mimic activity (catalytic activity)

The concentration of $KMnO_4$ was determined by titration with known concentration of sodium oxalate solution, then the concentration of $H_2O_2$ was determined by titration with known concentration of $KMnO_4$. Standard curve consisted of (0, 1, 2, 3, 4 and 5) $x10^{-5}$ M of $KMnO_4$ was prepared to find the concentration of color absorbed from $KMnO_4$ (as shown in Fig. 1).

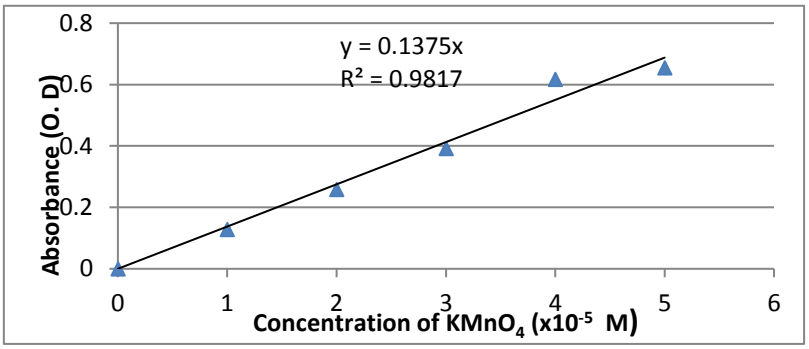



**Figure 1: Standard curve of $KMnO_4$ solution at 525 nm.**

Catalase mimic activity was determined by using the reaction with final concentration of manganese dioxide ($MnO_2$) solution (2 mM), and hydrogen peroxide (750 µM), (as the following reaction) [13]:

$$2H_2O_2 \xrightarrow{MnO2} O_2 + 2H_2O \quad \text{…..(4)}$$

After five minutes that acidic solution consist from potassium permanganate ($KMnO_4$) solution (300 µM as final concentration), acidity with some drops of sulphoric acid ($H_2SO_4$). The permanganate solution (purple color) will reacting with the excess of hydrogen peroxide ($H_2O_2$) (which no reacted with $MnO_2$), and reduced to manganese sulfate (color less), as product reaction as following equation:

$$3H_2SO_4 + 2KMnO_4 + 5H_2O_2 \longrightarrow 2MnSO_4 + K_2SO_4 + 5O_2 + 8H_2O \quad \text{…..(5)}$$

Hydrogen peroxide concentration which used is directly proportional to the concentration of potassium permanganate that used in the reaction. The decreasing in permanganate concentration (color) is measured calorimetrically at 525 nm by using standard curve concentration. The procedure of Catalase mimic activity was done according following steps in describing in table 1.

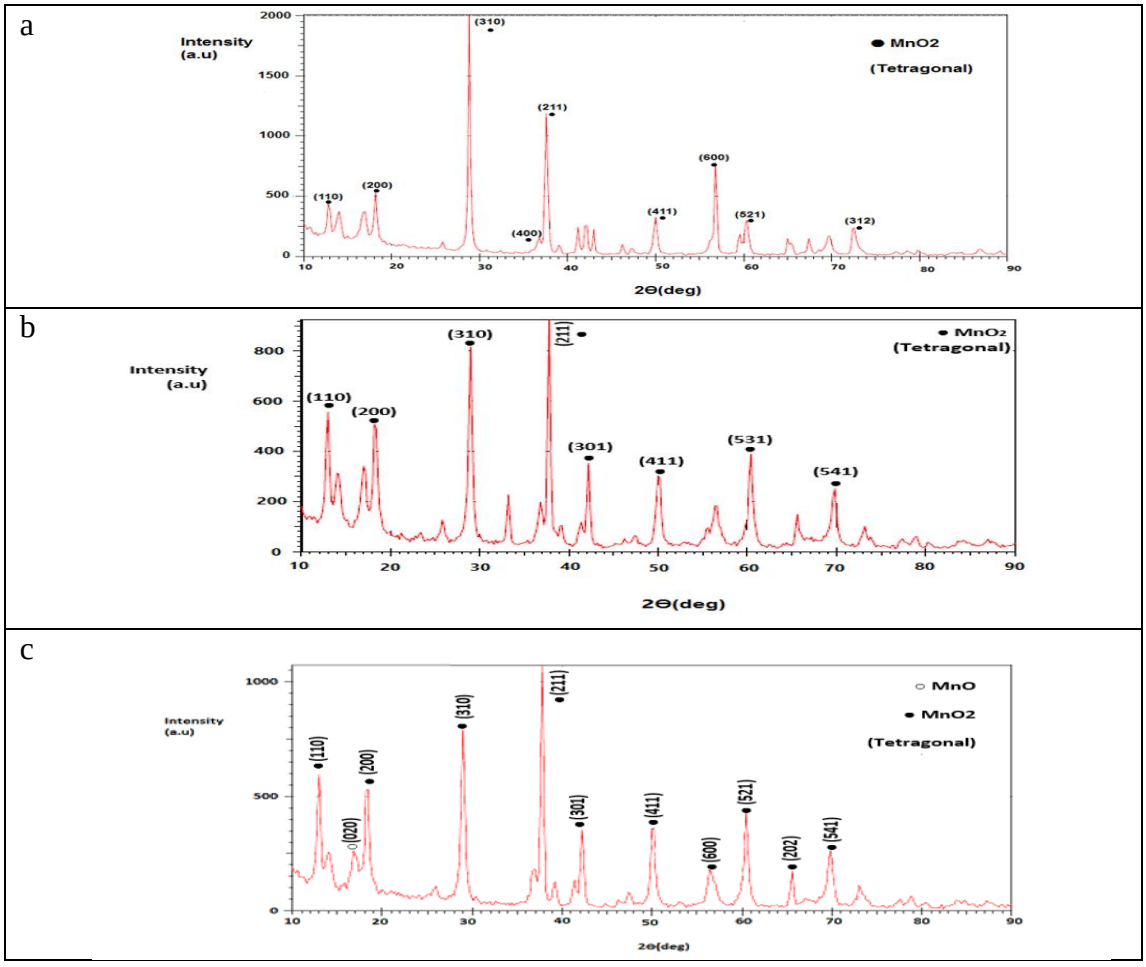
**Table 1. Shows the procedure that used for measurement of catalase activity.**

| Reagents | Test | Control |
|---|---|---|
| Metal oxide solution | 500 µl | - |
| Distilled water | 1000 µl | 2500 µl |
| Hydrogen peroxide | 1000 µl | 1000 µl |
| Mix with vortex and for 5 min, after that, add: | | |
| Acidic solution of potassium permanganate | 500 µl | 500 µl |
| Total volume | 3000 µl | 3000 µl |

## 4. Results and Discussion

The XRD pattern of $MnO_2$ nanostructure is illustrated in Fig.1 All the diffraction peaks are well indexed to the pure polycrystalline tetragonal α-$MnO_2$ phase which in a good agreement with (JCPDS Card No.44-0141) with lattice constants of (a = b = 9.78475 Å, c = 2.86302 Å) and (α=β=γ=90˚). Fig. 1-a shows the diffraction patterns of $MnO_2$ at annealing temperature 250˚C, the diffraction peaks for (211), (310), (200) planes at 2θ=37.57˚, 2θ=28.8˚ and 2θ=218.15˚refer to the tetragonal structure belonged to alpha phase. The new phase at 700˚C was identified as cubic $Mn_2O_3$ (JCPDS Card No.41 -1442) Fig.1-d.

Table -1 shows the X-ray diffraction patterns of prepared product ($Mn(OH)_2$) at different annealing temperatures (250, 400, 550 and 700˚C) for 120 min. The increase of annealing temperature from 250 to 550˚C increased the intensity of diffraction and increase the lattice constant is in agreement with the reference [15]. At annealing 700˚C the lattice constant is decrease because formation another new phase called $Mn_2O_3$ [16].



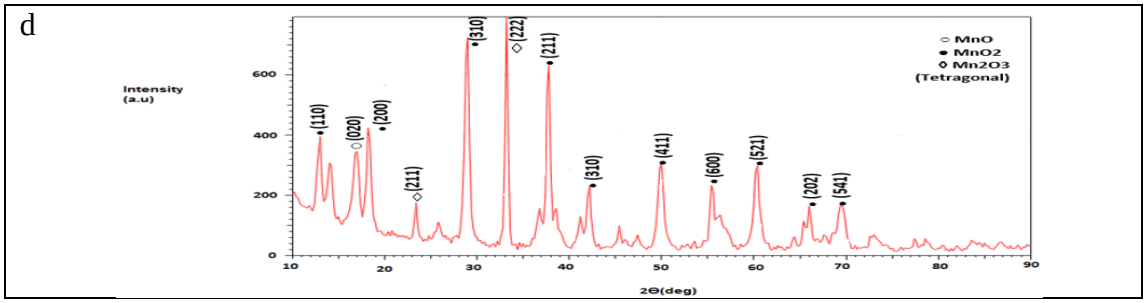
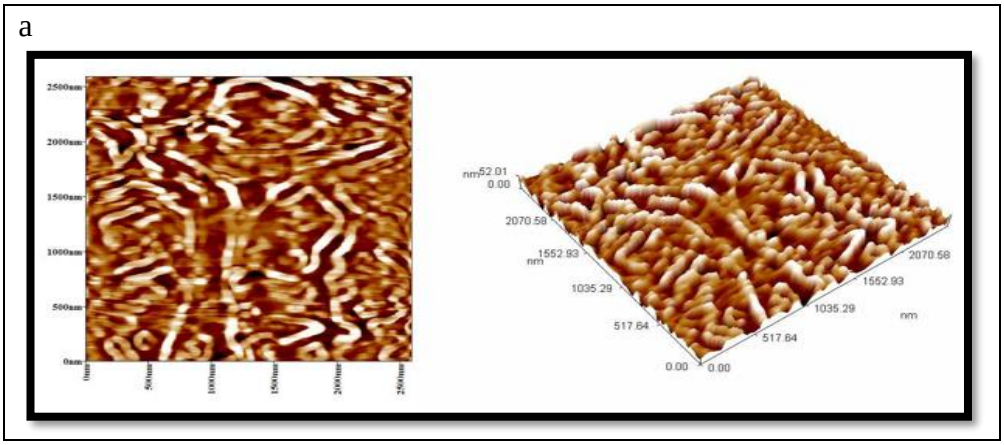



**Figure 2- XRD patterns for $MnO_2$ with annealing temperatures at: (a) 250˚C, (b) 400˚C, (C) 550˚C and (d) 700˚C for 120 min.**

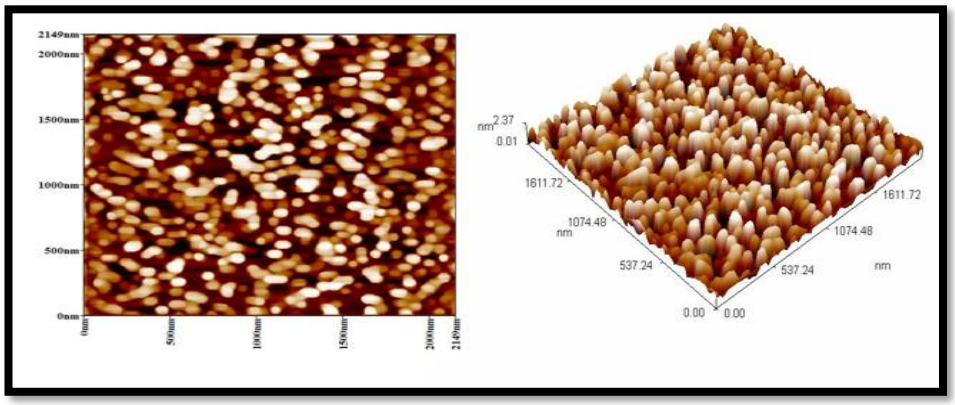
**Table 1- The obtained result of the XRD for $MnO_2$ at different annealing temperatures (250, 400, 550 and 700˚C) for 120 min.**

| $MnO_2$ annealing Temperature for 120 min | 2θ (deg) | hkl | FWHM (deg) | Grain size (nm) | d XRD (Å) | Lattice parameter | |
|---|---|---|---|---|---|---|---|
| | | | | | | a XRD (Å) | c XRD (Å) |
| $MnO_2$ 250˚C | 37.572 | 211 | 0.552 | 15.1928 | 2.391 | 9.7930 | 2.8555 |
| | 28.805 | 310 | 0.506 | 16.2018 | 3.096 | | |
| | 18.155 | 200 | 0.597 | 13.4781 | 4.882 | | |
| $MnO_2$ 400˚C | 37.788 | 211 | 0.545 | 15.3894 | 2.378 | 9.7316 | 2.8406 |
| | 28.991 | 310 | 0.636 | 12.8982 | 3.077 | | |
| | 18.356 | 200 | 0.724 | 11.1086 | 4.829 | | |
| $MnO_2$ 550˚C | 37.794 | 211 | 0.541 | 15.5119 | 2.378 | 9.7284 | 2.8404 |
| | 29.001 | 310 | 0.645 | 12.7226 | 3.076 | | |
| | 18.346 | 200 | 0.694 | 11.5879 | 4.831 | | |
| $MnO_2$ 700˚C | 37.715 | 211 | 0.621 | 13.5230 | 2.383 | 9.7673 | 2.8438 |
| | 28.883 | 310 | 0.699 | 11.7420 | 3.088 | | |
| | 18.245 | 200 | 0.672 | 11.9633 | 4.858 | | |

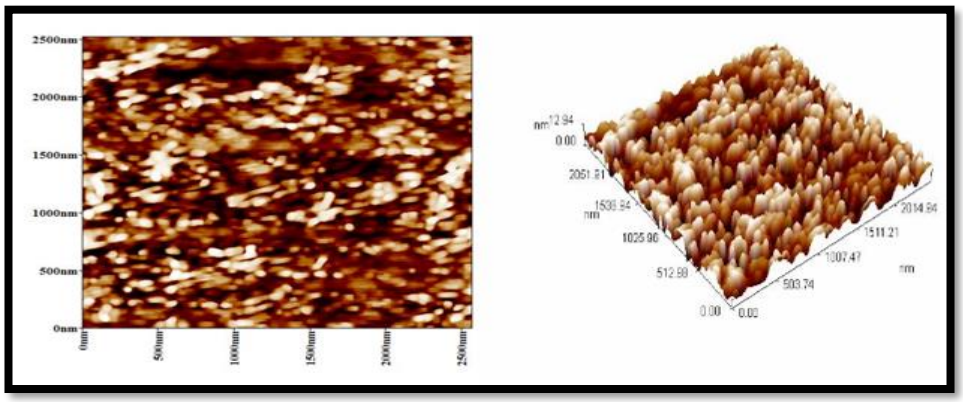
Fig. (3- a to d) show the AFM images and the granularity accumulation distribution chart of $MnO_2$ powders with annealing at (a-250, b-400, c-550, and d-700)˚C. The average grain size found to be (66.27 – 81.65 nm). AFM results show that the grain size increase by increasing temperature this is due to improving the crystalline of the powders.



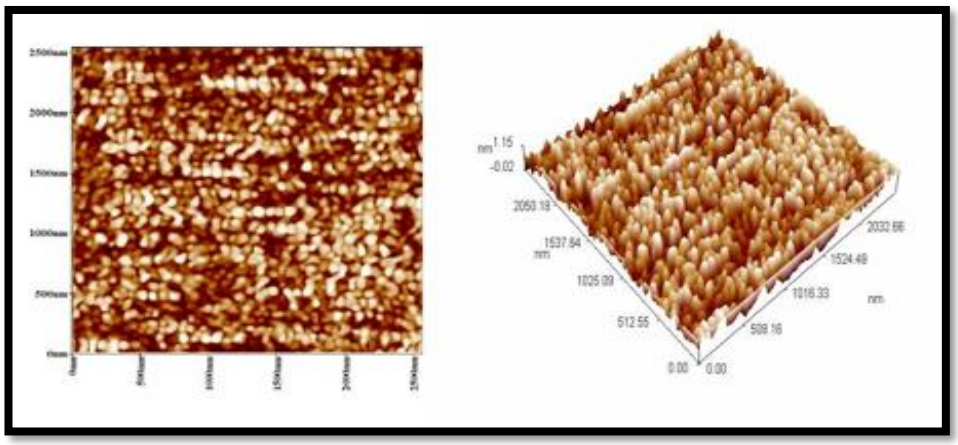
b



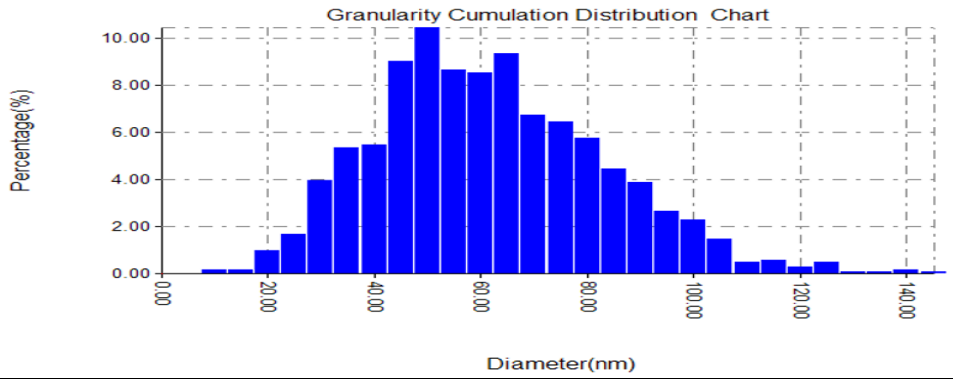
c



d



a


Granularity Cumulation Distribution Chart
Percentage(%)
10.00
8.00
6.00
4.00
2.00
0.00
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
Diameter(nm)

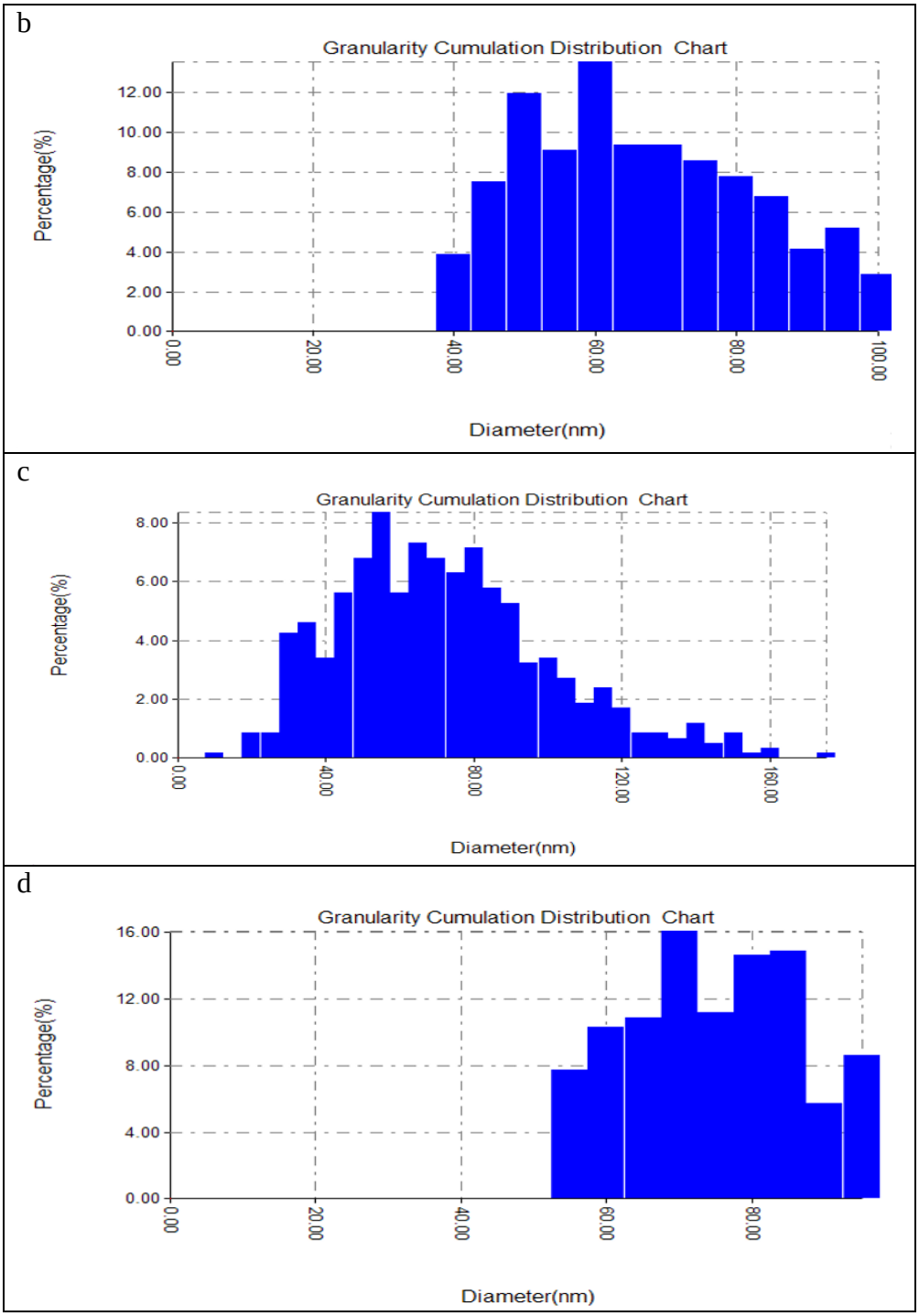
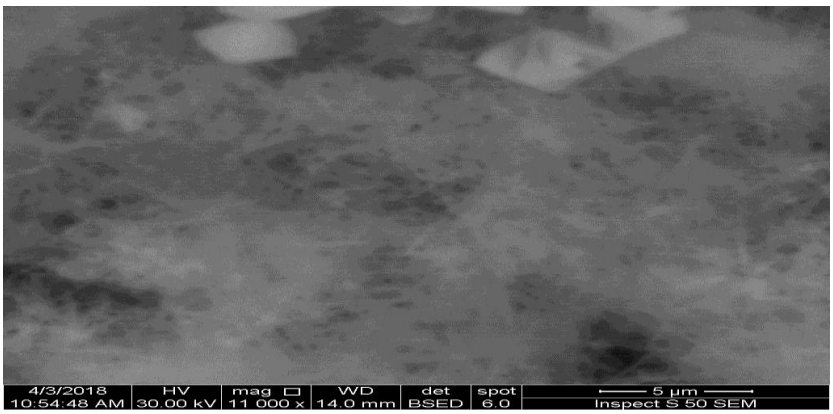



**Figure 3: Two and three dimensional AFM images and the morphology o for $MnO_2$ with annealing temperatures at: (a) 250˚C, (b) 400˚C, (C) 550˚C and (d) 700˚C for 120 min.**

For magnifications 5µm (Fig. 4- a to d ) , the morphology of the $MnO_2$ that prepared by hydrothermal method at different temperature (250-700)˚C was primarily investigated by SEM, according to the morphology of $MnO_2$ there are smooth and high-quality nanowires with diameter of 17.33 to 42.89 nm and several micrometers in length for average. These nanowires aggregate into spherical shape with diameter of about 4.069 to 6.955 µm.

a


4/3/2018
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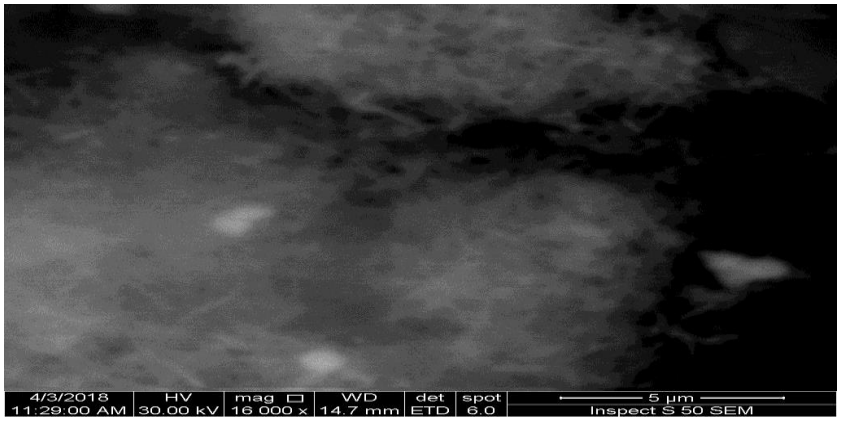
b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c

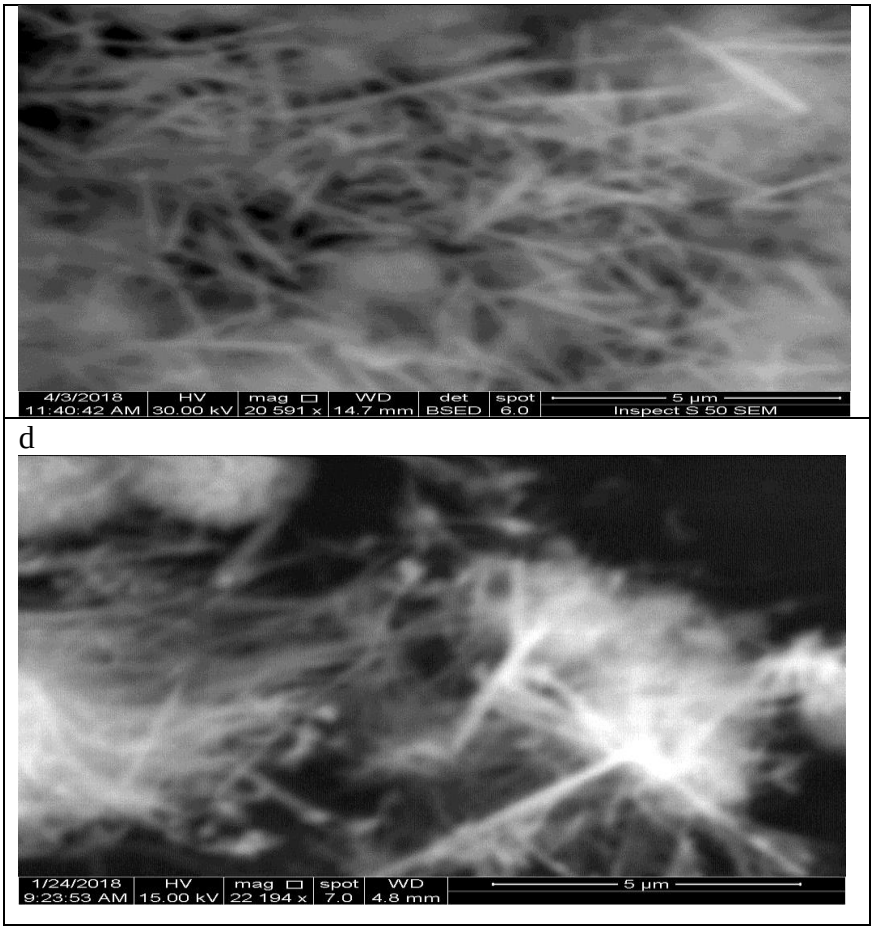


**Figure 4: SEM image for $MnO_2$ at (a) 250˚C, (b) 400˚C, (C) 550˚C and (d) 700˚C for 120 min.**

The following equation was used to calculate the rate reaction of catalase mimic activity of $MnO_2$ annealing at (As-prepared -700 ºC) for 2 h:

Rate of catalase activity ($sec.^{-1}$) = (2.303/t) x (log ($C_o$/C)) …..(6)

Where: t = time of reaction (seconds); $C_o$ and C are total concentration of hydrogen peroxide in cell reaction before and after reaction respectively. Our results show that the 400 ºC is the best rate reaction of catalase mimic activity (2.59 $x10^{-2}$ $S^{-1}$), these results are show in Fig. 6 and table 2.

**Figure 6- The rate of reaction as catalase mimic activity ($S^{-1}$) of $MnO_2$ annealing at (as-prepared - 700 ºC for 2 h.**

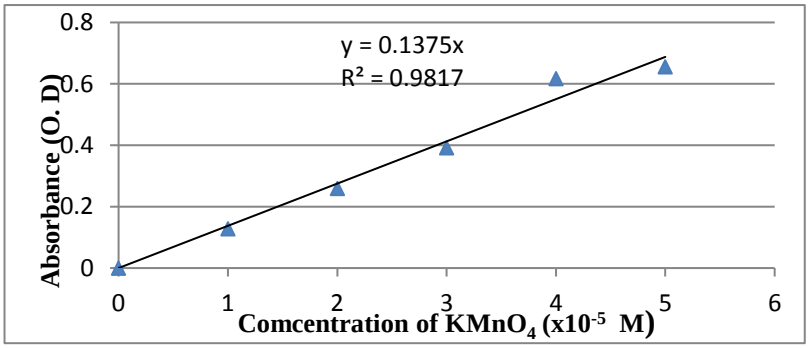

**Table 2: The rate of reaction as catalase mimic activity ($S^{-1}$) of $MnO_2$ annealing at (as-prepared - 700 ºC for 2 h.**

| Annealing Temperature (ºC) | K x$10^{-2}$ $S^{-1}$. Rate of reaction as catalase mimic activity ($S^{-1}$) |
|---|---|
| | $MnO_2$ |
| As-prepared | 1.69 |
| 250 | 2.10 |
| 400 | 2.59 |
| 550 | 1.97 |
| 700 | 1.49 |

## 5. Conclusions

$MnO_2$ nanostructures were prepared by hydrothermal method and annealing at different temperatures (250, 400, 550 and 700 ºC) for 2 h. The calculation rate of reaction (K) as catalase mimic activity against the low concentration of hydrogen peroxide (2 mM) has been done.

The result found annealing at 400 ºC were the highest activity (2.59 x$10^{-2}$ Sec.) among different annealing temperatures.

## Conflict of Interests

There are no conflicts of interest

## References

[1] X. Wang and Y. Li, "Synthesis and formation mechanism of manganese dioxide nanowires/nanorods," Chem. Eur. J., vol. 9, no. 1, pp. 300–306, 2003.

[2] X. Xia, H. Li, and Z. Chen, "The Study of Semiconduction Properties of γ-MnO2 with Different Degrees of Reduction," J. Electrochem. Soc., vol. 136, no. 1, pp. 266–271, 1989.

[3] A. A. Hlaing and P. P. Win, "The synthesis of α-MnO2 nanorods using hydrothermal homogeneous precipitation," Adv. Nat. Sci. Nanosci. Nanotechnol., vol. 3, no. 2, p. 25001, 2012.

[4] S. C. Pang, S. F. Chin, and C. Y. Ling, "Controlled synthesis of manganese dioxide nanostructures via a facile hydrothermal route," J. Nanomater., vol. 2012, p. 2, 2012.

[5] H. Aebi, "[13] Catalase in vitro," in Methods in enzymology, vol. 105, Elsevier, 1984, pp. 121–126.

[6] D. P. Nelson and L. A. Kiesow, "Enthalpy of decomposition of hydrogen peroxide by catalase at 25 C (with molar extinction coefficients of H2O2 solutions in the UV)," Anal. Biochem., vol. 49, no. 2, pp. 474–478, 1972.

[7] H. Aebi, "Catalase," in Methods of enzymatic analysis, Elsevier, 1974, pp. 673–684.

[8] F. Van Lente and M. Pepoy, "Coupled-enzyme determination of catalase activity in erythrocytes.," Clin. Chem., vol. 36, no. 7, pp. 1339–1343, 1990.

[9] Siqueira A. J. S., Remião J. O., Azevedo A. M. P., and Azambuja C. R. J., "A gasometric method to determine erythrocyte catalase activity", *Brazilian Journal of Medical Biological Research*, vol. 32, no. 9: pp.1089–1094. 1999.

[10] L. Goth, "A simple method for determination of serum catalase activity and revision of reference range," *Clin. Chim. acta*, vol. 196, no. 2–3, pp. 143–151, 1991.

[11] A. K. Sinha, "Colorimetric assay of catalase," *Anal. Biochem.*, vol. 47, no. 2, pp. 389–394, 1972.

[12] Hadwan M. H., "New Method for Assessment of Serum Catalase Activity". *Indian Journal of Science and Technology*, vol. 9, no. 4: pp. 1-5. 2016.

[13] R. Ragg, M. N. Tahir, and W. Tremel, "Solids go bio: inorganic nanoparticles as enzyme mimics," *Eur. J. Inorg. Chem.*, vol. 2016, no. 13-14, pp. 1906–1915, 2016.

[14] X. Wang, A. Yuan, and Y. Wang, "Supercapacitive behaviors and their temperature dependence of sol–gel synthesized nanostructured manganese dioxide in lithium hydroxide electrolyte," *J. Power Sources*, vol. 172, no. 2, pp. 1007–1011, 2007.

[15] R. Poonguzhali, N. Shanmugam, R. Gobi, N. Kannadasan, and G. Viruthagiri, "Effect of thermal annealing on the structural, morphological and super capacitor behavior of MnO2 nanocrystals," Mater. Sci. Semicond. Process., vol. 27, pp. 553–561, 2014.

[16] A. K. M. A. Ullah et al., "Synthesis of Mn3O4 nanoparticles via a facile gel formation route and study of their phase and structural transformation with distinct surface morphology upon heat treatment," J. Saudi Chem. Soc., vol. 21, no. 7, pp. 830–836, 2017.

# تحضير ودراسة الفعالية التحفيزية لثنائي اوكسيد المنغنيز النانوي المحضر بطريقة الضغط الحراري

**الخلاصة**

حضرثنائي اوكسيد المنغنيز النانوي بطريقة الضغط الحراري (الاوتوكليف). وتم تلدين ثنائي اوكسيد المنغنيز عند درجات حرارية مختلفة (250،400،550 و700م°). اخذت القياسات للمساحيق النانوية ولمتغيرات متعددة ومن ثم شخصت البنية التركيبية وطوغرافية الاسطح بوساطة فحص حيود الاشعة السينيه (XRD), مجهر القوه الذريه (AFM) و المجهر الاكتروني الماسح (SEM). درست فعالية ثنائي اوكسيد المنغنيز كعامل مقلد لانزيم الكتليز (الفعالية التحفيزية) ضد بيروكسيد الهيدروجين وباستخدام طريقة جديدة ووجد ان التلدين بدرجة حرارة 400°م هي الافضل.

**الكلمات الدالة:** مسحوق ثنائي اوكسيد المنغنيزالنانوي، الحرارة المائية، مقلد انزيم الكتليز.