Triple-enzymatic activity of CuMn$_2$O$_4$ nanoparticles: analytical applications for H$_2$O$_2$ and L-cysteine detection

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Abstract

CuMn$_2$O$_4$ NPs were prepared via co-precipitation method and characterized using microscopic and spectroscopic analyses. CuMn$_2$O$_4$ NPs exhibit a triple-enzymatic activity including peroxidase-, oxidase- and catalase-like activity. The effect of various parameters on the initial rate of the catalytic reaction of CuMn$_2$O$_4$ NPs with peroxidase- and oxidase-like activity was studied by UV-vis spectroscopy following the increasing absorption at 415 nm corresponding to the oxidation product of substrate o-phenylenediamine (OPD). Kinetic analyses indicate the Michaelis-Menten model for CuMn$_2$O$_4$ NPs for both peroxidase- and oxidase-like activity. Based on the high peroxidase-like activity of CuMn$_2$O$_4$ NPs, they were further studied as a colorimetric sensor for the detection of H$_2$O$_2$ with a linear range from 0.5 mM to 22 mM and detection limit of 0.11 mM. Inhibition of the high oxidase-like activity of CuMn$_2$O$_4$ NPs was utilized for colorimetric detection of L-cysteine with a linear range from 50 µM to 200 µM and a detection limit of 54.15 µM.

Keywords: Peroxidase; Oxidase; Catalase; CuMn$_2$O$_4$; H$_2$O$_2$; L-cysteine.

1. Introduction

Peroxidases and oxidases form a subcategory of a large group of enzymes called oxidoreductases. They catalyze transfer of electrons from one molecule as an electron donor to another as an electron acceptor, in oxido-reduction reactions. Peroxidases and oxidases act
on H$_2$O$_2$ and O$_2$ as an electron acceptor, respectively[1]. The use of natural enzymes is limited
due to their structural complexity, low stability, high cost of preparation and purification, and
substrate specificity[2, 3]. After pioneering work of Yan and co-workers on peroxidase-like
activity of Fe$_3$O$_4$ [4] many nanomaterials have been reported for mimicking enzymatic
activity in order to overcome the natural enzymes' drawbacks[5-9]. Nanomaterials with
enzymatic activity are divided into several categories such as carbon-based [10-12], metal-
based [13-16], metal oxide-based [17-19] and metal sulfide-based [20, 21]. In addition, bi-
and multi-metallic nanoparticles [22, 23], mixed metal oxide nanoparticles [24, 25], and
nanocomposites, in which metal or metal oxides are combined with other types of
nanomaterials [26, 27], are also rapidly developed for their synergistic effects. Enzymatic
activity of nanomaterials has been used in many applications from biosensing, immunoassay,
neuroprotection, cancer diagnosis and therapy to pollutant removal [6, 28-30].

In these typical reactions, the oxidation of substrate catalyzed by peroxidase-mimetic
nanomaterials are usually use for fabrication of sensors or biosensor in order to detect of
H$_2$O$_2$ and H$_2$O$_2$-related analytes such as glucose, cholesterol, xanthine and so on [26, 31, 32].
Also, sensors or biosensors can fabricate based on inhibition of peroxidase- and/or oxidase-
like activity which usually used for detection of metals, biothiols, antioxidants, etc. [33-36].

Mixed metal oxides in spinel structure with the formula AB$_2$O$_4$ (A is bivalent and B is
trivalent metal ions) have been attracted enormous interest among inorganic-based
nanomaterials because of their special electrochemical properties, high catalytic activities and
exceptional chemical stability [37]. Gao et al. have fabricated hollow MnCo$_2$O$_4$ nanofibers
with excellent oxidase-like activity in the oxidation of TMB. They used the inhibition effect
of sulfite and L-cysteine on oxidase-like activity for the detection of these species [24]. Vetr
et al. reported the peroxidase-like catalytic activity of MFe$_2$O$_4$ (M = Mn, Co, Ni, Zn) [38] and
mesoporous MnCo$_2$O$_4$ [39] in the chromogenic oxidation of OPD to OPDox which were used
for detection of \( \text{H}_2\text{O}_2 \). However, ferrites (MFe\(_2\)O\(_4\)) and cobaltites (MCo\(_2\)O\(_4\)) (M is bivalent metal ions) have been studied for their peroxidase and oxidase-like activities [24, 25, 31, 35, 36, 38-43] but little attention has been paid to enzymatic activity of manganites (MMn\(_2\)O\(_4\)). In this study, CuMn\(_2\)O\(_4\) NPs were prepared via a co-precipitation method without any surface modification, characterized by typical techniques and their enzymatic activity was studied. Results showed that CuMn\(_2\)O\(_4\) NPs can catalyze the oxidation of OPD both in the presence of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \), in a similar way to the action of peroxidase- and oxidase enzymes, respectively. The catalase-like activity of CuMn\(_2\)O\(_4\) NPs was monitored by the decomposition reaction of \( \text{H}_2\text{O}_2 \) into oxygen and water. Also, the kinetic and mechanism of peroxidase- and oxidase-like activity were investigated. Besides, based on the peroxidase-like activity of CuMn\(_2\)O\(_4\) NPs, a simple colorimetric sensor for \( \text{H}_2\text{O}_2 \) detection and based on the oxidase-like activity of CuMn\(_2\)O\(_4\) NPs, a simple and sensitive colorimetric sensor for L-cysteine detection were established.

2. Experimental section

2.1. Materials

Cu(CH\(_3\)COO)\(_2\)•3H\(_2\)O, Mn(CH\(_3\)COO)\(_2\)•4H\(_2\)O, Na\(_2\)CO\(_3\), NaOH, Citric Acid (CA), Na\(_2\)HPO\(_4\), \( \text{H}_2\text{O}_2 \) 30%, \( \sigma \)-phenylenediamine (OPD) were purchased from Sigma-Aldrich or Merck and used as received without further purification. Deionized water was used throughout the experiments.

2.2. Characterization

Powder X-ray diffraction (XRD) data were collected with a Philips PW 1830 diffractometer (Cu-K\(\alpha\) X-radiation, \( \lambda = 1.54 \ \text{Å} \)). The scanning electron microscopy (SEM) image was taken on a KYKY–EM3200 scanning electron microscope. The elemental analysis was recorded with an energy dispersive X-ray (EDX) analyzer, MIRA3 FEG–SEM series. FT–IR spectra of
the sample in the form of KBr pellet was recorded using an Alpha-Bruker FT-IR spectrophotometer. All absorbance measurements were recorded with a RAYLEIGH (UV-1800) ultraviolet-visible (UV-vis) spectrometer. The O\textsubscript{2} generation from H\textsubscript{2}O\textsubscript{2} decomposition was recorded by a portable water quality meter (Model: 8603, S/N: 1000258).

2.3. Preparation of CuMn\textsubscript{2}O\textsubscript{4} NPs

CuMn\textsubscript{2}O\textsubscript{4} nanoparticles were prepared via the co-precipitation procedure [44]. In this procedure, Cu(CH\textsubscript{3}COO)\textsubscript{2}.H\textsubscript{2}O (0.49 g, 2.5 mmol) and Mn(CH\textsubscript{3}COO)\textsubscript{2}.4H\textsubscript{2}O (1.22 g, 5 mmol) were dissolved in 10 mL distilled water. The mixture was gradually diluted to 50 mL. To achieve precipitate, the solution was maintained at 60–80 °C and pH was raised to about 8 by dropwise addition of sodium carbonate (2M) for 10 min. The precipitate was aged under constant conditions (80 °C, pH 8, 150 rpm stirring) for 2 h. The precipitate was then filtered, washed with boiling distilled water and alcohol to bring the pH of the sub-filtration to 7. The black precipitate was dried in vacuum for 16 h and calcined at 415 °C for 2 h.

2.4. Study of triple-enzymatic activity of CuMn\textsubscript{2}O\textsubscript{4} NPs

To study of peroxidase-like activity, H\textsubscript{2}O\textsubscript{2} (30 %, 50 µL) was added into a solution of OPD (10 mM) and the mixture was diluted to 5 mL. Then 0.5 mL dispersed solution of CuMn\textsubscript{2}O\textsubscript{4} NPs (100 µg/mL) was added. For investigating the oxidase-like catalytic activity, CuMn\textsubscript{2}O\textsubscript{4} NPs (100 µg/mL) was added into a fresh solution of OPD (10 mM) in absence of H\textsubscript{2}O\textsubscript{2} and the mixture was diluted to 5 mL. In the above two studies, the absorbance was recorded in the wavelength range from 350 nm to 500 nm with a time interval for 30 s up to 10 min at room temperature. To investigate the catalase-like activity of CuMn\textsubscript{2}O\textsubscript{4} NPs, H\textsubscript{2}O\textsubscript{2} (30 %, 50 µL) and CuMn\textsubscript{2}O\textsubscript{4} NPs (100 µg/mL) solution were added into 5 mL distilled water. The concentration of O\textsubscript{2} generated from H\textsubscript{2}O\textsubscript{2} decomposition was recorded by a dissolved oxygen meter.
The steady-state kinetic analysis of peroxidase- and oxidase-like activity of CuMn$_2$O$_4$ NPs was studied using a UV-vis detection for 200 s in a kinetic scan mode. The reaction was carried out at room temperature and the absorbance measurements at 415 nm were performed immediately after the aqueous solution containing desired concentrations of H$_2$O$_2$ and OPD mixed with optimum content of CuMn$_2$O$_4$ NPs (120 μg/mL) and optimum pH of buffer solution (pH 6.2). The kinetic analysis of peroxidase-like activity of CuMn$_2$O$_4$ NPs was performed with optimum concentration of OPD (15 mM) and varied concentration of H$_2$O$_2$ (0.5 – 40 mM) as substrate or vice versa optimum concentration of H$_2$O$_2$ (8 mM) and varied concentration of OPD (1 – 24 mM) as substrate in final volume of reaction mixture with 3 mL of buffer solution. Similarly, the kinetic analysis of the oxidase-like activity of CuMn$_2$O$_4$ NPs was performed with a fixed concentration of oxygen soluble and varied concentration of OPD (1 – 20 mM) as a substrate in a final volume of the reaction mixture of 3 mL buffer solution. Then, the reaction rates were determined from the concentration versus time plots by using the Lambert-Beer law (A= εbc where, A: Absorbance, ε: molar extinction coefficient, b: optical path length, c: concentration of substrate) in the initial stage of the catalytic reaction. The kinetic parameters were determined by the typical Michaelis-Menten equation: $V_0=V_{\text{max}} [S]/(K_m + [S])$ where $V_0$ is the initial rate of reaction, $V_{\text{max}}$ is the maximum reaction rate, $K_m$ is the Michaelis constant and [S] is the concentration of substrate. Ascorbic acid was used to identify the mechanism of catalytic oxidation reaction of OPD by CuMn$_2$O$_4$ NPs in the presence or absence of H$_2$O$_2$.

### 2.5. Colorimetric Detection of H$_2$O$_2$

A typical colorimetric analysis was realized as follows: different concentrations of H$_2$O$_2$ (0.5 – 32 mM) were added into mixtures of a fresh solution of OPD (15 mM) in buffer solution (pH 6.2). A dispersed solution of CuMn$_2$O$_4$ NPs (120 μg/mL) was added and the mixture was incubated at room temperature for 10 min. Then, H$_2$SO$_4$ was added into the mixture to stop
the reaction. Finally, the absorbance measurements for OPDox at 415 nm of yellowish-brown solutions carried out. The limit of detection (LOD) has been calculated by data analysis from the slope of a calibration curve.

2.6. Colorimetric Detection of L-cysteine

In this detection, different concentrations of L-cysteine (0 – 400 µM) were added into mixtures of fresh solutions of OPD (40 mM) in the mentioned buffer solution (pH 6.2). Then dispersed solution of CuMn$_2$O$_4$ NPs (120 µg/mL) was added. The mixture was incubated at room temperature for 3 min. Then, the absorbance measurements at 388 nm of brownish pale green solutions carried out. The limit of detection (LOD) has been calculated by data analysis from the slope of a calibration curve. To selectivity study and investigate the effect of other amino acids, the stock solution (1 mM) of 5 kinds of natural amino acids including arginine, glycine, methionine, tryptophan, and tyrosine were added under the same conditions.

3. Results and discussion

3.1. Characterization

The structure of the as-prepared catalyst was determined by powder X-ray diffraction (XRD). The XRD pattern in the range of $2\theta = 10$-80$^\circ$ is shown in Fig. 1. The peaks in the $2\theta = 18.3^\circ$, 31.4$^\circ$, 35.8$^\circ$, 43.9$^\circ$, 54.2$^\circ$, 58.1$^\circ$, 63.5$^\circ$ are corresponded to (111), (220), (311), (400), (422), (511), (440) planes, in a cubic crystal structure, respectively. These results confirm the spinel structure of CuMn$_2$O$_4$ NPs with high purity as previous studies [24, 35, 44]. The particle size of CuMn$_2$O$_4$ NPs estimated using the most intense peak (311) based on the Debye-Scherrer equation [45] ($D=\frac{k\lambda}{\beta\cos\theta}$ where, D: average crystalline size, k: Scherrer constant (0.89), $\lambda$: X-ray wavelength used, $\beta$: the angular line width at half maximum intensity and $\theta$ is the Bragg's angle in degrees' unit) was found to be of about 5 nm.
Figure 2 shows the FT-IR spectrum of the as-prepared CuMn$_2$O$_4$ NPs. The absorption bands around 400-600 cm$^{-1}$ are related to the oxygen coordinated with metal ions (Cu$^{2+}$ and Mn$^{3+}$) [46]. The absorption peaks at 3437 and 1637 cm$^{-1}$ is related to the vibration of O-H of water molecules adsorbed on the surface of the particles [39].

The morphology and size of as-prepared catalyst were characterized by scanning electron microscopy (SEM). The SEM image in Fig. 3a shows that the CuMn$_2$O$_4$ NPs have spherical shapes with an average diameter of about 30-80 nm. A moderate degree of agglomeration was also observed in SEM images. The energy dispersive X-ray (EDX) is used to measure the composition of participates which verified CuMn$_2$O$_4$ NPs contain copper, manganese, and oxygen without any impurities as shown in Fig. 3b.

3.2. The triple-enzymatic catalytic activity of CuMn$_2$O$_4$ NPs

To study the peroxidase-like activity of CuMn$_2$O$_4$ NPs in the presence of H$_2$O$_2$, OPD was used as a donor substrate. CuMn$_2$O$_4$ NPs can also oxidase OPD by dissolved oxygen. In two cases, a new band at 410–415 nm indicates to produce OPDox that could be observed by naked eyes [47, 48]. The control reaction in the absence of CuMn$_2$O$_4$ NPs showed little variation in the absorbance at 410-415 nm (Fig. 4a). There is more absorbance from OPDox in peroxidase-like activity than there was in the oxidase-like activity of CuMn$_2$O$_4$ NPs as shown in Fig. 4a. Furthermore, CuMn$_2$O$_4$ NPs can catalyze the decomposition of H$_2$O$_2$ to product O$_2$ as bubbles that could be also observed in the mixture solution of CuMn$_2$O$_4$ NPs and H$_2$O$_2$ (Fig. 4b).

3.3. Optimization
Several factors such as time, pH, CuMn$_2$O$_4$ NPs content, H$_2$O$_2$, and OPD concentration should be considered in the oxidation reaction study of OPD by CuMn$_2$O$_4$ NPs with peroxidase- or oxidase-like activity. The effect of pH was measured by incubating the suspension of CuMn$_2$O$_4$ NPs in pH of buffer solution from 3 to 7. The significant absorbance was observed from the supernatant solution in the leach test when the pH value of the buffer solution was around 3-5, which would be evidence that the NPs are unstable in acidic pH conditions and leached cations from CuMn$_2$O$_4$ NPs into the solution can oxidase the OPD. As shown in Fig. 5a, the oxidation reaction has a higher rate when the pH value of the buffer solution was around 3-5. Under the optimal pH 6.2, the peroxidase-like activity was enhanced directly with the increasing content of CuMn$_2$O$_4$ NPs at a constant concentration of OPD and H$_2$O$_2$ (Fig. 5b).

As CuMn$_2$O$_4$ NPs can oxidase OPD before adding H$_2$O$_2$ with oxidase-like activity and can decompose H$_2$O$_2$ before adding OPD with catalase-like activity, it is important that the reagents be added in order already mentioned. As shown in Fig. 6a the absorbance at 415 nm was increasing with the reaction time. The absorbance versus time plot is shown in Fig. 6b was indicated that the absorbance related to OPDox increased gradually and reached to a maximum at 200 s then fixed with little variation in the absorbance.

The optimal conditions for the maximum peroxidase-like catalytic activity of prepared CuMn$_2$O$_4$ NPs during the oxidation process including CuMn$_2$O$_4$ NPs content, H$_2$O$_2$, and OPD concentrations were set as 120 µg/mL, 8 mM, 15 mM, respectively.

3.4. Kinetic and mechanism analysis of peroxidase- and oxidase-like activity of CuMn$_2$O$_4$ NPs
The steady-state kinetics carried out to study the effect of each substrate concentration on reaction rate, achieve kinetic parameters and investigate reaction mechanism. To calculate the initial reaction rate, absorbance was converted to the corresponding concentration of OPD$_{ox}$ by using the Lambert-Beer law and related to the molar extinction coefficient of OPD$_{ox}$ value $\varepsilon = 16700 \text{ M}^{-1} \text{ cm}^{-1}$ (at 415 nm) [36]. Figure 7(a-d) shows the steady-state kinetic analysis of the oxidation reaction of OPD by CuMn$_2$O$_4$ NPs in the presence of H$_2$O$_2$. As shown in Fig. 7a and c reaction rates were found to be dependent on the concentrations of H$_2$O$_2$ and OPD and increased gradually with increasing the concentrations of H$_2$O$_2$ and OPD, then the reactions reached a maximum rate to saturation of the reactivity of CuMn$_2$O$_4$ NPs [36]. The $K_m$ and $V_{max}$ values for each OPD and H$_2$O$_2$ substrates were calculated by using the Lineweaver-Burk plots of double reciprocal of the Michaelis–Menten equation. The CuMn$_2$O$_4$ NPs possess higher peroxidase-like activity in oxidation of OPD compared to that of natural HRP enzyme [18] as well as those corresponding single metal oxides, CuO [49-51] and MnO$_2$ [18] with the $K_m$ and $V_{max}$ value of 0.08 mM and 10.8 mMs$^{-1}$ toward OPD and 0.59 mM and 16.9 mMs$^{-1}$ toward H$_2$O$_2$, respectively (Table 1).

The kinetic of oxidase-like activity of CuMn$_2$O$_4$ NPs was investigated as earlier described for peroxidase activity. The kinetic parameters, $K_m$ and $V_{max}$, were found to be 0.199 mM and 5.63 mMs$^{-1}$ toward OPD, respectively (Fig. 8a and b).

Furthermore, the mechanism of enzymatic (peroxidase and oxidase)-like activity of CuMn$_2$O$_4$ NPs was studied using ascorbic acid as a radical scavenger [52, 53]. As shown in Fig. 9, the...
absorption at 410 – 415 nm indicates the production of OPDox, which confirms CuMn$_2$O$_4$ NPs can oxidase OPD in the presence of H$_2$O$_2$ in spite of using ascorbic acid as a radical scavenger. This can be because the mechanism of the peroxidase-like activity of CuMn$_2$O$_4$ NPs is different from that of Fenton or Fenton-like reactions, which are related to the formation of free radicals from H$_2$O$_2$. Therefore, CuMn$_2$O$_4$ NPs as a mediator can facilitate the electron transfer process from absorbed OPD on the surface of the CuMn$_2$O$_4$ NPs to H$_2$O$_2$ directly. A similar mechanism has been suggested previously for Co$_3$O$_4$ [25] and NiCo$_2$O$_4$ [54]. Also, ascorbic acid was used in the oxidation reaction of OPD by CuMn$_2$O$_4$ NPs with oxidase-like activity. Figure 10 b shows no band at 410 – 415 nm that indicates OPD oxidation by CuMn$_2$O$_4$ NPs did not occur in the presence of ascorbic acid. This result indicates ascorbic acid has an inhibition effect on the oxidation reaction of OPD on CuMn$_2$O$_4$ NPs surface in absence of H$_2$O$_2$ [36].

3.5. Colorimetric detection of H$_2$O$_2$ based on peroxidase-like activity of CuMn$_2$O$_4$ NPs

Detection of H$_2$O$_2$ as a by-product of several biochemical reactions or as a contaminant in various branches of industry has attracted enormous interest [55]. There are many types of analysis methods for the detection and measurement of H$_2$O$_2$ [56, 57]. Since the chromogenic oxidation of OPD to OPDox in peroxidase-like activity system is dependent on H$_2$O$_2$ concentration, it can be used to determine the concentration of H$_2$O$_2$ in aqueous solution as a simple analysis method. In Fig. 10a, the absorbance at 415 nm for oxidized OPD increases with the concentration of H$_2$O$_2$. Figure 10b shows the corresponding linear calibration curve. The linear range is from 0.5 mM to 22 mM, and the detection limit is calculated to be 0.11 mM. Table 2 gives a comparison of the detection limit and efficiency of peroxidase-like activity of CuMn$_2$O$_4$ to oxidase of OPD in the detection of H$_2$O$_2$ with other catalytic systems.
reported in the literature. These results show that the detection limit for CuMn$_2$O$_4$/OPD system is the same order of magnitude as that of metal oxide nanoparticles such as mesoporous MnCo$_2$O$_4$.

<Figure 10>

<Table 2>

3.6. Colorimetric detection of L-cysteine concentration based on the oxidase-like activity of CuMn$_2$O$_4$ NPs

The normal concentration of cysteine in plasma samples from healthy human subjects is approximately in the range of 200–300 µM [58]. Deficiency or unnatural level of cysteine can directly cause slowed growth, liver damage, hair depigmentation, malabsorption syndromes, edema and lethargy [59, 60]. There are many colorimetric detections of L-cysteine based on peroxidase-like activity of inorganic-based nanomaterials [61-63] that high nucleophilicity of thiol side chain in cysteine can decrease affinity toward the substrate by shielding of CuMn$_2$O$_4$ NPs [33]. The colorimetric method for the detection of L-cysteine based on the oxidase-like activity of inorganic-based nanomaterials was also developed [24, 64]. Figure 11 shows the absorbance measurement for OPDox in the oxidase-like activity system depending on L-cysteine concentration in the wavelength range from 320 nm to 470 nm. In Fig. 12a, the absorbance at 388 nm for OPDox decreases with an increasing concentration of L-cysteine. Figure 12b shows the corresponding linear calibration curve. The linear range is from 50 µM to 200 µM, and the detection limit is calculated to be 56.15 µM. The effect of other amino acids including Arg, Gly, Met, Thr and Tyr on OPD oxidation by CuMn$_2$O$_4$ NPs was also tested. As shown in Fig. 13, amino acids tested did not have a significant effect on the oxidase-like activity of CuMn$_2$O$_4$ nanoparticles except L-cysteine. Compared with other inorganic-based nanomaterials with enzymatic activity for L-cysteine
detection by UV-vis detection (Table 3), CuMn$_2$O$_4$ NPs possess high potential for the
detection of L-cysteine in a wide range of concentration.

4. Conclusions

This work investigated the triple-enzymatic activity of CuMn$_2$O$_4$ NPs including peroxidase-, oxidase- and catalase-like activity. A detailed analyzing of the kinetic and mechanism by the UV-vis detection of the catalytic reaction confirms that CuMn$_2$O$_4$ NPs possess peroxidase-like activity superior to that of corresponding single metal oxides which can be originated from transferring of electrons from OPD to H$_2$O$_2$ by CuMn$_2$O$_4$ NPs as mediator. Based on the peroxidase-like activity of CuMn$_2$O$_4$ NPs, a colorimetric method was developed for the detection of H$_2$O$_2$. Colorimetric detection of L-cysteine based on inhibition of the oxidase-like activity of CuMn$_2$O$_4$ NPs was also developed, in which the affinity of CuMn$_2$O$_4$ NPs toward OPD decrease in the presence of L-cysteine. These results offer a simple, rapid, inexpensive and green method for analytical applications of CuMn$_2$O$_4$ as a versatile nanoparticle.

5. References


3 [14] Jv, Y., Li, B. and Cao R. "Positively-charged gold nanoparticles as peroxidase mimic and their  
4 application in hydrogen peroxide and glucose detection", Chem. Commun., 46 (42), pp. 8017-8019  
5 (2010).
8 (2013).
10 "Dual-enzyme characteristics of polyvinylpyrrolidone-capped iridium nanoparticles and their cellular  
11 protective effect against H₂O₂-induced oxidative damage", ACS Appl. Mater. Interfaces, 7 (15), pp.  
16 as both peroxidase and oxidase mimics", Analyst, 137 (19), pp. 4552-4558 (2012).
18 nanoparticles: promising peroxidase mimetics for H₂O₂ and glucose detection", Anal. Methods, 4 (10),  
22 3501-3506 (2012).
24 based on the intrinsic peroxidase-like activity of MoS₂ nanosheets", Nanoscale, 6 (20), pp. 11856-  
27 nanoparticles with the enhanced peroxidase-like activity for ultrafast colorimetric detection of H₂O₂",  
14


Vetr, F., Moradi-Shoeili, Z. and Özkar, S. "Oxidation of o-phenylenediamine to 2, 3-diaminophenazine in the presence of cubic ferrites MFe$_2$O$_4$ (M= Mn, Co, Ni, Zn) and the application in colorimetric detection of H$_2$O$_2$", *Appl. Organomet. Chem.*, 32 (9), e4465 (2018).


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Table 1. Comparison of kinetic parameters for substrate oxidation by inorganic-based nanomaterials with peroxidase-like activity.
<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substrate</th>
<th>$K_m$ (mM)</th>
<th>$V_{max} \times 10^7$ (Ms$^{-1}$)</th>
<th>$K_m$ (mM)</th>
<th>$V_{max} \times 10^7$ (Ms$^{-1}$)</th>
<th>Ref.</th>
</tr>
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<tr>
<td>HRP</td>
<td>OPD</td>
<td>0.6</td>
<td>0.46</td>
<td>0.34</td>
<td>0.94</td>
<td>[18]</td>
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<tr>
<td>HRP</td>
<td>TMB</td>
<td>0.43</td>
<td>0.1</td>
<td>3.7</td>
<td>0.87</td>
<td>[35]</td>
</tr>
<tr>
<td>BSA-MnO$_2$</td>
<td>OPD</td>
<td>0.31</td>
<td>0.82</td>
<td>0.12</td>
<td>0.57</td>
<td>[18]</td>
</tr>
<tr>
<td>MnFe$_2$O$_4$</td>
<td>OPD</td>
<td>27</td>
<td>1.04</td>
<td>-</td>
<td>-</td>
<td>[39]</td>
</tr>
<tr>
<td>CuO NPs</td>
<td>TMB</td>
<td>0.013</td>
<td>-</td>
<td>85.6</td>
<td>-</td>
<td>[49]</td>
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<tr>
<td>CuO nanosheets</td>
<td>TMB</td>
<td>-</td>
<td>-</td>
<td>15.8</td>
<td>0.077</td>
<td>[50]</td>
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<tr>
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<td>1.46</td>
<td>2.88</td>
<td>0.88</td>
<td>[51]</td>
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<td>CuMn$_2$O$_4$ NPs</td>
<td>OPD</td>
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<td>10.8</td>
<td>0.59</td>
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</table>

**Table 2.** Comparison of the various peroxidase-like activities of inorganic-based nanomaterials for the detection of H$_2$O$_2$. 

This work
<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Substrate</th>
<th>Detection limit (mol/L)</th>
<th>Linear range (mol/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnCo$_2$O$_4$</td>
<td>OPD</td>
<td>$2 \times 10^{-4}$</td>
<td>$5 \times 10^{-4} - 0.12$</td>
<td>[38]</td>
</tr>
<tr>
<td>MnFe$_2$O$_4$</td>
<td>OPD</td>
<td>$0.3 \times 10^{-4}$</td>
<td>$1 \times 10^{-4} - 0.015$</td>
<td>[39]</td>
</tr>
<tr>
<td>CuS</td>
<td>OPD</td>
<td>$0.11 \times 10^{-6}$</td>
<td>$1 \times 10^{-6} - 1 \times 10^{-3}$</td>
<td>[48]</td>
</tr>
<tr>
<td>NiCo$_2$O$_4$</td>
<td>TMB</td>
<td>$0.21 \times 10^{-6}$</td>
<td>$1 \times 10^{-5} - 4 \times 10^{-4}$</td>
<td>[54]</td>
</tr>
<tr>
<td>CuMn$_2$O$_4$</td>
<td>OPD</td>
<td>$1.1 \times 10^{-4}$</td>
<td>$5 \times 10^{-4} - 0.022$</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table 3. Comparison of various enzymatic activity of inorganic-based nanomaterials for the detection of L-cysteine.
<table>
<thead>
<tr>
<th>property</th>
<th>(µmol/L)</th>
<th>(µmol/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MnCo₂O₄</td>
<td>Oxidase-like</td>
<td>TMB</td>
<td>0.0343</td>
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<tr>
<td>CuMnO₂</td>
<td>Peroxidase-like</td>
<td>TMB</td>
<td>11.26</td>
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<tr>
<td>MoS₂-PPy-Pd</td>
<td>Peroxidase-like</td>
<td>TMB</td>
<td>0.08</td>
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<tr>
<td>Fe₃O₄</td>
<td>Peroxidase-like</td>
<td>TMB</td>
<td>0.028</td>
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<tr>
<td>Asp/Ce-NT</td>
<td>Oxidase-like</td>
<td>TMB</td>
<td>0.0332</td>
</tr>
<tr>
<td>CuMn₂O₄</td>
<td>Oxidase-like</td>
<td>OPD</td>
<td>56.15</td>
</tr>
</tbody>
</table>

Fig 1. XRD pattern of CuMn₂O₄ NPs.

Fig 2. FT–IR spectrum of CuMn₂O₄ NPs.

Fig 3. SEM image (a) and EDX spectrum (b) of CuMn₂O₄ NPs.
Fig 4. The absorbance measurement for OPDox in the wavelength range from 350 nm to 500 nm in different reaction systems: (a) i) OPD (10 mM), ii) OPD (10 mM) + H₂O₂ (30%, 50 µL), iii) OPD (10 mM) + CuMn₂O₄ NPs (100 µg/mL), iv) OPD (10 mM) + H₂O₂ (30%, 50 µL) + CuMn₂O₄ NPs (100 µg/mL); (b) Dependence of dissolved oxygen generation of catalase-like activity of CuMn₂O₄ NPs on CuMn₂O₄ NPs content, citric acid-Na₂HPO₄ buffer solution (pH 6.2); the concentration of H₂O₂ (30%, 50 µL).

Fig 5. Dependence of the enzymatic activity of CuMn₂O₄ NPs on (a) pH, (dispersed solution of CuMn₂O₄ NPs (120 µg/mL), the concentration of H₂O₂ (8 mM), OPD (15 mM)); (b) CuMn₂O₄ NPs content, (the concentration of H₂O₂ (8 mM), OPD (15 mM); citric acid-Na₂HPO₄ buffer solution (pH 6.2)).

Fig 6. The absorbance measurement for OPDox in (a) the wavelength ranges from 350 nm to 500 nm for 10 min, (b) a kinetic scan mode at 415 nm. Conditions: H₂O₂ (8 mM), OPD (15 mM) and dispersed solution of CuMn₂O₄ NPs (120 µg/mL) and citric acid-Na₂HPO₄ buffer solution (pH 6.2).

Fig 7. The steady-state kinetic analysis of oxidation reaction of OPD by CuMn₂O₄ NPs in the presence of H₂O₂ (a) the concentration of OPD was optimum (15 mM) and the H₂O₂ concentration was varied (0.5 – 40 mM); (b) Lineweaver-Burk plots of peroxidase-like activity of CuMn₂O₄ NPs in varied concentration of H₂O₂; (c) The concentration of H₂O₂ was optimum (8 mM) and the OPD concentration was varied (1 – 24 mM); and (d) Lineweaver-Burk plots of peroxidase-like activity of CuMn₂O₄ NPs in varied concentration of OPD; Conditions: dispersed solution of CuMn₂O₄ NPs (120 µg/mL), in citric acid-Na₂HPO₄ buffer solution (pH 6.2).

Fig 8. The steady-state kinetic analysis of oxidation reaction of OPD by CuMn₂O₄ NPs in absence of H₂O₂ (a–b). (a) The OPD concentration was varied (0 – 5 mM); (b) Lineweaver-Burk plot of peroxidase-like activity of CuMn₂O₄ NPs with different concentrations of OPD. Conditions: dispersed solution of CuMn₂O₄ NPs (120 µg/mL), in citric acid-Na₂HPO₄ buffer solution (pH 6.2).

Fig 9. The absorbance measurement for OPDox in the wavelength range from 350 nm to 500 nm in the presence of ascorbic acid and (a) with H₂O₂, (b) without H₂O₂.

Fig 10. (a) The absorbance measurement for OPDox at 415 nm in the presence of H₂O₂ at different concentrations (0 – 40 mM), (b) Linear calibration plot for H₂O₂ determination using CuMn₂O₄ NPs.

Fig 11. The absorbance measurement for OPDox in the wavelength range from 320 nm to 470 nm, Conditions: OPD (40 mM) and dispersed solution of CuMn₂O₄ NPs (120 µg/mL), in citric acid-Na₂HPO₄ buffer solution (pH 6.2).
Fig 12. (a) The absorbance measurement for OPDox at 388 nm in the presence of L-cysteine at different concentrations (50 – 450 µM), (b) Linear calibration plot for L-cysteine determination using CuMn$_2$O$_4$ NPs.

Fig 13. Changes of relative absorbance intensity at 415 nm in the OPD oxidation reaction catalyzed by CuMn$_2$O$_4$ NPs in the presence of 1 mM amino acids including Cys, Arg, Gly, Met, Thr, and Tyr.
Figure 2

Figure 3

(a) 0/8

(b) 0/9

Wavelength [nm]

Absorbance

O₂ mg/L

[CuMnO₄ NPs] µg/mL
Figure 4

(a) 

(b)

Figure 5

Figure 6
Figure 7

(a) $V_v \times 10^7 \text{mM/s}$ vs. $[\text{H}_2\text{O}_2]$ mM

(b) $1/V_v \times 10^7$ vs. $1/[\text{H}_2\text{O}_2]$ with the linear fit $y = 0.0056x + 0.1005$, $R^2 = 0.9542$

Figure 8

(c) $V_v \times 10^{-7}$ mM/s vs. [OPD] mM

(d) $1/V_v \times 10^7$ vs. $1/[\text{OPD}]$ with the linear fit $y = 0.1434x + 0.0823$, $R^2 = 0.9975$

(a) $V_v \times 10^{-7}$ mM/s vs. [OPD] mM

(b) $1/V_v$ vs. $1/[\text{OPD}]$ with the linear fit $y = 0.0355x + 0.1776$, $R^2 = 0.9324$
Figure 9

Figure 10

Figure 11
Figure 12

Figure 13