Received: 28 April 2017 | Revised: 4 July 2017 | Accepted: 6 July 2017
DOI: 10.1002/aoc.4018

FULL PAPER

WILEY Applied Organometallic Chemistry

# Synthesis and catalytic evaluation of $Fe_3O_4$/MWCNTs nanozyme as recyclable peroxidase mimetics: Biochemical and physicochemical characterization

Navvabeh Salarizadeh[1] | Minoo Sadri[1] | Reza H. Sajedi[2]

[1] Department of Biochemistry and Biophysics, Education and Research Center of Science and Biotechnology, Malek Ashtar University of Technology, Tehran, Iran

[2] Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

**Correspondence**
Minoo Sadri, Department of Biochemistry and Biophysics, Education and Research Center of Science and Biotechnology, Malek Ashtar University of Technology, Tehran, Iran.
Email: sadri.mn313@gmail.com

**Abstract**

In this research, the nanocomposite of multiwalled carbon nanotubes and magnetic metal oxide nanoparticles ($Fe_3O_4$/MWCNTs), as enzyme mimetic, was synthesized using an *in situ* chemical reduction method. The structure, composition and morphology of the prepared $Fe_3O_4$/MWCNT nanocomposite materials were characterized using X-ray diffraction, FT-IR and scanning electron microscopy with energy dispersive X-ray spectroscopy, respectively. The magnetic properties of the nanocomposite were investigated by the vibrating sample magnetometer. A colorimetric system involving nanozyme, phenol/4-aminoantipyrine and $H_2O_2$ was utilized for the determination of peroxidase mimetic catalytic assay. The obtained results confirmed that the synthesis of $Fe_3O_4$/MWCNTs nanostructures was successful. It was found that $Fe_3O_4$/MWCNTs nanohybrid exhibited peroxidase-like activity without any pH limitation. Colorimetric data demonstrated that the prepared nanocatalyst had higher catalytic activity toward $H_2O_2$ than MWCNTs. The kinetic parameters of the nanozyme, $K_m$ and $V_{max}$, were estimated to be 8.3 mM and 1.4 mM min$^{-1}$, respectively. The $Fe_3O_4$/MWCNTs nanostructures were also successfully applied for glucose detection. In addition, peroxidase-like activity of the nanozyme increased in the presence of butyl-imidazolium bromide ionic liquid. These biomimetic catalysts have some advantages, such as simplicity, stability, reusability and cost effectiveness, which makes them great candidates to be used in various fields of biotechnology applications.

**KEYWORDS**

4-aminoantipyrine, characterization, $Fe_3O_4$/MWCNTs nanostructure, nanozyme, peroxidase-like activity

## 1 | INTRODUCTION

Peroxidases have wide biotechnological applications in environment bioremediation, immunoassay, bimolecular detections and industrial catalysts.[1–5] Horseradish peroxidase (EC 1.11.1.7) catalyzes the oxidation process of aromatic compounds by hydrogen peroxide or alkyl hydroperoxide. This enzyme was widely applied in the synthesis of polymers and the bioremediation of toxic organic compounds like phenolic structures in wastewaters.[1,2,6] Despite the importance of natural enzymes, due to their sensitivity against environmental changes, low stability, high costs of purification and storage, their usages are not cost-effective.[7–9] Therefore, artificial catalysts can be designed and synthesized as enzyme mimetics, including oxidase, peroxidase, dehydrogenases, catalase, nuclease and proteases with sufficient stability.[10–14] Peroxidases, as one of the enzyme mimics, have been investigated by some researchers, recently.[15–19] These peroxidase mimetics have been used for glucose and $H_2O_2$ detection.[20] DNAzymes,[16] nanozymes and complexes of organic nanostructures such as heme, hemin, porphyrine and cyclodextrines as peroxidase mimetics have been studied.[4,15,21] Nanozymes are nano-based materials with similar activity to natural enzymes.[4,9] Compared with natural enzymes, nanozymes possess advantages, including thermal stability, low cost, easy production and different range pH-tolerable.[22] It has been found that iron and iron oxide nanoparticles possess intrinsic peroxidase and oxidase-like activities.[2,4,23,24] Research has been reported where magnetic nanostructures have enzyme-like activity,[9,25] but the self-aggregation of magnetic nanoparticles decreases the catalytic activity of nanoparticles. Therefore, loading these nanoparticles on nanostructures, with large specific surface areas, like carbon nanotubes, grapheme, and their combination with surfactants reduces their self-aggregation and dissolution.[24,26–29] Recently, carbon nanotubes (CNTs) have been extensively considered by researchers due to their exceptional mechanical, thermal and electrical properties.[30] The combination of CNTs with metal oxide nanoparticles magnetic types has attracted much attention due to their unique properties, including electrical and chemical conductivity, mechanical and structural properties of CNTs, and magnetic properties and chemical stability of metal oxide nanoparticles.[24,31–33] CNTs have been used in imaging techniques, such as Raman spectroscopy, near-infrared fluorescence and ultrasonography.[30,31] A high ratio of surface area to weight in nanotubes is an important factor for designing of nanocomplexes. These carbon-based nanocomposites have been used in various fields, like drug delivery, biosensing, catalysis, fuel cells, capacitors and decontamination purposes.[7,32,34] Peroxidase-like catalytic activities of $Fe_3O_4$, CNTs and carbon-based nanostructures have been approved.[32–37] The synthesis of CNT-coated $Fe_3O_4$ nanocomplex was reported by some researchers, and their applications for $H_2O_2$ detection and Orange II degradation were investigated.[2,9,35] The earth-abundant element-based nanoparticles are low-cost materials in the design of novel enzyme mimetics. Because of the disadvantages of natural peroxidases, this research is focused on the artificial peroxidase mimics.

In this research, $Fe_3O_4$/MWCNTs nanocomposites were prepared via a novel method and they were utilized as catalysts. The characterization of the nanocomposites was achieved using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX), X-ray diffraction (XRD) and FT-IR. Finally, biochemical characterizations of the nanocatalysts were evaluated using phenol/4-aminoantipyrine (4-AAP) as a new colorimetric assay for peroxidase mimetics. A wide range of substrates, such as phenol, aminophenols, indophenols, diamines and a number of other compounds have been used for HRP spectrophotometric assay.[38] In most studies, $H_2O_2$ was determined by peroxidase artificial enzymes on 3,3′,5,5′-tetramethylbenzidine (TMB) and ABTS as substrate.[17] Also, a combination of the peroxidase-like catalytic activity of the $Fe_3O_4$/MWCNTs nanohybrids with glucose oxidase (GOX) allowed the system of a sensitive colorimetric assay for glucose monitoring. $Fe_3O_4$/MWCNT nanocatalysts exhibited good peroxidase-like catalytic activity. These magnetic nanocatalysts can be suitable candidates in future enzyme-based applications, such as glucose and $H_2O_2$ detection.

## 2 | EXPERIMENTAL

### 2.1 | Materials

Multiwalled CNTs (MWCNTs) with diameters of 5–20 nm, lengths of 6–15 mm and purities of 97% were provided from Research Institute of Petroleum Industry of Iran (RIPI). Ammonium iron (II) sulfate, sodium acetate, 4-AAP, GOX and hydrazine hydrate were purchased from Sigma-Aldrich (USA). Phenol, TMB, dimethyl sulfoxide, $H_2O_2$, acetic acid, $KH_2PO_4$ and $K_2HPO_4$ were obtained from Merck (Germany).

### 2.2 | Synthesis of $Fe_3O_4$/MWCNT nanocomposites

In order to synthesis the nanocomposites; MWCNTs were modified with a mixture of nitric acid/sulfuric acid as oxidative reagents at 150 °C for 2 h. Then, the treated MWCNTs were washed, filtered and sonicated for 15 min. In order to synthesis the $Fe_3O_4$/MWCNTs nanocomposites, 0.57 g ammonium ferrous sulfate (hexahydrate) was dissolved in 10 ml of deionized water. Next, 0.1 g of the modified MWCNTs was added to the previous solution, and they were sonicated with 3 min intervals for 15 min. Then, 1.25 ml of hydrazine hydrate was added to the mixture, which was followed by adding step by step of 2 M NaOH solution under ultrasonic stirring for 15 min. When the pH of suspension reached 11–12, the suspension was refluxed at the boiling temperature of the solvent. The magnetic hybrid product was separated with a magnet, and then washed with deionized water and ethanol several times, and the final product was dried in the vacuum oven at 50 °C for 24 h.

## 2.3 | Characterization of $Fe_3O_4$/MWCNTs nanocomposites

The morphology and elemental composition of nanocomposites were investigated by SEM/EDX (Zeiss Sigma 500 VP SEM/Oxford Instrument Detector). XRD [Philips PW1730, with Cu $K_\alpha$ radiation ($\lambda = 1.540598$ Å)] was used for determination of nanomaterial structures. FT-IR (Bruker Equinox, Germany) was applied to study the changes of chemical structure. The magnetic properties of $Fe_3O_4$/MWCNTs nanocomposites were investigated by the vibrating sample magnetometer (VSM; BHV-55, Riken, Japan).

## 2.4 | Colorimetric determination of peroxidase-like activity of $Fe_3O_4$/MWCNTs nanozyme

Colorimetric evaluation of the nanocomposites was performed by adding 2 mg ml$^{-1}$ nanozyme to 2.5 mM 4-AAP, 25 mM phenolic compound in 200 mM potassium phosphate buffer (pH 7.0) and $H_2O_2$ at room temperature. The absorbance changes were recorded at 510 nm using a µQuant™ microplate spectrophotometer (BioTek Instruments, Winooski, USA). This reaction mixture was chosen as nanozyme standard assay. Also, the colorimetric assay of nanocomposites was investigated in the presence of TMB-$H_2O_2$ substrates in acetate buffer (pH 4.5) at room temperature, and the absorbance was measured at 652 nm. The control experiments were also performed under the same conditions to compare relative catalytic activity of nanocatalysts.

## 2.5 | Kinetic measurements of peroxidase-like activity of nanozymes

The kinetic measurements were performed at appropriate concentrations of $H_2O_2$ substrate according to the assay conditions (like standard assay conditions) as described above. The nanozyme's kinetic parameters, Michaelis–Menten constant ($K_m$) and $V_{max}$, were determined using Lineweaver–Burk plot.

## 2.6 | Optimum pH determination

Because the enzyme's activity is affected by environmental conditions, the dependency of peroxidase-like activity of nanozyme on pH value was investigated at the pH range of 4.0–9.0. The pH profile of nanozyme was determined under standard assay reaction.

## 2.7 | Effect of ionic liquids on catalytic activity of nanozyme

Ionic liquids, known as green catalysts, have been used in bio-electrochemistry and biocatalysis as stable solvents.[39] Because peroxidase-like activity of these catalysts has been demonstrated,[40] the synergistic effect of imidazolium-based ionic liquid (butyl-imidazolium bromide) with $Fe_3O_4$/MWCNTs nanocatalysts was investigated. For this purpose, 200 µl butyl-imidazolium bromide was mixed with 2 mg ml$^{-1}$ $Fe_3O_4$/MWCNTs and the obtained mixture was stirred for 20 min. Then, catalytic activity assay was measured at 510 nm and the results were compared with those obtained from ionic liquid and nanocatalyst.

## 2.8 | Glucose detection

Detection of glucose was performed with reaction mixture consisting of 100 µl of 1 mg ml$^{-1}$ GO and 1 ml of different concentrations of glucose solutions, which were incubated (pH 7.0) at 37 °C for 20 min. Then, 1 ml of 4-AAP/phenol solution and 50 µl of nanocatalyst were added into the above mixture. Finally, the absorption spectrum of the obtained solution was recorded by the UV–Vis spectrophotometer at the wavelength range from 300 to 700 nm.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Characterization of $Fe_3O_4$/MWCNTs nanostructure

Physicochemical characterization of $Fe_3O_4$/MWCNTs nanocatalyst is shown in Figure 1. The morphology of synthesized nanostructures was investigated with SEM. It was clearly obvious that the $Fe_3O_4$ nanoparticles were well attached and distributed on the surface of MWCNTs. The average size of nanocomposites was estimated to be about 25 nm using SEM analysis. The EDX spectrum in Figure 1b shows the presence of carbon, iron and oxygen in magnetic $Fe_3O_4$/MWCNTs. Also, the weight ratios of Fe, O and C were obtained as 19.9%, 15% and 65%, respectively, by EDX analyses. The carbon, iron and oxygen signals corresponded to CNTs and $Fe_3O_4$ nanoparticles, respectively.

FT-IR spectroscopy of $Fe_3O_4$/MWCNTs, $Fe_3O_4$ and MWCNTs throughout the range of 400–4000 cm$^{-1}$ was also performed to confirm the structural changes. The position of bands at 584 and 1618 cm$^{-1}$ can be attributed to the Fe–O–Fe stretching and bending modes in $Fe_3O_4$ nanoparticles.[17] It was observed that the bands at 1100, 1412, 1631, 2928 and 3424 cm$^{-1}$ were assigned to the stretching modes of C–O, C–C, C = O, C–H and –OH in

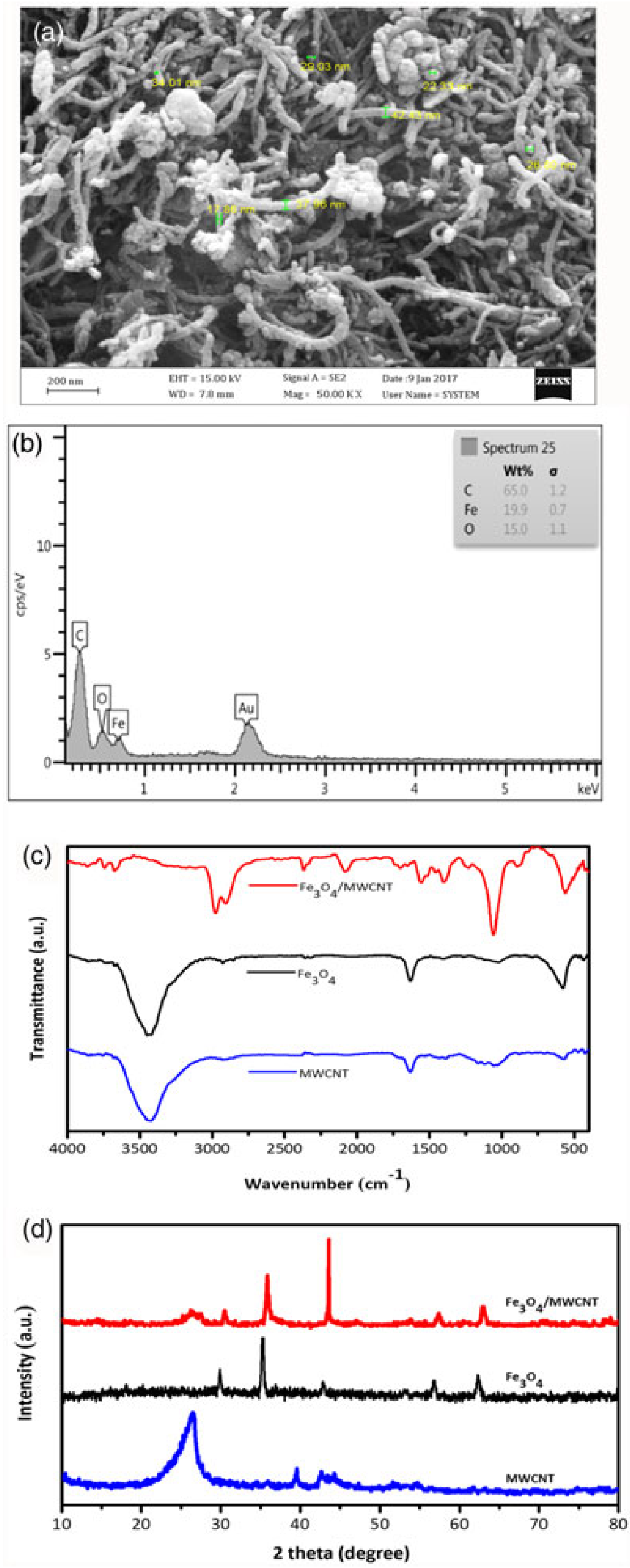


**FIGURE 1** (a, b) typical SEM/EDX images of $Fe_3O_4$/MWCNT nanocomposites. (c) FT-IR spectra of $Fe_3O_4$/MWCNT nanohybrids, $Fe_3O_4$ and MWCNT. (d) XRD patterns of $Fe_3O_4$/MWCNT, $Fe_3O_4$ and MWCNT

the functional groups of the MWCNTs, respectively. In $Fe_3O_4$ graph, the peak at 3420 $cm^{-1}$ related to the hydroxyl group (−OH) of adsorbed water. In the FT-IR spectra of $Fe_3O_4$/MWCNTs, apart from the main peaks of $Fe_3O_4$, additional peaks were also observed that were attributed to the functional groups of the MWCNTs but, as expected, the intensity of the peaks decreased significantly. The reduction of –OH peak intensity in $Fe_3O_4$/MWCNTs spectrum at 3423 $cm^{-1}$ demonstrated that the binding of $Fe_3O_4$ nanoparticles to MWCNTs was successful. These observations clearly confirm that $Fe_3O_4$ nanoparticles have successfully supported onto the surface of the MWCNTs.

The crystalline structures of the synthesized $Fe_3O_4$/MWCNTs nanohybrids and MWCNTs were confirmed with powder XRD measurements, and the main peaks of $Fe_3O_4$/MWCNTs crystals were clearly presented. The reduction of peak intensity at $2\theta = 26.6°$ that related to MWCNTs indicated that the MWCNTs structure was changed in the synthesis process. Peaks at $2\theta = 30.48°$, 35.82°, 43.54°, 53.86°, 57.40° and 62.98° are devoted to (220), (311), (400), (422), (511) and (440) crystal planes, respectively, which are the major peaks of $Fe_3O_4$/MWCNTs. These characteristic peaks originated from $Fe_3O_4$ nanoparticles. These results are consistent with previous studies.[17] Also, no diffraction peak due to any other new phase was observed. The average crystallite size of nanohybrids calculated by the Debye–Scherer equation was about 18 nm.

The hysteresis curves of the $Fe_3O_4$-coated MWCNTs were recorded at room temperature with a VSM. The saturation magnetization Ms, the remanent magnetization Mr and the coercivity Hc were the main technical parameters to characterize the magnetism of ferromagnetic materials. These nanocomposites were magnetically separated and reused after the completion of reaction. With magnetic separation, it was not required to recover the catalyst using filtration and centrifugation methods. The resulting data showed that the saturation magnetization of $Fe_3O_4$/MWCNTs nanohybrids was lower than $Fe_3O_4$ nanoparticles.[41,42] This was due to the low content of magnetic nanoparticles in the carbon-based nanocomposites, as the MWCNTs exhibited no magnetic characteristics (Figure 2).

## 3.2 | Colorimetric assay of peroxidase-like activity of $Fe_3O_4$/MWCNTs nanozyme

In this study, the peroxidase-like catalytic activity of $Fe_3O_4$/MWCNTs nanocomposites, prepared according to the described method, was investigated using a different process compared with the peroxidase mimetics reported in previous studies. The colorimetric assay of the

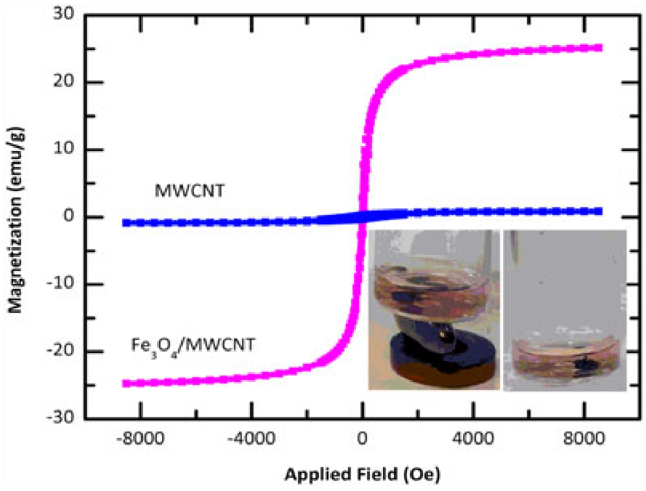


**FIGURE 2** Hysteresis loops of $Fe_3O_4$/MWCNTs nanocomposite

nanocomposites was performed in the presence of 4-AA/phenol and $H_2O_2$ as substrates at room temperature. A solution of $Fe_3O_4$/MWCNTs could catalyze the oxidation of 4-AAP in the presence of $H_2O_2$ to produce a pink-colored quinoneimine dye resulting from oxidation of 4-AAP, which showed its maximum absorbance at 510 nm (Figure 3a).

4-Aminoantipyrine/phenol/$H_2O_2$ reaction without nanocatalysts and $H_2O_2$/nanozyme system without AAP exhibited no catalytic activity (Figure 4). 4-Aminoantipyrine/phenol/nanozyme system showed a weak color variation, but significant color variations were observed in the presence of nanozyme and two substrates. Therefore, the nanocatalyst in the absence of two substrates exhibited no significant absorption peak in the range of 350–700 nm (Figure 4). These data demonstrated that both the substrates were required for reaction progress. Also, colorimetric evaluation was performed with TMB substrate (Figure 3b). TMB is a chromogenic substrate for peroxidase enzymes, and it is used as a hydrogen donor and converts to oxidized TMB product with a blue color solution that can be read at 652 nm. According to obtained data from two reaction systems, no such absorption spectrum was observed from a solution in the absence of the nanocatalysts. When nanocatalysts were added to the solution, the maximum absorption was obtained at 510 and 652 nm as a strong response. Figure 4b shows the catalytic activity of nanozymes at 510 nm in different situations.

The catalytic activity of the reaction system also depends on the amount of nanocatalyst (Figure 5). It was found that the peroxidase-like catalytic activity was increased by increasing the amount of nanocatalyst. Our findings reveal that the prepared $Fe_3O_4$/MWCNTs can be used as a peroxidase mimetic with high activity

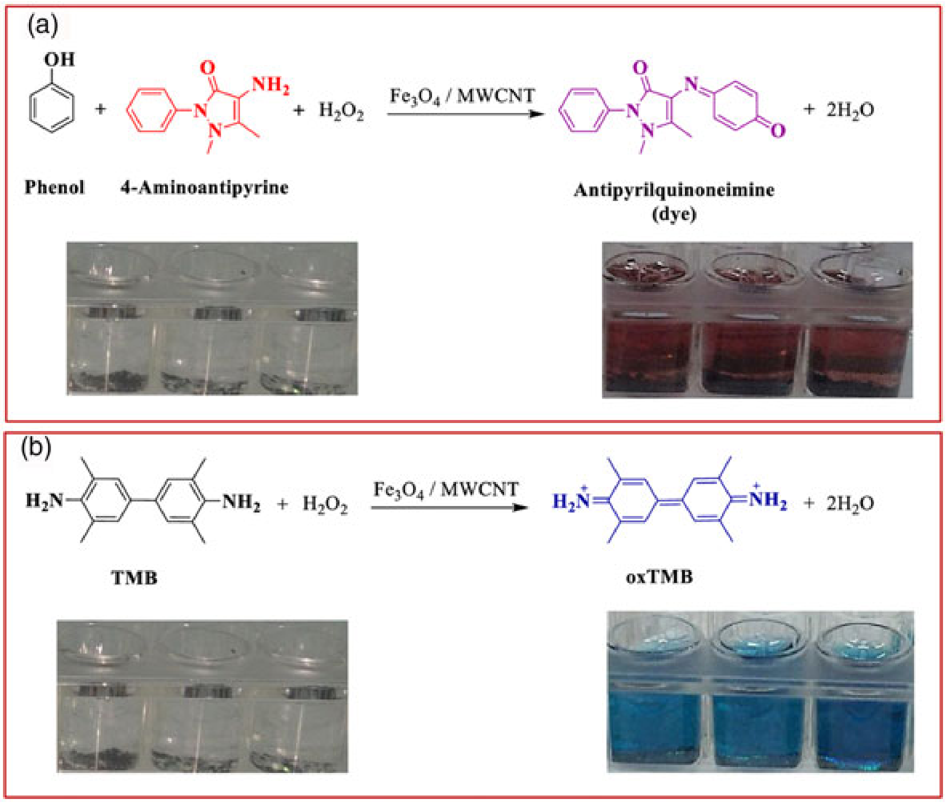


**FIGURE 3** Colorimetric investigations of $H_2O_2$ concentrations with 4-AAP/phenol and TMB substrates using $Fe_3O_4$/MWCNTs nanozyme

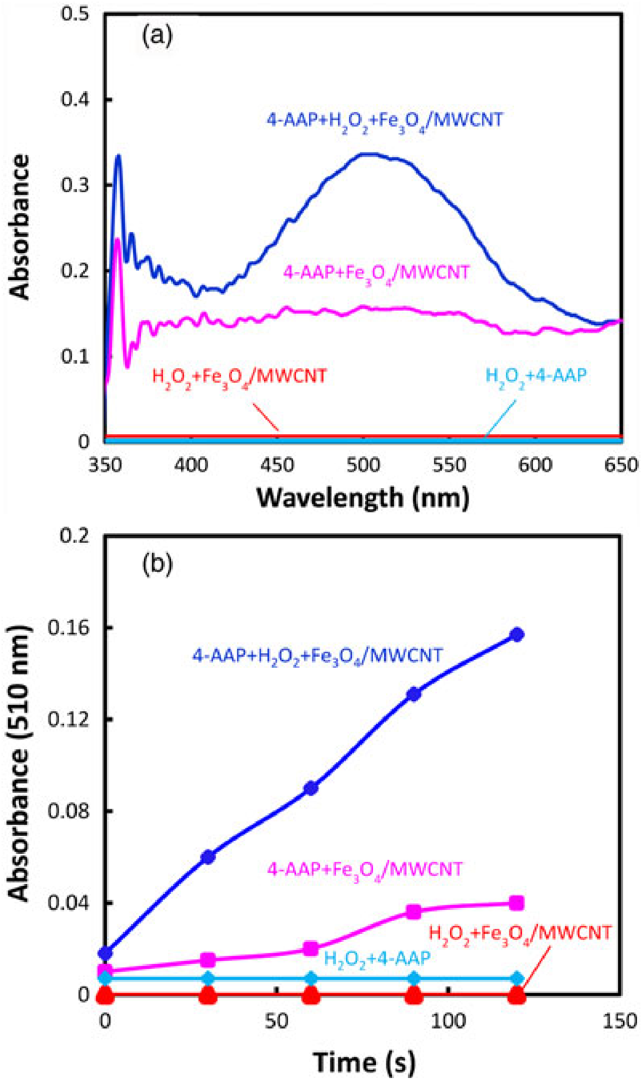


**FIGURE 4** (a) UV–vis absorption spectra of the oxidized 4-AAP/phenol in phosphate buffer for different systems. (b) the relative activity of $Fe_3O_4$/MWCNTs nanocatalyst in different situations

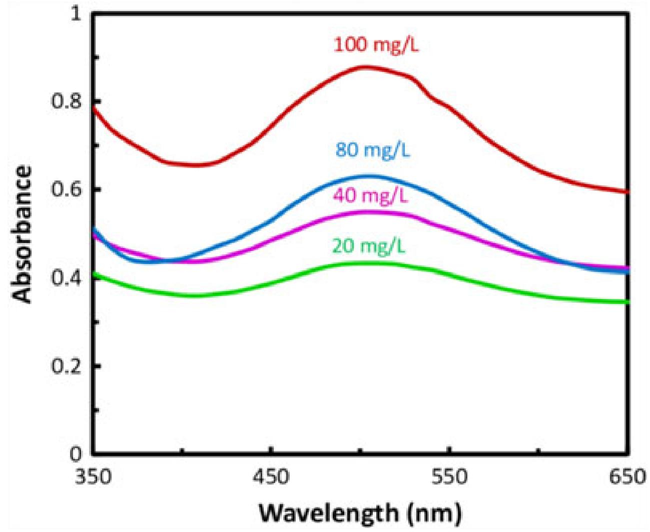


**FIGURE 5** Typical absorption spectra of phenol/4-AAP reaction solutions (90 µl phenol/4-AAP + 90 µl $H_2O_2$) catalytically oxidized by different concentrations of $Fe_3O_4$–MWCNTs nanozyme

compared with similar systems.[2,20] According to the peroxidase-like activity of $Fe_3O_4$ and CNT nanomaterial, as shown in previous studies, the combination of $Fe_3O_4$ and CNT nanostructures results in a peroxidase mimetic with noticeably higher efficiency compared with $Fe_3O_4$ and CNT.

Peroxidase-like activities of $Fe_3O_4$/MWCNTs in the degradation of methylene blue,[17] GOCNT–Pt nanocomposites (platinum nanocatalysts loaded on graphene oxide-carbon nanotubes),[8] $Fe_3O_4$ nanoparticles loaded on GO-dispersed carbon nanotubes,[2] CNTs and $Fe_3O_4$ have been reported using TMB and ABTS as substrates by other researchers. Prepared nanocomposite exhibited higher catalytic activity compared with pure CNTs and $Fe_3O_4$ nanostructures. Compared with GOCNT–Pt and GCNT–$Fe_3O_4$ nanocomposites, our nanocatalysts exhibited lower catalytic activity. Because the catalytic activities of nanocatalysts could be shape- and size-dependent,[2] these properties may be associated with the spread graphene sheet and special surface morphology of nanocomposites. The preparation of $Fe_3O_4$–MWCNTs nanocomposites for the purpose of diarylpyrimidinones synthesis[43] and orange II degradation[9] has previously been done (Figure 6).

Peroxidase mimetics activity of MWCNTs and $Fe_3O_4$/MWCNTs nanocatalysts was compared in the presence of TMB and 4-AAP/phenol substrates. It was found that the peroxidase-like catalytic activity of $Fe_3O_4$/MWCNTs was higher than MWCNTs in both reaction systems. Moreover, both nanostructures exhibited higher catalytic activity in the TMB/$H_2O_2$ reaction mixture compared with the 4-AAP/phenol/$H_2O_2$ system. It can be concluded that the affinity of nanocatalysts to TMB substrate is greater than that of 4-AAP/phenol.

## 3.3 | Kinetic parameters

The kinetic parameters of nanozyme were calculated based on Michaelis–Menten and Lineweaver–Burk plots. The $Fe_3O_4$/MWCNTs nanozyme indicated Michaelis–Menten-type kinetics. The kinetic parameters of $K_m$ and $V_{max}$ were determined to be 8.3 mM and 1.4 mM min$^{-1}$, respectively. In comparison with HRP, which was reported,[44] $Fe_3O_4$/MWCNTs exhibited good peroxidase-like activity. The kinetic parameters of Fe/CNT-based similar nanocatalysts in previous studies for $H_2O_2$ substrate are shown in Table 1. The $K_m$ value of this nanocatalyst is smaller than that one for $Fe_3O_4$/

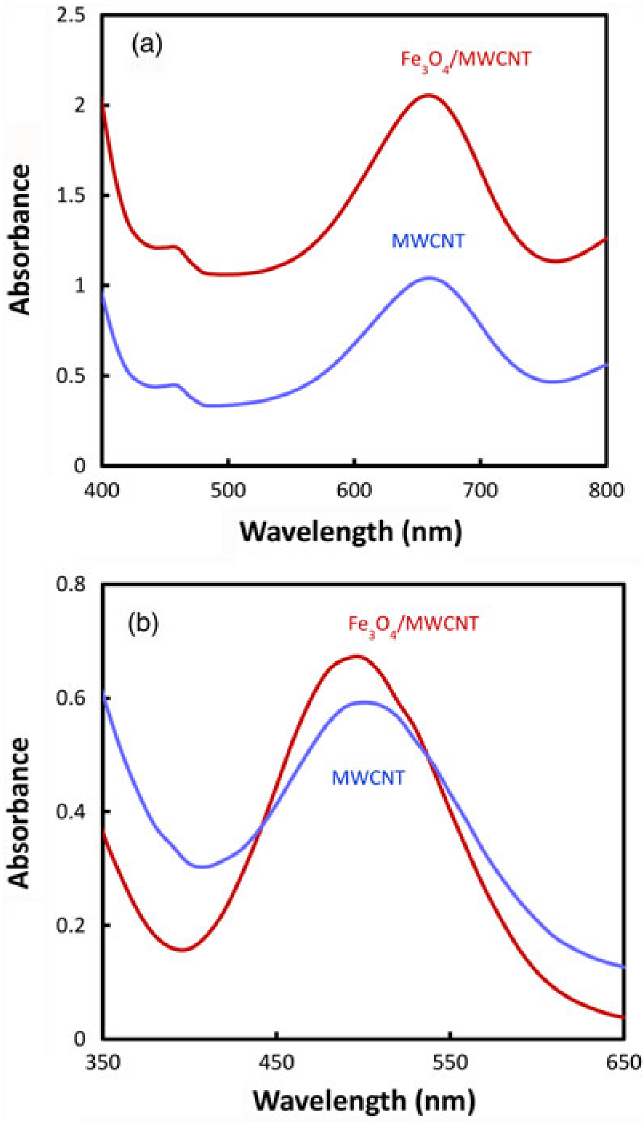


**FIGURE 6** UV–vis absorption spectra of: (a) oxidized 4-AAP in phosphate buffer pH 7; and (b) oxidized TMB in acetate buffer for MWCNT and $Fe_3O_4$/MWCNTs

MWCNTs, which suggests that the $Fe_3O_4$/MWCNTs have a lower affinity for $H_2O_2$ than the other mentioned nanocatalysts according to assay conditions. Colorimetric assay and $V_{max}$ value showed that the catalytic rate of $Fe_3O_4$/MWCNTs to 4-AAP oxidation was much greater than the reported nanocatalysts.

The kinetic parameters were measured for the various concentrations of $H_2O_2$ with a constant concentration of 4-AAP/phenol and $Fe_3O_4$/MWCNTs nanozyme. In another study, the kinetics assay was performed in the presence of TMB substrate and kinetic parameters were obtained. In this experiment, it was shown that the nanozyme catalytic activity had a direct relation with the concentrations of TMB and $H_2O_2$ (the data are not shown; Figure 7).

## 3.4 | Effect of ionic liquids in catalytic activity of nanozyme

Ionic liquids have good properties, such as catalytic activity, high thermal stability and ionic conductivity. Ionic liquids have been used in catalytic reactions as solvents, catalysts and catalyst supports. Recently, many researchers have been interested in magnetic nanohybrids, especially CNT–ionic liquid nanostructures, for magnetic separation processes.[45] In this research, the effect of butyl-imidazolium bromide ionic liquid on the catalytic activity of $Fe_3O_4$/MWCNTs was investigated. As shown in Figure 10, the absorbance of $Fe_3O_4$/MWCNTs

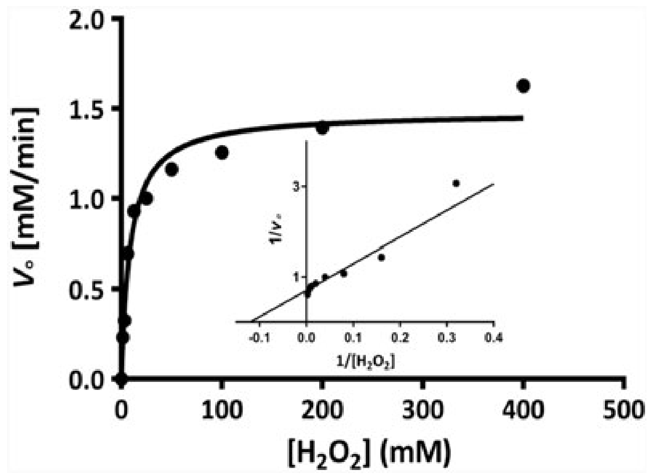


**FIGURE 7** Michaelis–Menten curve for $Fe_3O_4$/MWCNTs nanozyme. The experiment was performed in phosphate-buffered saline (0.2 M, pH 7.0) at a fixed concentration of nanozyme, 4-AAP/phenol and different concentrations of $H_2O_2$. Inset: Lineweaver–Burk plot for $Fe_3O_4$/MWCNTs

**TABLE 1** Kinetic parameters of $Fe_3O_4$/MWCNT nanozyme for $H_2O_2$ substrate in comparison with HRP and other Fe/CNT-based similar peroxidase mimetics

| Catalyst | $V_{max}$ (m s$^{-1}$) | $K_m$ (mM) | Temperature | Ref. |
|---|---|---|---|---|
| $Fe_3O_4$/MWCNT | $2.3 \times 10^{-5}$ | 8.3 | Room temperature | Present study |
| GCNT-$Fe_3O_4$ | $0.387 \times 10^{-8}$ | 2.52 | Room temperature | 2 |
| Hemin–SWCNT | $4.79 \times 10^{-8}$ | 0.08 | 37 °C | 22 |
| HRP | $8.7 \times 10^{-8}$ | 3.7 | — | 2 |

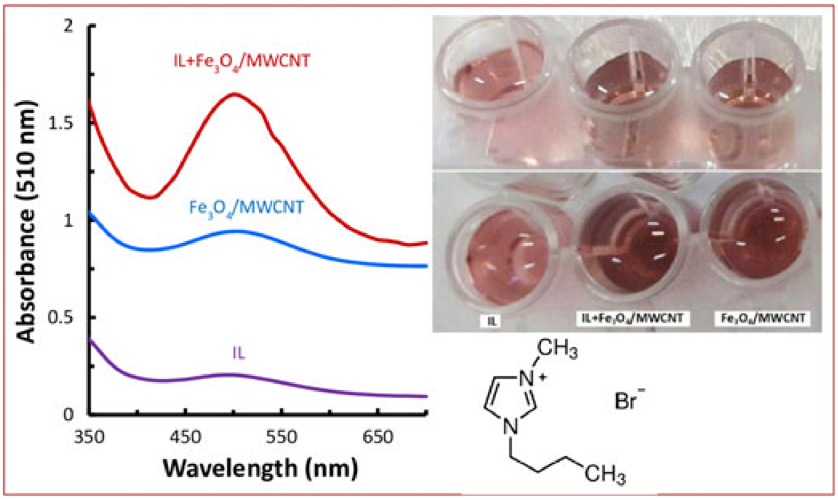


**FIGURE 8** The effect of imidazolium-based ionic liquid on $Fe_3O_4$/MWCNTs nanozyme

nanozyme increased in the presence of ionic liquid. According to our data and previous studies, for a synergistic effect and high efficiency of nanocatalysts, it can be proposed that the ionic liquids are immobilized on magnetic nanostructures. Also, ionic liquids can improve electrochemical responses due to their ionic conductivity,[45] therefore it is expected that the deposition of ionic liquids and nanostructures on the electrode surface can play an effective role in the design of glucose sensors. In this study, for the first time, the combination of ionic liquids and nanomaterials as peroxidase mimetics was examined (Figure 8).

## 3.5 | Effect of pH on nanozyme activity

To determine the optimum pH for the $Fe_3O_4$/MWCNTs assay method, peroxidase-like catalytic activity was investigated in the pH range of 4.0–9.0, and the nanozyme activity was measured according to the mentioned procedure. As shown in Figure 9, there is not a considerable difference in peroxidase activity rate at the different pH values. Compared with synthesized $Fe_3O_4$/MWCNTs nanocatalysts in reported studies, this nanozyme exhibited catalytic activity over a wide pH range. Similar results were obtained from Wang *et al.*[17] Because GOX is more stable at pH 7–8, it is suggested that this nanozyme can be a suitable peroxidase mimetics for designing non-enzymatic sensors for glucose detection.

## 3.6 | Detection of glucose

$H_2O_2$ is the main product of the GOX-catalyzed reaction; therefore, the nanocatalysts with peroxidase-like activity were also applied in the colorimetric and quantitative detection of glucose with final concentrations of 1.25, 2.5, 5, 10 and 20 mM under standard assay conditions (Figure 10). According to Equation 2, at a wavelength of

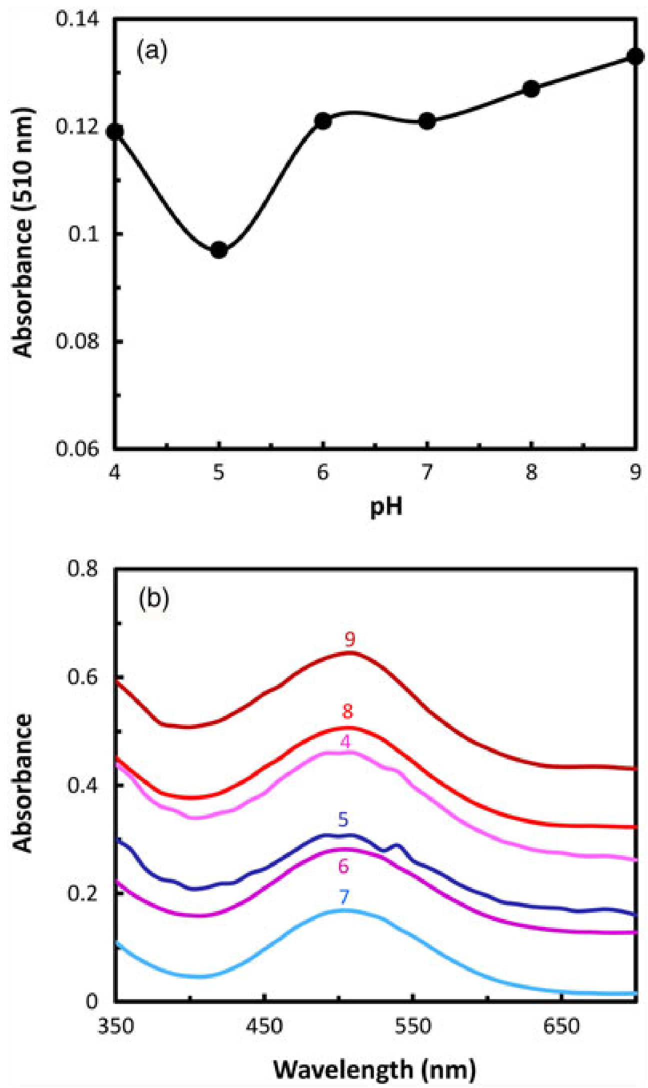


**FIGURE 9** The effect of pH on activity of $Fe_3O_4$/MWCNTs nanozyme in phosphate solution with 4-AAP substrate

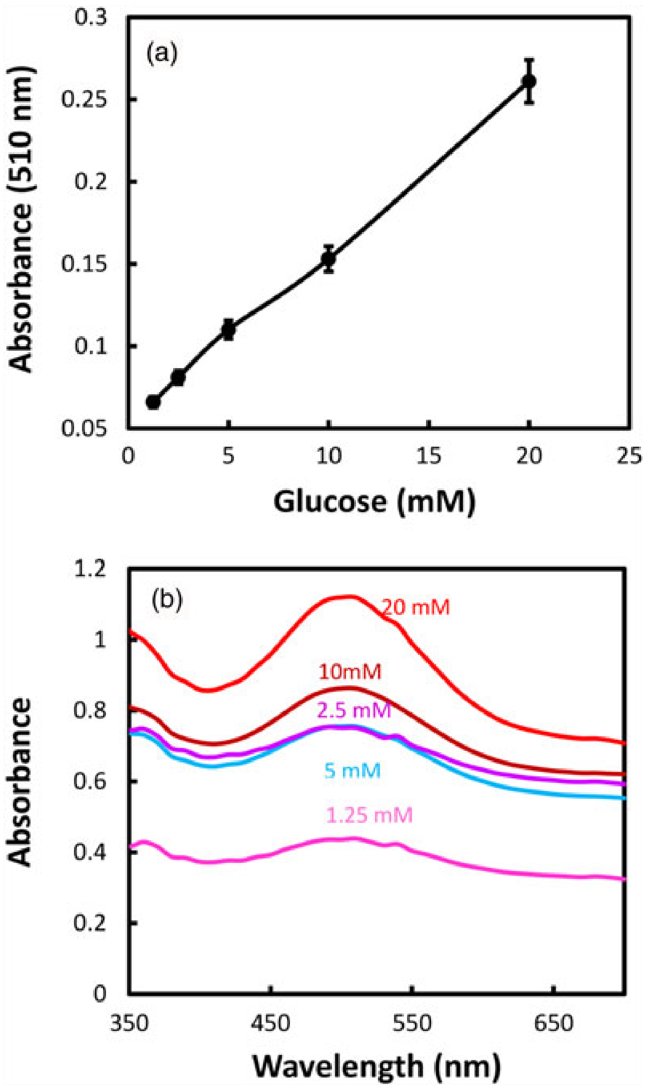


**FIGURE 10** (a) absorption spectrum of glucose detection by $Fe_3O_4$/MWCNTs nanocatalyst in the presence of glucose at various concentrations (1.25, 2.5, 5, 10 and 20 mM). (b) the absorbtion intensity at 510 nm against different concentrations of glucose

510 nm, the absorbance has a direct relation with the glucose concentration.

$$\text{Glucose} + O_2 \xrightarrow{\text{FAD-GOX}} H_2O_2 + \delta\text{-D-gluconolactone} \quad (1)$$

$$\text{Phenol} + \text{4-AAP} + 2H_2O_2 \xrightarrow{Fe_3O_4/\text{MWCNT}} \text{quinoneimine dye} + 4H_2O \quad (2)$$

According to Figure 10a and b, the absorbance increased by increasing the glucose concentration. A proportional change of color densities for 4-AAP chromogens was obtained for $H_2O_2$ and glucose.

## 4 | CONCLUSIONS

In summary, *in situ* assembly of $Fe_3O_4$ on MWCNTs nanostructures was performed by chemical reduction with hydrazine as peroxidase mimetics. This nanozyme exhibited higher peroxidase mimic behavior than MWCNTs. Colorimetric assays and kinetic parameters revealed that peroxidase mimetic activity of prepared nanocatalysts to TMB and 4-AAP oxidation was very high in comparison with other reported systems. The synergistic effect of $Fe_3O_4$ and MWCNTs enhances the enzyme-like activity of nanostructure. On the other hand, CNT supports the improvement of dispersivity of $Fe_3O_4$ nanoparticles and decreases agglomeration. These magnetic enzyme mimics exhibit some advantages over natural protein enzymes due to their characteristics, such as magnetic separation and reusability, multi-functionality, and direct electrochemistry to substrates.

The nanozyme showed a good sensitivity for glucose detection. Also, in the TMB/nanozyme colorimetric assay system, without $H_2O_2$, TMB oxidation was observed. We further explored the investigation and application of magnetic nanostructures in biocatalysis and biotechnology. This nanocatalyst with $H_2O_2$-induced oxidation can play an important role in reducing environmental pollution. It is suggested that this nanozyme with high catalytic activity can catalyze other organic substrates such as aromatic compounds even in the absence of $H_2O_2$. Also, the combination of nanocatalysts with ionic liquids and deep eutectic solvents can be applied in the design of new peroxidase mimetic systems.

## ACKNOWLEDGEMENTS

Financial support provided by the Research Council of the Malek-Ashtar University of Technology is gratefully appreciated. The authors would like to thank Dr Hassan Hosseini from Nanoscience Center of Imam Hossein University for the helpful comments.

## ORCID

*Navvabeh Salarizadeh* http://orcid.org/0000-0002-8243-3832
*Minoo Sadri* http://orcid.org/0000-0001-8540-7464

## REFERENCES

[1] K. Zhao, W. Gu, S. Zheng, C. Zhang, Y. Xian, *Talanta* **2015**, *141*, 47.

[2] H. Wang, S. Li, Y. Si, Z. Sun, S. Lia, Y. Lin, *J. Mater. Chem. B* **2014**, *2*, 4442.

[3] J. Wei, X. Chen, S. Shi, S. Mo, N. Zheng, *Nanoscale* **2015**, *7*, 19018.

[4] H. Wei, E. Wang, *Chem. Soc. Rev.* **2013**, *42*, 6060.

[5] E. Torres, M. Ayala, *Biocatalysis Based on Heme Peroxidases: Peroxidases as Potential Industrial Biocatalysts*, 6th ed., Springer, Berlin **2010**.

[6] A. Sukan, S. Sargin, *J. Biomater. Nanobiotechnol.* **2013**, *4*, 300.

[7] S. Zhu, X. E. Zhao, J. You, G. Xu, H. Wang, *Analyst* **2015**, *140*, 6398.

[8] H. Wang, Y. Lin, S. Li, Y. Si, N. Zhang, Z. Sun, H. Wu, *Nanoscale* **2014**, *6*, 8107.

[9] J. Deng, X. Wen, J. Li, *Environ. Technol.* **2016**, *4*, 1.

[10] J. Shu, Z. Qiu, Q. Wei, J. Zhuang, D. Tang, *Sci. Rep.* **2015**, 5.

[11] B. Li, D. Chen, J. Wang, Z. Yan, L. Jiang, D. Duan, J. He, Z. Luo, J. Zhang, F. Yuan, *Sci. Rep.* **2014**, *24*, 4.

[12] G. L. Wang, L. Y. Jin, Y. M. Dong, X. M. Wu, Z. J. Li, *Biosens. Bioelectron.* **2015**, *64*, 523.

[13] V. Kohler, N. J. Turner, *Chem. Commun.* **2015**, *51*, 450.

[14] Y. Lin, Z. Li, Z. Chen, J. Ren, X. Qu, *Biomaterials* **2013**, *34*, 2600.

[15] Z. Yang, Y. Cao, J. Li, M. Lu, Z. Jiang, X. Hu, *ACS Appl. Mater. Interfaces* **2016**, *8*, 12031.

[16] J. Yang, L. Zheng, Y. Wang, W. Li, J. Zhang, J. Gu, Y. Fu, *Biosens. Bioelectron.* **2016**, *77*, 549.

[17] H. Wang, H. Jiang, S. Wang, W. Shi, J. He, H. Liu, Y. Huang, *RSC Adv.* **2014**, *4*, 45809.

[18] H. A. Kermani, A. Shockravi, Z. Moosavi-Movahedi, A. Khalafi-Nezhad, S. Behrouz, G. Y. Tsai, G. H. Hakimelahi, A. Seyedarabi, A. A. Moosavi-Movahedi, *J. Iran. Chem. Soc.* **2013**, *10*, 961.

[19] Z. Moosavi-Movahedi, H. Gharibi, H. Hadi-Alijanvand, M. Akbarzadeh, M. Esmaili, M. S. Atri, Y. Sefidbakht, M. Bohlooli, K. Nazari, S. Javadian, J. Hong, *J. Biomol. Struct. Dyn.* **2015**, *10*, 1.

[20] X. Wang, Y. Hu, H. Wei, *Inorg. Chem. Front.* **2016**, *3*, 41.

[21] J. Kosman, B. Juskowiak, *Anal. Chim. Acta.* **2011**, *707*, 7.

[22] Y. Zhang, C. Xu, B. Li, *RSC Adv.* **2013**, *3*, 6044.

[23] L. Luo, Y. Zhang, F. Li, X. Si, Y. Ding, D. Deng, T. Wang, *Anal. Chim. Acta* **2013**, *788*, 46.

[24] L. Li, C. Zeng, L. Ai, J. Jiang, *J. Alloys Compd.* **2015**, *639*, 470.

[25] T. Liu, K. Zhao, L. Jin, J. Zhu, Y. Dong, Y. Yan, P. Wang, D. He, *Gen. Chem.* **2016**, *2*, 44.

[26] Y. Shi, J. Huang, J. Wang, P. Su, Y. Yang, *Talanta* **2015**, *143*, 457.

[27] N. A. Zubir, C. Yacou, J. Motuzas, X. Zhang, J. C. da Costa, *Sci. Rep.* **2014**, *4*, 4594.

[28] M. Zhu, G. Diao, *Nanoscale* **2011**, *3*, 2748.

[29] L. Lin, X. Song, Y. Chen, M. Rong, T. Zhao, Y. Wang, Y. Jiang, X. Chen, *Anal. Chim. Acta* **2015**, *869*, 89.

[30] W. J. Stark, P. R. Stoessel, W. Wohlleben, A. Hafner, *Chem. Soc. Rev.* **2015**, *44*, 5793.

[31] X. Zuo, C. Peng, Q. Huang, S. Song, L. Wang, D. Li, C. Fan, *Nano Res.* **2009**, *2*, 617.

[32] R. Cui, Z. Han, J. J. Zhu, *Chem. A Eur. J.* **2011**, *17*, 9377.

[33] G. L. Turdean, I. C. Popescu, A. Curulli, G. Palleschi, *Electrochim. Acta* **2006**, *51*, 6435.

[34] M. Liang, K. Fan, Y. Pan, H. Jiang, F. Wang, D. Yang, D. Lu, J. Feng, J. Zhao, L. Yang, X. Yan, *Anal. Chem.* **2012**, *85*, 308.

[35] J. W. Lee, H. J. Jeon, H. J. Shin, J. K. Kang, *Chem. Commun.* **2012**, *48*, 422.

[36] Y. Song, X. Wang, C. Zhao, K. Qu, J. Ren, X. Qu, *Chem. A Eur. J.* **2010**, *16*, 3617.

[37] C. Singh, S. Bansal, V. Kumar, S. Singhal, *Ceram. Int.* **2015**, *41*, 3595.

[38] V. Vojinović, A. M. Azevedo, V. C. Martins, J. M. Cabral, T. D. Gibson, L. P. Fonseca, *J. Mol. Catal. B: Enzym.* **2004**, *28*, 129.

[39] J. L. Carter, C. C. Santini, L. J. Blum, B. Doumèche, *RSC Adv.* **2015**, *5*, 23715.

[40] S. N. Azizi, M. J. Chaichi, P. Shakeri, A. Bekhradnia, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2015**, *146*, 277.

[41] Z. Xu, L. Ding, Y. Long, L. Xu, L. Wang, C. Xu, *Anal. Methods* **2011**, *3*, 1737.

[42] P. Zhang, Z. Mo, Y. Wang, L. Han, C. Zhang, G. Zhao, Z. Li, *RSC Adv.* **2016**, *6*, 39348.

[43] J. Safari, S. Gandomi-Ravandi, *RSC Adv.* **2014**, *4*, 11486.

[44] A. Molaei Rad, H. Ghourchian, A. A. Moosavi-Movahedi, J. Hong, K. Nazari, *Anal. Biochem.* **2007**, *362*, 38.

[45] Z. Zarnegar, J. Safari, *New J. Chem.* **2016**, *40*, 7986.


**How to cite this article:** Salarizadeh N, Sadri M, Sajedi RH. Synthesis and catalytic evaluation of $Fe_3O_4$/MWCNTs nanozyme as recyclable peroxidase mimetics: Biochemical and physicochemical characterization. *Appl Organometal Chem*. 2017;e4018. https://doi.org/10.1002/aoc.4018