

Synthesis and *physicochemical* characterization of $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanostructures as enzyme mimetics with peroxidase-like catalytic activity

Navvabeh Salarizadeh¹, Minoos Sadri^{1,*}, Hassan Hosseini² and Reza. H. Sajedi³

¹Department of Biochemistry and Biophysics, Education and Research Center of Science and Biotechnology, Malek Ashtar University of Technology, Tehran 16765-3454, Iran

²Department of Chemistry, Faculty of Sciences, Imam Hossein University, Tehran 16575-347, Iran

³Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran 14115-175, Iran

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*Corresponding Author

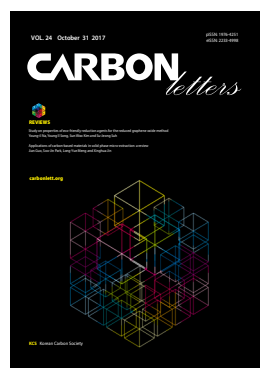
E-mail: sadri.mn313@gmail.com

Tel: +98-21-22974619

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Abstract

Carbon-based magnetic nanostructures in several instances have resulted in improved physicochemical and catalytic properties when compared to multi-wall carbon nanotubes (MWCNTs) and magnetic nanoparticles. In this study, magnetic MWCNTs with a structure of $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ as peroxidase mimics were fabricated by the one-pot hydrothermal method. The structure, composition and morphology of the nanocomposites were characterized with X-ray diffraction (XRD), Fourier transform infrared spectroscopy and transmission electron microscopy. The magnetic properties were investigated with a vibrating sample magnetometer. The peroxidase-like catalytic activity of the nanocomposites was investigated by colorimetric and electrochemical tests with 3,3',5,5'-tetramethylbenzidine (TMB) and H_2O_2 as the substrates. The results show that the synthesis of the nanocomposites was successfully performed. XRD analysis confirmed the crystalline structures of the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanohybrids and MWCNTs. The main peaks of the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ s crystals were presented. The $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$ and $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanocatalysts showed nearly similar physicochemical properties, but the $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanocatalyst was more appropriate than the $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanocatalyst in terms of the magnetic properties and catalytic activity. The optimum peroxidase-like activity of the nanocatalysts was obtained at pH 3.0. The $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanocatalyst exhibited a good peroxidase-like activity. These magnetic nanocatalysts can be suitable candidates for future enzyme-based applications such as the detection of glucose and H_2O_2 .

Key words: magnetic carbon nanotubes, peroxidase-like activity, characterization, biometric catalysts

1. Introduction

Natural enzymes have broad biotechnological applications and are used for various purposes in the pharmaceutical, chemical, food process, and agriculture industry as well as in the design of biosensors [1-4]. Despite the importance of enzymes, due to their sensitivity against environmental changes and low stability and high costs for purification and storage, their application economically is not cost-effective [1-5]. Therefore, the design and synthesis of catalysts as enzyme mimetics have been developed which could be applied to various potential fields [1,6]. These alternative catalysts must have activities like natural enzymes. Peroxidase mimetics, specially nano-based compositions, have been recently studied by many researchers [3,7-19]. Peroxidases are involved in the oxidation of various substrates [20]. These enzymes have broad applications in biotechnological fields including environment bioremediation, immunoassays, detection of bio-

molecules, industrial catalysts and so on [21]. DNAzymes, nanozymes and complexes of organic nanostructures such as heme, porphyrin, and cyclodextrins as peroxidase mimetics have been studied [2,9,17,22-24]. Compared with natural enzymes, nanocatalysts possess advantages such as thermal stability, low-cost, easy production and high tolerance in acidic and alkaline environments [25,26]. Moreover, the range of reactions catalyzed by artificial enzymes is larger than that of their natural counterparts [27]. Among these, metal nanoparticles have been considered by researchers due to their low toxicity, small size and high enzyme activity [28]. Carbon-based nanostructures such as carbon nanotubes (CNTs), graphene, fullerene, carbon nitride sheets, activated carbon and amorphous carbon due to their unique structural and chemical properties have been deemed important [29,30]. Recently, CNTs have been extensively considered by researchers due to their exceptional mechanical, thermal, and electrical properties [31]. The combination of metal oxides with other nanomaterials has been a remarkable subject. These combination nanostructures have many applications in electronic and magnetic instruments, catalysis, diagnosis, therapeutics and so on because they have the properties of both substances. Among these, the combination of CNTs with metal oxide nanoparticles, especially magnetic nanoparticles, have attracted much attention due to their unique properties as electrical and chemical conductors as well as the mechanical and structural properties of the CNTs and the magnetic properties and chemical stability of the metal oxide nanoparticles [13,14,32,33]. CNTs have been used in imaging techniques such as Raman spectroscopy, near-infrared fluorescence and ultrasonography [13]. The high ratio of surface area to volume in nanotubes is an important factor in the design of Nano complexes. These carbon-based nanocomposites have been used in various application such as drug delivery, biosensing, catalysis, fuel cells, decontamination and capacitors [2,8,15]. Preparation of Fe_3O_4 , multi-wall carbon nanotube (MWCNT) nanocomposites for purposes such as the synthesis of diaryl pyrimidinones [34] and the degradation of Orange II [22] has been performed previously.

The peroxidase-like catalytic activity of helical CNTs and carbon-based nanostructures has been shown previously [2,14-17]. It has been found that magnetic nanostructures such as Fe_3O_4 , Fe_2O_3 , Fe_2O_4 , CoFe_2O_4 , ZnFe_2O_4 , MnFe_2O_4 , and $\text{Co}_x\text{Ni}_{1-x}\text{Fe}_2\text{O}_4$ possess an activity for the oxidation of H_2O_2 [9,18]. Synthesis of nanoferrites of MFe_2O_4 ($\text{M} = \text{Mn}, \text{Co}, \text{Zn}$) and $\text{Ni}_{1-x}\text{M}_x\text{Fe}_2\text{O}_4$ with various metal substitutions leads to different catalytic and magnetic properties. In 2012, Su et al. [35] reported that ZnFe_2O_4 magnetic nanoparticles can be used for glucose detection. Moreover, the peroxidase-like activity of the $\text{ZnFe}_2\text{O}_4/\text{MWCNT}$ nanocomposite in immunoassays was investigated [36]. Preparation and characterization of ferrite nanoparticles in combination with CNTs was carried out. It has been reported that $\text{NiZnFe}_2\text{O}_4/\text{CNT}$ and $\text{NiCoFe}_2\text{O}_4/\text{CNT}$ complexes have more paramagnetic properties than that of single nanoferrites [19]. Synthesis of $\text{NiFe}_2\text{O}_4/\text{MWCNT}$ for sensor applications was reported by Ensafi et al. [37,38]. According to reported studies, it is expected that the combination of these nanoferrites with CNTs will possess an oxidase-like activity; however, the application of $\text{NiZnFe}_2\text{O}_4/\text{MWCNT}$ nanostructures in enzyme mimics has not

been reported yet.

In this study, highly dispersed carbon-based nanostructures, $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$ and $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$, were synthesized and characterized. For the first time, we report the peroxidase-like activities of nanocatalysts through the oxidation of the peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB), in the presence of H_2O_2 . Earth abundant element-based nanoparticles are low cost materials for the design of novel enzyme mimics. Due to the disadvantages of natural peroxidases, there is great interest in the current research on artificial peroxidase mimics. Moreover, by combining peroxidase-like mimics with glucose oxidase, a simple and sensitive colorimetric assay for the detection of glucose concentrations was developed.

2. Experimental

2.1. Materials and methods

MWCNTs with a diameter of 5–20 nm and a length of 6–15 μm with a 97% purity were obtained from the Iran Research Institute of Petroleum Industry (Iran). H_2O_2 , ammonium iron (II) sulfate and hydrazine hydrate were purchased from Sigma-Aldrich (USA). $\text{Ni}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, TMB, dimethyl sulfoxide (DMSO), acetic acid, sodium acetate, KH_2PO_4 and Na_2HPO_4 were obtained from Merck (Germany).

2.2. Synthesis of the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanocomposites

In a typical synthesis, MWCNTs were modified with a mixture of nitric acid/sulfuric acid as oxidative reagents at 150°C for 2 h. Then, the MWCNTs were washed, filtered and sonicated for 15 min. For the synthesis of the $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$, modified MWCNTs were mixed with solutions of 1 M $\text{Ni}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$ and $\text{Fe}(\text{NO}_3)_3$ at a molar ratio of 0.5:0.5:2 and then gently mixed followed by the slow addition of 1 M NaOH. The mixture was stirred for 1 h in the alkaline medium. Then, this precursor was placed in a Teflon-lined autoclave at 300°C for 3 h. The final product was washed with deionized water and dried in an oven at 100°C for 12 h. $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanostructures were fabricated with solutions of 1 M $\text{Ni}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$ and $\text{Fe}(\text{NO}_3)_3$ at a molar ratio of 0.25:0.25:2 using a similar procedure as described above.

2.3. Characterization of the nanocomposites

The morphology of the modified MWCNTs and nanocomposites were investigated by transmission electron microscopy (TEM; HT-7700, Hitachi High-Tech, Japan). X-ray diffraction (XRD; Philips PW1730, with $\text{Cu K}\alpha$ radiation [$\lambda = 1.540598 \text{ \AA}$]) was used to determine the nanomaterial structures. Fourier transform infrared spectrometer (FTIR; Bruker Equinox, USA) was applied to study the changes in the chemical structure. The magnetic properties of the ferrite/CNT nanocomposites were investigated with a vibrating sample magnetometer (VSM; BHV-55, Riken, Japan).

2.4. Colorimetric assay of the peroxidase-like activity of the nanocomposites

To investigate the peroxidase-like catalytic activity, a colorimetric assay was performed for the nanocomposites in the presence of TMB and H₂O₂ as the substrates at room temperature. The catalytic assay was performed using a 5 mM TMB solution, 2 mg mL⁻¹ nanomaterials and 200 mM H₂O₂ in acetate buffer pH 3.0. The color of the solution turned to blue with a characteristic absorbance peak at 652 nm. Control experiments were also done under the same conditions to compare the relative catalytic activity of the nanocatalysts.

The effect of pH on the catalytic reaction was evaluated in a pH range of 3–12 with the standard assay conditions mentioned above, and the optimized pH for the nanocatalyst activity in the TMB colorimetric system was then tested. Additionally, the dependence of the peroxidase-like activity was tested in various concentrations of H₂O₂.

2.5. Electrochemical analysis

Electrochemical detection of H₂O₂ was measured with a Ni_xZn_xFe₂O₄/MWCNT-coated electrode in an electrochemical working station (μ AutolabIII electrochemical analyzer). Three electrodes consisting of a glassy carbon (GC) working electrode, an Ag/AgCl reference electrode, and a platinum counter electrode were used. The GC electrode was polished with alumina and modified with the nanocatalysts in the presence and absence of TMB. Nanocatalysts were dissolved in PBS buffer pH 7.0 and dispersed with a bath sonicator, and then, 2 μ L from the solution was coated onto the GC electrode. This step was repeated three times, and after each coating phase, electrode was dried. Then, electrochemical voltammetry measurements were performed in a solution of H₂O₂ and TMB with final concentrations of 10 and 2 mM in a scanning range of -0.6 to +0.4 V at a scan rate of 0.10 V s⁻¹.

3. Results and Discussion

3.1. Characterization of the Ni_xZn_xFe₂O₄/MWCNT nanocomposites

The magnetic nanotubes were prepared with the hydrothermal method at 300°C for 3 h. The crystalline structures of the resulting Ni_xZn_xFe₂O₄/MWCNT nanocomposites and MWCNTs were confirmed with powder XRD measurements, and the main peaks of the Ni_xZn_xFe₂O₄/MWCNT crystals were clearly presented. The peaks at 2 θ =35.66° and 62.69° indexed to the (311) and (531) crystalline planes are the major peaks of Ni_{0.25}Zn_{0.25}Fe₂O₄. These data are similar to the reported ones by Cao et al. [39]. Moreover, the diffraction peaks at 2 θ =18.56°, 30.35°, 43.24°, 54.10°, 57.17° and 74.05° correspond to Ni_{0.25}Zn_{0.25}Fe₂O₄. The sharp diffraction peaks at 2 θ =35.74°, 62.61° and the minor peaks at 2 θ =18.56°, 30.31°, 33.35°, 43.24°, 54.10°, 57.03° and 74.18° are associated with Ni_{0.5}Zn_{0.5}Fe₂O₄ (Fig. 1). In the XRD pattern of Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT, the absence of a peak at 25–30°, which is related to the MWCNTs, indicates that the MWCNT

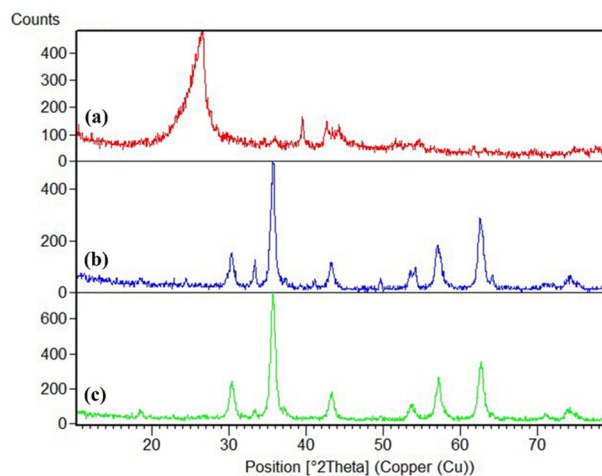


Fig. 1. X-ray diffraction patterns of the MWCNT (a), Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT (b), and Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT (c) nanohybrids. MWCNT, multi-wall carbon nanotube.

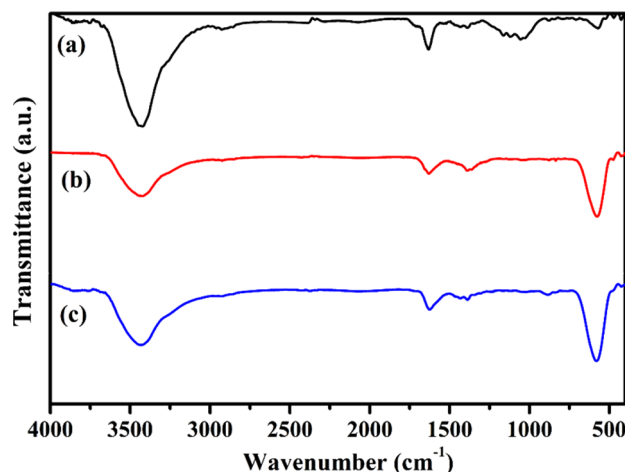


Fig. 2. FT-IR spectra of the MWCNT (a), Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT (b), and Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT (c) nanohybrids. FT-IR, Fourier transform infrared spectroscopy; MWCNT, multi-wall carbon nanotube.

structure is thoroughly changed by the hydrothermal process. Additionally, no diffraction peaks due to any other new phases are observed. In the XRD spectrum of Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT, the peak at 2 θ =33.3° and 49.62° might correspond to γ -Fe₂O₃ in the sample or FeNi₃ which may be due to the Ni and Fe released from the nanoferrites [38,39]. The average crystallite size of the Ni_{0.5}Zn_{0.5}Fe₂O₄ and Ni_{0.25}Zn_{0.25}Fe₂O₄ nanoferrites calculated by the Debye-Scherrer equation (Eq. 1) is about 17 and 25 nm, respectively.

$$d = \frac{k\lambda}{\beta \cos \theta} \quad (1)$$

Here, d is the crystallite size; β is the full width at half maximum of the most intense peak, and k is an instrumental constant.

FTIR spectroscopies of the Ni_xZn_xFe₂O₄/MWCNT nanohybrids and MWCNT for the range of 400–4000 cm⁻¹ were also

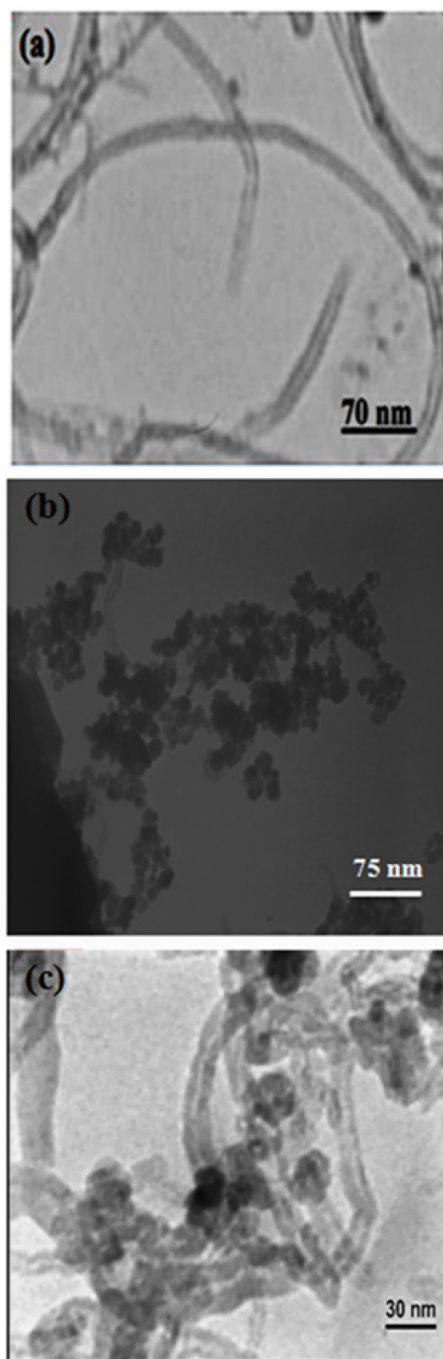


Fig. 3. Transmission electron microscopy images of MWCNT (a), $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$ (b), and $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$ (c). MWCNT, multi-wall carbon nanotube.

recorded to confirm structural changes (Fig. 2). The position of bands at 580.23 cm^{-1} can be attributed to the Fe-O stretching modes (the tetrahedral clusters) due to the interaction between the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4$ nanoparticles and the MWCNTs, indicating the presence of $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4$ in the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ s. The bands at 3433 and 1626 cm^{-1} are attributed to the stretching vibration and bending vibration of the OH groups of the absorbed

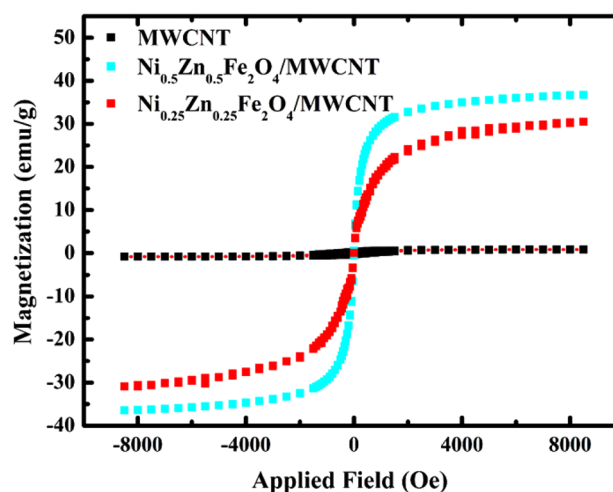


Fig. 4. Hysteresis loops of the $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$, $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$ and MWCNT nanostructures. MWCNT, multi-wall carbon nanotube.

Table 1. Vibrating sample magnetometer analysis of the synthesized nanocomposites

Nanocatalyst	M_s ($\text{A} \cdot \text{m}^2/\text{kg}$)	M_r ($\text{A} \cdot \text{m}^2/\text{kg}$)	H_c (A/M)
$\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4/\text{MWCNT}$	36.751	0.516	-392.32
$\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4/\text{MWCNT}$	30.51	0.01	-35.81

MWCNT, multiwall carbon nanotubes. M_s , saturation magnetization; M_r , remanent magnetization; H_c , coercivity.

water (Fig. 2b and c). These results are in agreement with data that have been reported by other researchers [36,39,40]. It was observed that the bands at 1620 and 3424 cm^{-1} are assigned to the stretching bands of C=O and -OH in the functional groups of the MWCNT (Fig. 2a).

The morphology of the resulting nanostructures was investigated with TEM (Fig. 3). It was seen that the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4$ nanoferrites are attached and distributed on the surface of the MWCNTs. The size of the $\text{Ni}_{0.25}\text{Zn}_{0.25}\text{Fe}_2\text{O}_4$ and $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ nanoferrites was estimated as $10\text{--}25$ and $5\text{--}15\text{ nm}$, respectively. The size of the nanoferrites is approximately consistent with the XRD results.

The magnetic properties of the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4$ -coated MWCNTs were measured in fields of $\pm 795.77\text{ A/M}$ using a vibrating sample magnetometer at room temperature. The hysteresis loops of the decorated MWCNTs are presented in Fig. 4. The saturation magnetization, the remanent magnetization, and the coercivity are the main technical parameters to characterize the magnetism of the prepared nanomaterials (Table 1). These nanocomposites were magnetically separated and reused after the completion of the reaction. With the magnetic separation, it is not required to recover the catalyst by filtration and centrifugation methods. The data showed that the saturation magnetization of the $\text{Ni}_x\text{Zn}_x\text{Fe}_2\text{O}_4/\text{MWCNT}$ nanocomposites were higher than that of the $\text{Fe}_3\text{O}_4/\text{MWCNT}$ nanostructures [36,41]. The obtained data proved

that the magnetic properties of the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT nanocomposite were better than that of the Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT. The increased saturation magnetization could be a result of the increased amounts of Ni and Zn.

3.2. Colorimetric assay of the peroxidase-like activity of the Ni_xZn_xFe₂O₄/MWCNT nanocomposites

In this study, for the first time, we report that Ni_xZn_xFe₂O₄/MWCNT nanocomposites prepared according to the described method possess an intrinsic peroxidase-like activity. To investigate the peroxidase-like catalytic activity, a colorimetric assay for the nanocomposites was performed in the presence of TMB and H₂O₂ as substrates at room temperature. A solution of Ni_xZn_xFe₂O₄/MWCNT could catalyze the oxidation of a peroxidase substrate, TMB, in the presence of H₂O₂. TMB is a chromogenic substrate that is used as a hydrogen donor for the reduction of H₂O₂ to water by horseradish peroxidase enzymes or peroxidase mimetics (Fig. 5).

The color of the solution turned blue due to the oxidized TMB product can be read at 370 or 652 nm. For the endpoint assay, the reaction was stopped with acid forming a yellow product that is read at 450 nm. In a continuous assay, the absorption characterizations of the oxidized TMB product with an absorbance peak at 652 nm monitored the catalytic reaction (Fig. 6), which suggested that the oxidation of TMB occurred. No color changes were observed in the solution containing TMB or H₂O₂ alone.

A high peroxidase-like catalytic activity of ZnFe₂O₄/MWCNT for immunosensor purposes was shown by Liu et al. [36]. Colorimetric investigations of peroxidase-like activities of helical MWCNT, GOCNT–Pt (platinum nanocatalysts loaded onto graphene oxide-carbon nanotubes) [19] and Fe₃O₄ nanoparticles loaded onto graphene oxide-dispersed carbon nanotubes (GCNT–Fe₃O₄) [2] have been reported by other researchers. According to Fig. 6, the peak intensity of the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT is higher than that of the Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT nanocatalysts at 652 nm. These differences can be attributed to the amount of Ni, Zn and Fe elements and the morphology and size of the nanostructures. The average crystallite size of the Ni_{0.5}Zn_{0.5}Fe₂O₄ ferrites was

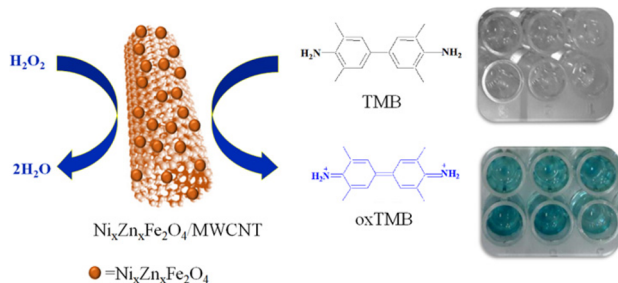


Fig. 5. Schematic diagram of the catalyzed reaction by the peroxidase-like activity of Ni_xZn_xFe₂O₄/MWCNT. Prepared Ni_xZn_xFe₂O₄/MWCNT as a nanocatalyst can be used to catalyze the oxidation of a peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB), by H₂O₂ to the oxidized colored product which provides a colorimetric detection of H₂O₂. MWCNT, multi-wall carbon nanotube.

smaller than that of the Ni_{0.25}Zn_{0.25}Fe₂O₄ ferrites which were estimated to be about 15 nm. Additionally, it has been suggested that the presence of Fe, Ni, and Zn transition metals has a more effective role in the peroxidase-like activity. The absorption changes of the reaction mixture were investigated under different conditions (Fig. 6). No such absorption spectrum was observed from a solution in the absence of the nanocatalysts. When the nanocatalysts were added to the solution, the absorption maximum at 652 nm appeared as a strong response. Moreover, in the absence of H₂O₂, no absorption peak at 652 nm was observed.

These data demonstrate that both of the substrates are required for the reaction to progress, and the nanocatalysts exhibit no absorption peak in the range of 400–800 nm. Our findings show that the Ni_xZn_xFe₂O₄/MWCNT can be used as a peroxidase mimetic.

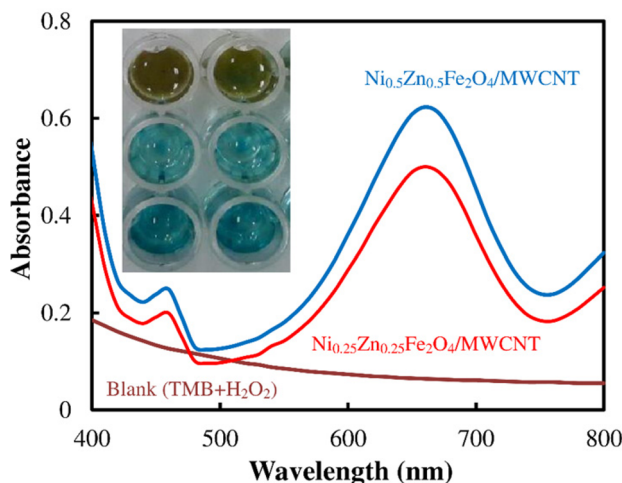


Fig. 6. Ultraviolet-visible absorption spectra of the oxidized 3,3',5,5'-tetramethylbenzidine (TMB) in acetate buffer by the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT and Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT. MWCNT, multi-wall carbon nanotube.

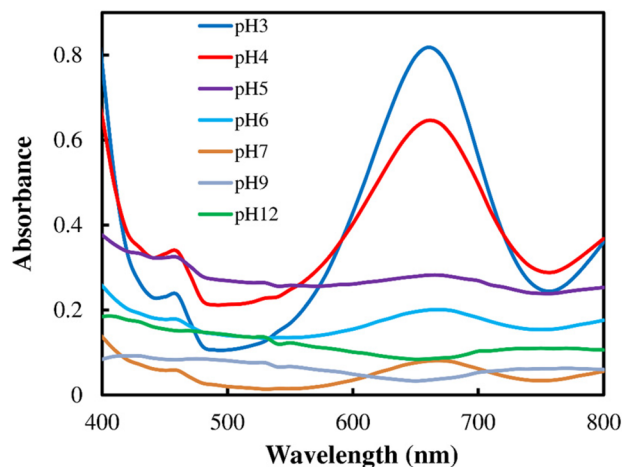


Fig. 7. Ultraviolet-visible absorption spectra of 3,3',5,5'-tetramethylbenzidine (TMB) using the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT nanocomposite with different pH values. MWCNT, multi-wall carbon nanotube.

3.3. The effect of pH on the nanocatalyst activity

To determine the optimum pH for the nanocatalyst assay method, the absorbance was measured for a pH range of 3.0–12.0. The optimum pH for the nanocatalyst activity was measured at pH 3.0. This result shows that the prepared nanostructures have a higher catalytic activity in acidic conditions (Fig. 7).

3.4. Effect of the H₂O₂ amount

Because H₂O₂ is a co-substrate for peroxidase-like reactions to catalyze the oxidation of various substrates, it has an important role in an enzyme mimetic system because the oxidation efficiency of TMB increases in the presence of higher concentrations of H₂O₂. First, hydroxyl radicals are formed during the catalysis of H₂O₂ by catalysts, and then, these radicals facilitate the TMB oxidation. As seen in Fig. 8, as the amount of H₂O₂ was increased, the activity increased; however, a reduction was observed in the absorbance spectrum at a H₂O₂ concentration of 800 mM. This reduction may be due to the inhibitory effect of the substrate concentration at high values.

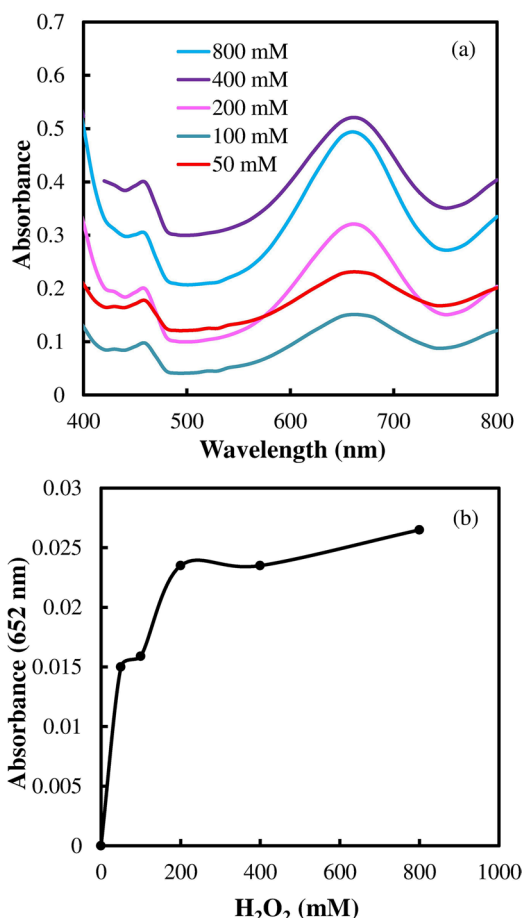


Fig. 8. (a) The absorption spectra of the TMB–H₂O₂ mixed solution in the presence of the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT nanocomposite and different concentrations of H₂O₂. (b) The absorption intensity at 652 nm against different concentrations of H₂O₂. TMB, 3,3',5,5'-tetramethylbenzidine; MWCNT, multi-wall carbon nanotube.

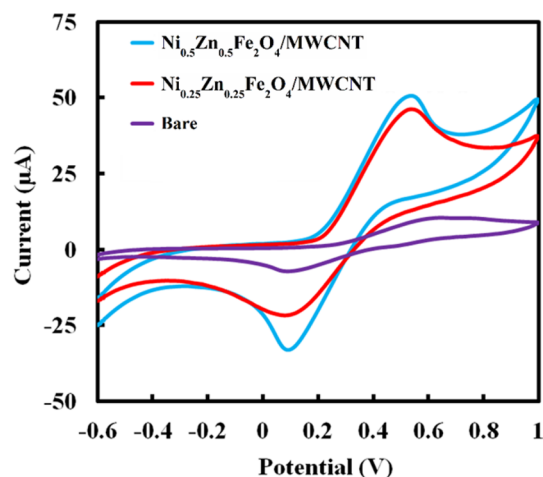


Fig. 9. Comparison of the electron transferring abilities of different modified glassy carbon electrodes with Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT, Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT and bare electrodes in the presence of the H₂O₂ and 3,3',5,5'-tetramethylbenzidine (TMB) substrates. MWCNT, multi-wall carbon nanotube.

3.5. Electrochemical analysis of the electrocatalysis activity of the Ni_xZn_xFe₂O₄/MWCNT nanocomposites

The electrocatalysis behavior of the nanocomposites in the presence of TMB and H₂O₂ was evaluated. Cyclic voltammetry was used to investigate the electron transferring ability of different coated electrodes. The Ni_xZn_xFe₂O₄/MWCNT nanocatalysts were loaded onto the GC electrodes to conduct direct electrocatalysis to TMB oxidation in the presence of H₂O₂. From the electrochemical test results, it was concluded that the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT electrode exhibited a better redox response to H₂O₂, which was ~1.5 times larger than that of the Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT (Fig. 9). This is likely attributed to the higher electromagnetic properties of the Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT which consequently lead to better electron transfer at the electrode/solution interface compared to the Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT nanocatalyst. These results are consistent with the spectrophotometer tests. The results suggest that the nanocatalysts have a peroxidase like activity with TMB and H₂O₂ as the substrates.

4. Conclusions

In summary, Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT and Ni_{0.25}Zn_{0.25}Fe₂O₄/MWCNT nanocomposites as stable and novel nanocatalysts with an intrinsic peroxidase mimicking activity were prepared with the hydrothermal method. The Ni_{0.5}Zn_{0.5}Fe₂O₄/MWCNT nanocatalyst shows good sensitivity for H₂O₂ detection. It is concluded that compositions consisting of transition metals like Ni, Zn, and Fe can be used as peroxidase mimetics. Considering the magnetic characteristics of the Ni_xZn_xFe₂O₄ nanoferrites, these nanocomposites can be magnetized as well as have the ability to separate from solution which leads to high reusability. It was found that the activity is maximum at pH 3.0. The electrochemical signal can be elevated toward H₂O₂ and TMB with the

combination of Ni_xZn_xFe₂O₄ and MWCNT nanomaterials. The stability, direct electrochemistry and multifunctionality of the nanostructures are better than that of natural peroxidases. These nanomaterial-based peroxidase mimetics can be suitable candidates for artificial enzymes and could be used in a wide range of applications such as biocatalysis, biosensing, and so forth.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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