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RESEARCH ARTICLE

Preparation and characterization of a carbon-based magnetic nanostructure via co-precipitation method: Peroxidase-like activity assay with 3,3′,5,5′-tetramethylbenzidine

Navvabeh Salarizadeh; Minoo Sadri*

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

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ABSTRACT

Objective(s): Natural and artificial enzymes have shown important roles in biotechnological processes. Recently, design and synthesis of artificial enzymes especially peroxidase mimics has been interested by many researchers. Due to disadvantages of natural peroxidases, there is a desirable reason of current research interest in artificial peroxidase mimics.

Methods: In this study, magnetic multiwall carbon nanotubes with a structure of Fe_3O_4/MWCNTs as enzyme mimetic were fabricated using in situ co-precipitation method. The structure, composition, and morphology of Fe_3O_4/MWCNTs nanocomposite were characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM). The magnetic properties were investigated by the vibrating sample magnetometer (VSM). Peroxidase-like catalytic activity of nanocomposite was investigated using colorimetric and electrochemical tests with 3,3′,5,5′-tetramethylbenzidine (TMB) substrate.

Results: The obtained data proved the synthesis of Fe_3O_4/MWCNTs nanocomposite. The average crystallite size of nanostructures was estimated about 12 nm by Debye–Scherer equation. It was found that Fe_3O_4/MWCNTs nanocomposite exhibit peroxidase-like activity. Colorimetric and electrochemical data demonstrated that prepared nanocomplex has higher catalytic activity toward H_2O_2 than pure MWCNT nanocatalyst. From electrochemical tests concluded that the Fe_3O_4/MWCNTs electrode exhibited the better redox response to H_2O_2, which is ~ 2 times larger than that of the MWCNTs.

Conclusions: The synthesis of Fe3O4 nanoparticles on MWCNTs was successfully performed by in situ co-precipitation process. Fe_3O_4/MWCNTs nanocatalyst exhibited a good peroxidase-like activity. These biomimetic catalysts have some advantages such as simplicity, stability and cost effectiveness that can be used in the design of enzyme-based devices for various applied fields.

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INTRODUCTION

Enzymes are catalysts have been applied in various fields including pharmaceutical and chemical industry, food industry, agriculture industry and biosensing [1-3]. Despite the importance of natural enzymes, their application is not economically cost-effective due to the sensitivity against the environmental changes and low stability, high costs of purification and storage [1-6].Therefore, artificial catalysts can be designed

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* Corresponding Author Email: *sadri.mn313@gmail.com*

and synthesized as enzyme mimetics including oxidases, peroxidase, dehydrogenases, esterases, and proteases that provide sufficient stability [1,7-11]. These enzyme mimetics possess activities comparable with natural enzymes [3,12]. Peroxidase is an enzyme mimic that has been recently investigated by some researchers [1-7,12-18]. Peroxidases have a wide range of biotechnological applications in environment bioremediation, immunoassay, detection of biomolecules, industrial catalysts and so on [12,13]. It has been found that DNA-based molecules (DNAzymes) and nano-based materials (nanozymes) possess peroxidase-mimicking activity. A wide variety of nanozymes consisting of carbon-based, metal based, metal oxide-based and other nanomaterials have been studied as peroxidase mimetics [4-7,12,14,19]. Compared to natural enzymes, nanozymes have considerable potentials including thermal stability, low-cost, easy production, and different range pH-tolerable [20]. It has been found that ferromagnetic and paramagnetic nanoparticles such as Fe_3O_4 nanoparticles possess intrinsic oxidase-like activity [2,12,21]. However, their activity as peroxidase-mimetic has been ignored, since Fe_3O_4 nanozymes have been conjugated to HRP in order to present peroxidase activity in a number of applications, such as commercial magnetic enzyme linked immunosorbent assay (ELISA) kits [22]. Researchers have reported that magnetic nanoparticles have enzyme-like Activity [6,23], but magnetic nanoparticles tend to aggregate which decreases their catalytic activity. Loading these nanoparticles on other nanostructures or their combination with surfactants reduce the aggregation [24, 25]. Carbon-based nanostructures such as carbon nanotubes, graphene, fullerene, carbon nitride sheets, activated and amorphous carbon are a great deal of importance due to their unique structural and chemical properties [26,27]. Peroxidase-like activity of Fe_3O_4, CNT, and carbon-based nanostructures such as graphene-iron nanoparticles, poly(styrene sulfonate)/Pt-modified graphene nanosheets and Fe_3O_4–MWCNT nanocomposites has been demonstrated [28-35]. These combinational nanostructures, have many applications in electronic and magnetic instruments, catalysis, and so on. Among various nanocomposites, the combination between carbon nanotubes and metal oxide nanoparticles especially magnetic types have been considered due to their unique properties [30-32]. Carbon nanotubes 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 the design of nanocomplexes. These carbon-based nanocomposites have been used in various applications such as drug delivery, biosensing, catalysis, fuel cells, capacitors, and decontamination purposes [4,31,36]. Due to the disadvantages of natural peroxidases, there is a desirable reason of current research interest in artificial peroxidase mimetics. These nanocatalysts with H_2O_2-induced oxidation can play an important role to reduce the environmental pollution. Moreover, by combining of peroxidase-like mimics with other compounds such as glucose oxidase, a simple and sensitive assay for detection of chemical materials is developed.

In this research, Fe_3O_4/MWCNTs nanocomposite was fabricated, then characterized using X-ray diffraction, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and vibrating sample magnetometer. Finally, the catalytic activity of the nanocomposite was determined in the presence of TMB and H_2O_2 as substrates using spectrophotometry and cyclic voltammetry.

MATERIALS AND METHODS

Multiwalled carbon nanotubes (MWCNTs) with diameter of 5-20nm, length of 6–15mm, purity of 97% were obtained from Research Institute of Petroleum Industry (RIPI, Iran). H_2O_2, Ammonium iron (II) sulfate, NaOH and were purchased from Sigma-Aldrich (USA). TMB, dimethyl sulfoxide (DMSO), tris-hydroxymethyl aminomethane (Tris), KH_2PO_4 and K_2HPO_4 were obtained from of Merck (Germany).

Synthesis of Fe_3O_4/MWCNTs nanocomposite

Fe_3O_4/MWCNTs nanocomposite was synthesized according to Gong et al method with some modifications [37]. In order to synthesize of nanocomposite, MWCNTs were modified with a mixture of nitric acid/ sulfuric acid as oxidative reagents at 150 °C for 2 h. Then, MWCNTs washed, filtered, and sonicated for 15 min. Functionalized MWCNTs mixed with 100 ml solutions containing ammonium ferrous sulfate and ammonium ferric sulfate (the molar ratio of Fe^{2+}: Fe^{3+} is 1:2) followed by the slow addition of 3 mL of 2 $molL^{-1}$ NaOH solution at a constant temperature of 60°C under ultrasonic stirring for 20 min. The mixture stirred for 30 min in alkaline medium. The final product washed with deionized water and dried in the oven at 70°C for 18 h.

Characterization of Fe_3O_4/MWCNTs nanocomposite

The morphology of modified MWCNTs and nanocomposite were investigated by TEM (HT-7700). XRD analysis (Philips PW1730, with Cu Kα radiation (λ=1.540598 Å) was used for determination of nanomaterial structures. Fourier transform infrared spectrophotometer (FTIR, Bruker Equinox) was applied to study of chemical structure changes. Magnetic properties of Fe_3O_4/MWCNTs nanocomposite were investigated by VSM (BHV-55, Riken).

Colorimetric assay of peroxidase-like activity of nanozyme

To evaluate the colorimetric assay of nanocomposite 2 $mgml^{-1}$ nanocatalyst was added to 90 µl TMB (DMSO solution) and 90 µl H_2O_2 substrate in Tris solution (0.2 M, pH 6) at room temperature for 5-10 min. Subsequently, monitoring of oxidized TMB was measured at 652 nm using UV-Visible Spectrophotometer (Epoch ™ Microplate reader). Control experiments were also carried out under the same conditions to compare the relative catalytic activity of nanocatalysts.

Electrochemical analysis

Electrochemical detection of H_2O_2 was done using Fe_3O_4/MWCNTs-coated electrode in an electrochemical working station (µAutolabIII electrochemical analyzer). Cyclic voltammetry analysis was performed through a three electrode setup consisting of glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum counter electrode. The GC electrode was polished with alumina and modified with nanozyme. Nanozyme was dissolved in PBS buffer at pH 7 and dispersed with ultrasonic, then 3 µl of solution coated on GC electrode. After coating phase, electrode dried. Then, electrochemical voltammetry measurements were performed in a solution of H_2O_2 and TMB substrates in scanning range -0.6 to 1 V at a scan rate of 0.10V s^{-1}. The electrochemical changes of the reaction mixture investigated under different conditions in the presence and absence of H_2O_2.

RESULTS AND DISCUSSION

Characterization of Fe_3O_4/MWCNTs nanostructure

The crystalline structures of the synthesized Fe_3O_4/MWCNTs nanocomposite and MWCNTs were confirmed with powder XRD measurements, and the main peaks of Fe_3O_4/MWCNTs crystals were clearly presented. Peaks in 2θ= 30.54°, 35.87°, 43.65°, 54.03°, 57.61° and 63.12° are devoted to (220), (311), (400), (422), (511) and (440) crystal planes are major peaks of Fe_3O_4/MWCNT (Fig. 1s). These data are consistent with reported studies [34,38]. The absence of the peak at 25°-30° that related to MWCNT indicating MWCNT structure is changed in the synthesis process. Also, no diffraction peak due to any other new phase is observed. The average crystallite size of ferrite calculated by Debye–Scherer equation is about 12 nm.

FTIR spectroscopy of Fe_3O_4/MWCNTs nanocomposite and MWCNTs throughout the range of 400–4000 cm^{-1} was also performed to confirm structural changes (Fig. 2) Position of bands at 584 cm^{-1} can be attributed to the Fe–O–Fe stretching and bending modes, indicating the presence of Fe_3O_4 in the Fe_3O_4/MWCNTs [38]. It was observed that the bands at 1100 cm^{-1}, 1412 cm^{-1}, 1631 cm^{-1}, 2920 cm^{-1} and 3438 cm^{-1} are assigned to the stretching and bending modes of C–O, C–C, C=O, $-CH_2$ and –OH in the functional groups of the MWCNTs.

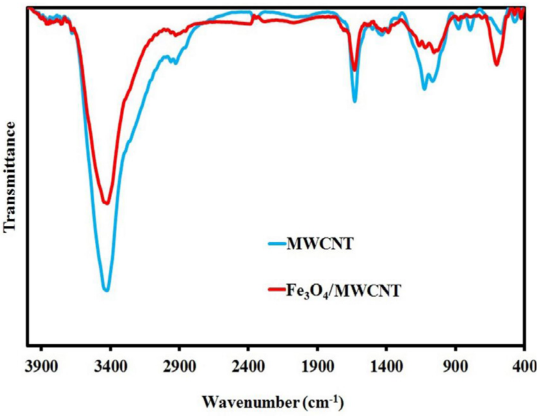

Fig. 2. FT-IR spectra of Fe_3O_4/MWCNTs nanocomposite and MWCNTs

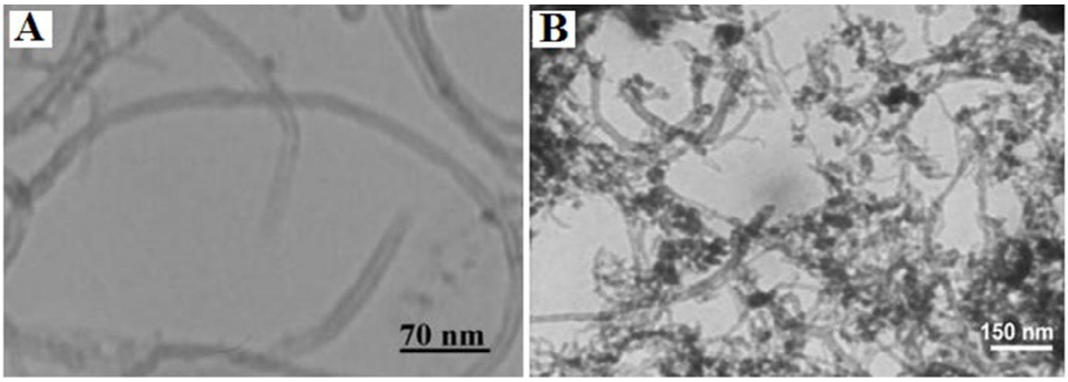

Fig. 3. Typical TEM images of (A) MWCNTs, (B) Fe_3O_4/MWCNTs nanocomposite

The morphology of synthesized nanostructures was investigated with TEM (Fig. 3). It is clearly seen that Fe_3O_4 nanoparticles are well attached and distributed on the surface of MWCNTs. The size of the nanocomposite was estimated about 12-15 nm using TEM analysis.

The hysteresis curves of the Fe_3O_4-coated MWCNTs were recorded at room temperature with a vibrating sample magnetometer. The saturation magnetization Ms, the remanent magnetization Mr, and the coercivity Hc are the main technical parameters to characterize the magnetism of ferromagnetic materials. This nanocomposite magnetically could be separated and reused after the completion of the reaction. With the magnetic separation, it is not required to recover catalyst by filtration and centrifugation methods. Resulted data showed that the saturation magnetization of Fe_3O_4/MWCNTs nanocatalyst was lower than Fe_3O_4 nanoparticles [38-40].

Colorimetric assay of peroxidase-like activity of Fe_3O_4/MWCNTs nanocomposite

In this study, we are reporting the Fe_3O_4/MWCNTs nanocomposite prepared according to described method possessing intrinsic peroxidase-like activity. In order to investigate the peroxidase-like catalytic activity, the colorimetric assay of the nanocomposite was performed in the presence of TMB and H_2O_2 as substrates at room temperature. A solution of Fe_3O_4/MWCNTs could catalyze the oxidation of a peroxidase substrate, TMB, in the presence of H_2O_2 to produce a blue color, as shown on Fig. 5s. It was found in the higher concentrations of reaction mixture components was exhibited more deep color changes (Fig. 6s).

Fabrication of graphene-Fe_2O_3 hybrids (GO-Fe_2O_3) via co-precipitation method with peroxidase-like activity [5], Fe_3O_4 nanoparticles loaded on graphene oxide-dispersed carbon nanotubes [2], CNTs and Fe_3O_4 have been reported by other researchers. Compared to GOCNT–Pt and GCNT–Fe_3O_4 nanocomposite, our nanocatalyst exhibited the lower catalytic activity. Since the catalytic activities of nanocatalyst could be shape- and size-dependent [2], these properties may be associated with the spread graphene sheet and special surface morphology of nanocomposite.

Preparation of Fe_3O_4–MWCNTs nanocomposite for purposes such as synthesis of diarylpyrimidinones [38] and degradation of orange II [6] has been previously performed.

The oxidized TMB solution, which originated from the oxidation of TMB, showed a maximum absorbance at 652 nm. The absorption changes of the reaction mixture were investigated under different conditions (Fig. 7). In the TMB/Nanocatalysts (Fe_3O_4/MWCNTs and MWCNTs) systems exhibit no absorption peak in the range of 400-800 nm. Also, in the absence of H_2O_2 without nanocatalysts, no absorption peak was observed at 652 nm. When nanozymes were added to the solution, the absorption maximum at 652 nm appeared as a strong response. These data demonstrate that both of substrate required for reaction progress (Fig. 7B). Also, prepared nanocomposite exhibited higher catalytic activity compared to pure MWCNTs.

The Fig. 8 shows nanozymes catalytic activity at Abs652 nm in different situations. In the TMB/H_2O_2 reaction without nanocatalyst, TMB/Nanozyme and H_2O_2/Nanozyme systems were observed no catalytic activity (Fig. 8). But in the presence of nanozyme and two substrates, indicated significant color variations.

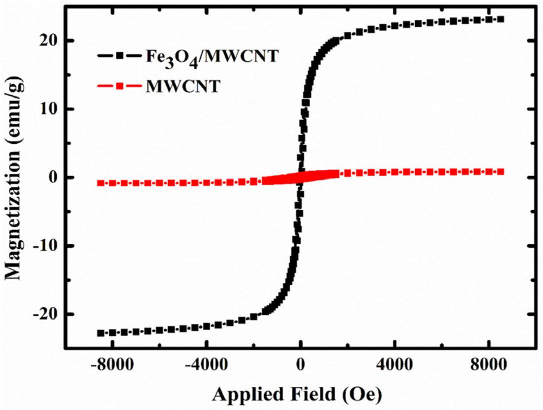

Fig. 4. Hysteresis loops of Fe_3O_4/MWCNTs nanocomposite

Our findings reveal that Fe_3O_4/MWCNTs can be used as a peroxidase mimetic. According to peroxidase-like activity of Fe_3O_4 and CNT nanomaterial's that has been proved in previous studies, the combination of Fe_3O_4 and CNT nanostructures, results in a peroxidase mimetic with noticeably higher efficiency compared to Fe_3O_4 and CNT.

Effect of H_2O_2 concentrations

Since H_2O_2 is a co-substrate of peroxidase to catalyze the oxidation of various substrates, it plays an important role in the enzyme mimetic system because the oxidation efficiency of TMB increased in the presence of higher concentration of H_2O_2. Firstly, hydroxyl radicals are formed during the catalysis of H_2O_2 by catalysts and then these radicals facilitate the TMB oxidation (Fig. 9). According to Fig 8, the absorbance increased by increasing the H_2O_2 concentration.

Electrochemical analysis of electrocatalysis activity of Fe_3O_4/MWCNTs nanocomposite

Electrocatalytic behavior of nanostructures was evaluated in the presence of TMB and H_2O_2. Cyclic voltammetry was used to investigate the electrons transferring ability of different coated materials. The Fe_3O_4/MWCNT and MWCNT nanocatalysts were loaded onto the GC electrodes to conduct direct electrocatalysis to TMB oxidation in the presence of H_2O_2. The resulted data show that peak height is increased by modification of electrodes with Fe_3O_4/MWCNT compared to MWCNT-modified electrodes (Fig. 10C). This increased current represents an increasing in the catalytic activity that could be attributed to the increase in specific surface area due to the presence of Fe_3O_4/MWCNTs and the synergistic effect arising from Fe_3O_4 nanoparticles and carbon nanotubes [41].

The direct electrocatalysis of TMB using the MWCNT and Fe_3O_4/MWCNT-modified electrodes was also performed. Fig. 10A shows that no significant reduction response observed in the modified electrodes in the absence of TMB. The electron transfer is facilitated on Fe_3O_4/MWCNT-modified electrode compared to CNT-modified one in the presence of TMB alone [2]. Also, the direct electrocatalysis of H_2O_2 on different electrodes shows no remarkable voltammetric response (Fig. 10B).

The comparison of Fig. 10A, B and C shows, CV peak heights were intensified by addition of H_2O_2. According to electrochemical tests, the Fe_3O_4/MWCNTs nanocatalyst exhibited better redox response to H_2O_2 reduction and organic substrate oxidation in the presence of both substrates. These results are consistent with the spectrophotometer tests. It is concluded that Fe_3O_4/MWCNTs nanocatalyst possesses higher affinity toward TMB than H_2O_2.

CONCLUSIONS

In summary, the synthesized Fe_3O_4/MWCNT nanocomposite according to mentioned method exhibited a significant peroxidase-like activity. The electrochemical signal elevated toward H_2O_2 and TMB substrates in compare to MWCNTs nanostructure. This magnetic nanozyme may exhibit some advantages compared with natural protein enzymes due to characteristics of magnetic separation and reusability, multifunctionallity and direct electrochemistry to substrates. Since active site of natural enzymes has within the pocket of the enzyme molecule and is not exposed to the surface of the enzyme, nanozymes may indicate better responses in direct electrochemistry to substrates in compare to natural enzymes. We further explore the investigation and application of this magnetic nanostructure in biocatalysis, biotechnology, etc. Also, this nanocatalyst with H_2O_2-induced oxidation can play an important role to reduce the enviromental pollutions.

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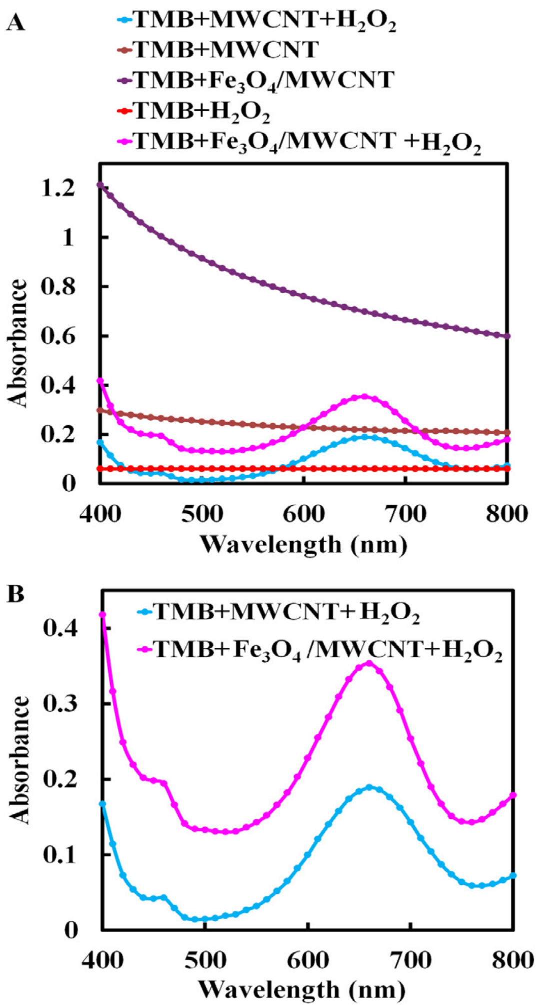

Fig. 7. UV-Vis absorption spectra of the oxidized TMB in Tris solution pH 6 for different

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.nanomedicine-rj.com/jufile?ar_sfile=312339

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