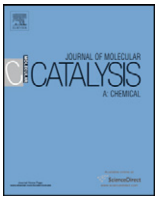
Journal of Molecular Catalysis A: Chemical 360 (2012) 71–77

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata



Peroxidase-like activity and amperometric sensing of hydrogen peroxide by Fe_2O_3 and Prussian Blue-modified Fe_2O_3 nanoparticles

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ARTICLE INFO

Article history:
Received 1 December 2011
Received in revised form 27 February 2012
Accepted 22 April 2012
Available online 28 April 2012

Keywords:
Fe_2O_3 nanoparticles
Prussian Blue modified Fe_2O_3
Peroxidase mimic
Electrocatalytic activity
Amperometric sensor

ABSTRACT

Nano-sized crystals of iron oxide (Fe_2O_3) was synthesized from a single-source precursor complex $[Fe_3(\mu_3\text{-}O)(O_2CCH_2Cl)_6(H_2O)_3]NO_3\cdot H_2O$ by solvothermal process. Afterward it was chemically modified with electro-active Prussian Blue (PB). The resulting Fe_2O_3 and PB-Fe_2O_3 nanoparticles (NPs) were characterized by X-ray diffraction analysis (XRD), transmission electron microscopy (TEM), UV–vis and IR spectroscopic techniques. Structural analyses showed that the average diameter of hexagonal Fe_2O_3 (maghemite) and PB-Fe_2O_3 NPs are 40 and 46 nm, respectively. The as-synthesized nanocomposite (PB-Fe_2O_3 NPs) showed superior catalytic activity toward oxidation of peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in presence of H_2O_2 and follow typical Michaelis–Menten kinetics. Furthermore, the nanocomposite, immobilized on surface of a glassy carbon electrode (GCE), exhibited electrocatalytic activity toward reduction of hydrogen peroxide and can be used for its amperometric detection.

1. Introduction

There has been considerable interest in the synthesis of transition metal oxide-based NPs because of their important physico-chemical properties that could be harnessed to design electro-optical devices, catalyst and sensors [1–5]. Of particular interest are magnetic metal oxides NPs, which find wide applications in information storage and retrieval [6,7], magnetic separation devices [8,9], photo catalysis [10,11], biomedicine [12], biosensing [13–15] and so on. In addition, magnetic iron oxide nanoparticles have showed attractive prospects due to its high stability, relatively low cost and *n-type* semi conductive properties with a small band gap. The important applications are in the area of gas sensing, lithium-ion battery production [16], catalysis, water splitting, water purification, solar energy conversion to pigmentation [17], biomedical applications [18,19], magnetic targeting and drug delivery [20,21], cancer hyperthermia [22,23] and magnetic resonance imaging (MRI) [24–26]. Recently, the unique catalytic activities, homogeneous or heterogeneous, have been reported for iron oxide nanoparticles [27,28]. Although the intrinsic peroxidase-like activity of Fe_3O_4 NPs was studied extensively [29] however the peroxidase activity of Fe_2O_3 is still undeveloped due to lack of ferrous ions in it. Therefore to explore the peroxidase activity of Fe_2O_3, it is needed for modification by augmenting impregnated with Prussian Blue (PB) [30,31]. This will provide the necessary ferrous ions which help the catalytic reaction. In addition, Prussian Blue exhibits excellent electrochemical behavior that can accelerate the electron transfer [32] and good catalytic property which helps to catalyze the reduction of hydrogen peroxide [33].

In this article, we report a simple approach to synthesize Fe_2O_3 NPs from a trinuclear iron (III) complex as a single source precursor. We have modified the surface of Fe_2O_3 with electro active PB to enhance its catalytic activity. These Fe_2O_3 and resultant nanocomposite (PB-Fe_2O_3 NPs) have been found to act as an artificial peroxidase nano-mimetics toward oxidation of TMB in the presence of H_2O_2. Furthermore, the PB-Fe_2O_3 NPs was immobilized onto the surface of a glassy carbon electrode for amperometric sensing of hydrogen peroxide.

2. Experimental

2.1. Materials

All chemicals were of reagent grade and used without further purification. $Fe(NO_3)_3\cdot 9H_2O$, chloroacetic acid, $NaHCO_3$, $FeCl_3\cdot 6H_2O$, potassium ferricyanide, $Na_2HPO_4\cdot 2H_2O$, orthophosphoric acid, anhydrous sodium acetate, acetic acid, dimethyl

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http://dx.doi.org/10.1016/j.molcata.2012.04.011

sulfoxide (DMSO), hydrogen peroxide (30%) and glutaraldehyde (25% aq soln) were purchased from Spectrochem Pvt. Ltd. (India). Ethylenediamine (EN), polyvinylpyrrolidone (PVP), 3,3′,5,5′-tetramethyibenzidine (TMB) were purchased from Sigma–Aldrich. Solvents were used as received.

2.2. Synthesis of the precursor complex $[Fe_3(\mu_3\text{-}O)(O_2CCH_2Cl)_6(H_2O)_3]NO_3{\cdot}H_2O$

A solution of $ClCH_2COOH$ (4.7 g, 0.05 mol) in distilled water (50.0 ml) was mixed with solid $NaHCO_3$ (4.2 g, 0.05 mol) and the mixture was stirred at ∼80 °C for a few minutes. To this solution, Fe $(NO_3)_3{\cdot}9H_2O$ (10 g, 0.025 mol) in distilled water (20.0 ml) was added dropwise, when a deep red solution was obtained. The solution was cooled to room temperature and stirring was continued for 3 h. It was then filtered and kept for several days. A crystalline product that deposited was collected by filtration, washed with cold methanol and dried in air. Yield 3.0 gm (41%). Found: C, 16.45; H, 2.36; N, 1.52. $C_{12}H_{20}Cl_6Fe_3NO_{20}$ requires: C, 16.38; H, 2.27; N,1.59. IR data (KBr, υ/cm^{-1}): 3562 (m), 3260 (w, br), 1618 (s), 1428 (s), 1383(s), 1266 (m), 797 (m), 687 (m), 572 (s). UV–vis [in MeCN, λ_{max}/nm (ε/M^{-1} cm^{-1})] 300 (8680).

2.3. Preparation of Fe_2O_3 NPs

To vigorously stirred ethylenediamine (25 ml) were added in succession the 1.0 g precursor complex $[Fe_3(\mu_3\text{-}O)(O_2CCH_2Cl)_6(H_2O)_3]NO_3{\cdot}H_2O$, 1.2 g PVP and 1 ml of deionized water. When all the materials dissolved, the clear red brown solution was transferred into a Teflon-lined stainless steel autoclave with a capacity of 50 ml. The temperature of the autoclave was maintained at 150 °C for 36 h in a thermo stated air oven after which it was brought to room temperature over a period 4 h. The black NPs that deposited were collected by centrifugation, washed several times with methanol and finally dried in air.

2.4. Preparation of PB-Fe_2O_3 NPs

The as-prepared Fe_2O_3 NPs were suspended in 10 ml aqueous solution of 0.10 M K_3 [Fe (CN) $_6$] containing 0.01 M HCl and ultrasonicated for about 20 min. To this, 10 ml of 0.10 M $FeCl_3$ solution containing 0.01 M HCl was added and the resultant mixture was again ultrasonicated for 30 min. The NPs were collected by centrifugation and washed repeatedly with 0.01 M HCl solution and finally with deionized water. The material was dried in vacuum at room temperature for several hours.

2.5. Fabrication of PB-Fe_2O_3-modified glassy carbon electrode

A glassy carbon electrode (GCE, 3 mm in diameter) was cleaned successively with 1.0, 0.3 and 0.05 μm alumina slurry (CH Instrument) followed by rinsing thoroughly with doubly distilled water and then dried at room temperature. The electrode was fixed on the working table in bottom-up position. PB-Fe_2O_3 (5 mg) were dispersed in 5 ml water and ultrasonicated for 1 h. 10 μl of colloidal suspension was dropped on the GCE surface and dried at room temperature for overnight. Afterward, 5 μl of 1% glutaraldehyde was slowly coated onto the PB-Fe_2O_3 modified GCE surface and allowed to dry in the same condition for 1 h.

2.6. Physical measurements

Elemental (C, H and N) analyses were performed on a Perkin-Elmer 2400II elemental analyzer. Powder XRD patterns were obtained on a Philips PW 1140 parallel beam X-ray diffractrometer with Bragg–Bretano focusing geometry and monochromatic CuKα radiation (λ = 1.540598 Å). TEM measurements were made on a JEOL JEM-2100 microscope using an accelerating voltage of 200 kV. FT-IR spectra were recorded using KBr disks on a JASCO FTIR-460 plus spectrophotometer. Absorption spectra and Peroxidase-like activities were studied spectrophotometrically using Agilent-8453 diode-array spectrophotometer. Electrocatalytic activity and amperometric measurements were carried out on CHI620D electrochemical analyzer using three electrode systems.

2.7. Peroxidase-like activity measurements

The peroxidase-like activities of the Fe_2O_3 and PB-Fe_2O_3 NPs were investigated through the catalytic oxidation of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in presence of H_2O_2 to produce a blue color reaction which was quenched by adding H_2SO_4 (Fig. S1).

To examine the capability of Fe_2O_3 or PB-Fe_2O_3 NPs as catalyst on the oxidation of TMB, 2.4 μl of 0.125 M TMB (dissolved in DMSO) in 3.0 ml acetate buffer (0.1 M and pH 4.0) was successively treated with (i) 20 μg Fe_2O_3 or PB-Fe_2O_3 NPs, (ii) 4 μl of 30% H_2O_2, (iii) 4 μl of 30% H_2O_2 with 20 μg Fe_2O_3 or PB-Fe_2O_3 NPs. All the reactions were monitored spectrophotometrically in time-scan mode at 653 nm. The kinetic analysis with TMB as the substrate was performed using 20 μg PB-Fe_2O_3 NPs with fixed concentration of H_2O_2 (13 mM) and varying concentration of TMB (0, 8.3, 10.4, 12.5, 14.4, 20, 40, 60, 80, 120, 160 and 200 μM). Similarly, the kinetic analysis with H_2O_2 as the substrate was performed using 20 μg PB-Fe_2O_3 NPs with fixed concentration of TMB (100 μM) and varying concentration of H_2O_2 (0, 6.5, 13, 19, 26, 32, 39, 48, 58 and 68 mM). Kinetic parameters were calculated based on the Michaelis–Menten equation (1).

$$V_0 = V_{max}\frac{[S]}{[S]+K_M} \tag{1}$$

The Michaelis–Menten equation describes the relationship between the rates of substrate conversion by an enzyme and the concentration of the substrate. In this equation, V_0 is the rate of conversion, V_{max} is the maximum rate of conversion, [S] is the substrate concentration, and K_M is the Michaelis constant which is equivalent to the substrate concentration at which the rate of conversion is half of V_{max} and denotes the affinity of the enzyme for the substrate.

2.8. Electrocatalytic activity measurements

The electrocatalytic activity of the PB-Fe_2O_3 NPs, modified on GCE, was studied using cyclic voltammetric technique. H_2O_2 was taken as a model compound because the identification of H_2O_2, a product of enzymatic reactions catalyzed by a large number of oxidases, is important in the field of biosensor development [34]. Electrocatalytic measurements were carried out in 0.025 M phosphate buffer solution (PBS) (pH 6.0) with PB-Fe_2O_3/GC working electrode at room temperature using a platinum wire auxiliary and an Ag/AgCl reference electrode. The amperometric response of PB-Fe_2O_3/GC was carried out at −0.15 V *vs.* Ag/AgCl at optimum conditions (pH and temperature) and compared with that of bare GC and Fe_2O_3/GC. The current response due to the addition of H_2O_2 was monitored. The apparent Michaelis–Menten constant, K_M^{app} and the maximum current of Fe_2O_3/GC and PB-Fe_2O_3/GC electrodes can be determined from the Michaelis–Menten equation [35].

$$I = \frac{I_{max}[S]}{K_M^{app}+[S]} \tag{2}$$

where I is the steady-state current, I_{max} the maximum current measured under conditions of enzyme saturation, [S] is the concentration of substrate. Rearrangement of Michaelis–Menten equation