Electronic Supplementary Information

A Selective Colorimetric Strategy for Probing Dopamine and Levodopa through the Mussel-Inspired Enhancement of Fe_3O_4 Catalysis

Mengyuan Yin [a], Shuai Li [a], Yuqi Wan [a], Luping Feng [b], Xiaoting Zhao [a], Sheng Zhang [a], Shuhui Liu [a], Peng Cao [a], Hua Wang *[,a,b]

[a] Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, P. R. China.

[b] School of Chemistry and Chemical Engineering, Harbin Institute of Technology, Harbin, Heilongjiang 150090, P. R. China.

* Corresponding Author: E-mail addresses: huawangqfnu@126.com; Tel: +86 537 4456306;

Fax: +86 537 4456306; Web: http://wang.qfnu.edu.cn.

Abstract

Supplementary information includes the experimental details of the materials and instruments, the synthesis of mesoporous Fe_3O_4 nanoparticles (NPs), and colorimetric analysis tests. Figures or Tables are provided for the comparable dynamic studies on the catalysis activities of Fe_3O_4 NPs with DA, the optimization of colorimetric analysis conditions, and the environmental robustness and storage stability of Fe_3O_4 NPs, and the results of comparable dynamic studies on the catalysis activities of Fe_3O_4 NPs with and without DA.

Experimental Section

Materials and reagents

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ethylene glycol (EG), ethanol, acetic acid, and hydrogen peroxide (H_2O_2) were purchased from Sinopharm Chemical Reagent Co. (China). Sodium acetate (NaAc), sodium chloride (NaCl), and sodium hydroxide (NaOH) were obtained from Tianjin Kermel Chemical Reagent Co., Ltd. Dopamine (DA) and levodopa (LDA) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). 3,3,5,5-tetramethylbenzidine (TMB), tyrosine, tyramine, adenosine triphosphate disodium salt (ATP), glycine (Gly), phenylalanine

(Phe), lysine (Lys), cysteine (Cys), glutathione (GSH), ascorbic acid (AA), tryptophan (Try), glutamic acid (Glu), urea, histidine (His), histamine, glucose were purchased from Sigma-Aldrich (Beijing, China). They were used as received without further purification. All other reagents are of analytical grade. Deionized water (18 MΩ) was obtained from an ultra-pure water system (Pall, USA).

Apparatus

High-resolution transmission electron microscopy (TEM, Tecnai G20, FEI) and scanning electron microscope (SEM, Hitachi E-1010, Horiba Ex-250) were employed to characterize the prepared catalytic materials like Fe_3O_4 NPs. Moreover, colorimetric measurements were performed using Infinite M 200 PRO (TECAN, Switzerland) and UV-3600 spectrophotometer (Shimadzu, Japan).

Fabrication of catalytic Fe_3O_4 NPs

Mesoporous Fe_3O_4 NPs were fabricated by the solvothermal route using $FeCl_3 \cdot 6H_2O$ and NaAc in EG solution. Briefly, 3.46 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 70 mL EG to form a clear solution by intense stirring, followed by the addition of 4.62 g of NaAc. Further, the mixture was vigorously mixed by ultrasonication to give a homogeneous solution, and then transferred into a Teflon-lined stainless steel autoclave (100 mL capacity) to be heated at 200 °C for 8 h. After the autoclave was allowed to cool down to room temperature, the resulting black products of magnetite particles were collected by magnetic separation, and washed for several times under sonication separately with water and ethanol. Subsequently, the yielded mesoporous Fe_3O_4 NPs were dried under vacuum at 60 °C for 12 h to be stored at room temperature.

Colorimetric analysis

Colorimetric investigations of the peroxidase-like catalysis activities of mesoporous Fe_3O_4 NPs in the absence and presence of DA were conducted using TMB-H_2O_2 substrates in HAc-NaAc buffer (pH 3.6). Briefly, 5.0 mg Fe_3O_4 NPs was dispersed in 1.0 mL water under ultrasonic conditions. An aliquot of the Fe_3O_4 NPs suspensions, which concentration should be optimized, was added into 200 μL of DA or LDA solutions with different concentrations to be mixed

thoroughly by vortex, and then incubated for 10 min. Furthermore, a magnetic field was applied to ensure the magnetic separation of DA or LDA-coated Fe_3O_4 NPs. After being washed for three times with buffer, an aliquot of 200 μL TMB-H_2O_2 substrates were introduced to be incubated for 20 min at 37 ^oC. Furthermore, colorimetric measurements were performed for the reaction products with absorbance values recorded. The optimization of catalytic reaction conditions was carried out with 96-well plates by following the same procedure under different reaction conditions, including different amounts of Fe_3O_4 NPs (0.00050, 0.0010, 0.0025, 0.0050, 0.0075, 0.010 mg/mL), reaction temperature (4, 25, 37, 45, 60 °C), pH values (3.0, 4.0, 5.0, 6.0, 7.0, 8.0), and ionic strengths in NaCl concentrations (50, 100, 150, 200, 250, 300 mM). Moreover, comparable studies were conducted to explore the peroxidase-like activities of Fe_3O_4 NPs (0.005 mg/mL) using different small molecules (DA, LDA, Tyrosine, ATP, Gly, Phe, Lys, Cys, GSH, AA, Try, Glu, Urea, His, Glucose, and UA) and different ions (Cu^{2+}, Pb^{2+}, Ni^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Ag^{+}, Cr^{3+}， Co^{2+}, Al^{3+}, Na^{+}, K^{+}, Ca^{2+}, S^{2-}, Br^{-}, $H_2PO_4^{-}$, SO_4^{2-}, NO_3^{-}, HCO_3^{-}, HPO_4^{2-}, CO_3^{2-}, $C_2O_4^{2-}$, I^{-}, Ac^{-}, and Cl^{-} ions), each with the concentration of 1.0×10^{-5} M.

Moreover, steady-state kinetic studies were performed with 96-well plates for comparably exploring the catalysis activities of Fe_3O_4 NPs in the absence and presence of DA, of which the H_2O_2-TMB reaction substrates were used alternatively at a fixed concentration of one substrate (10 mM H_2O_2 or 5×10^{-4} M TMB) and varying concentrations of the second substrate of TMB (10, 50, 100, 200, 400, 600, 800 μM) or H_2O_2 (5.0, 10.0, 30.0, 50.0, 100, 150, 200 mM). Further, the dynamic catalysis parameters of Fe_3O_4 NPs and DA-stimulated Fe_3O_4 NPs were calculated by double-reciprocal plottings, including the Michaelis constant (K_m) and the maximal reaction velocity (V_{max}). In addition, colorimetric analysis experiments were carried out separately for different concentrations of DA or LDA in buffer (HAc-NaAc, pH 3.6) (0.010 to 10 μM) and spiked in urine (1.0 nM to 12 μM), so as to obtain the calibration detection curves for different DA or LDA concentrations in samples.

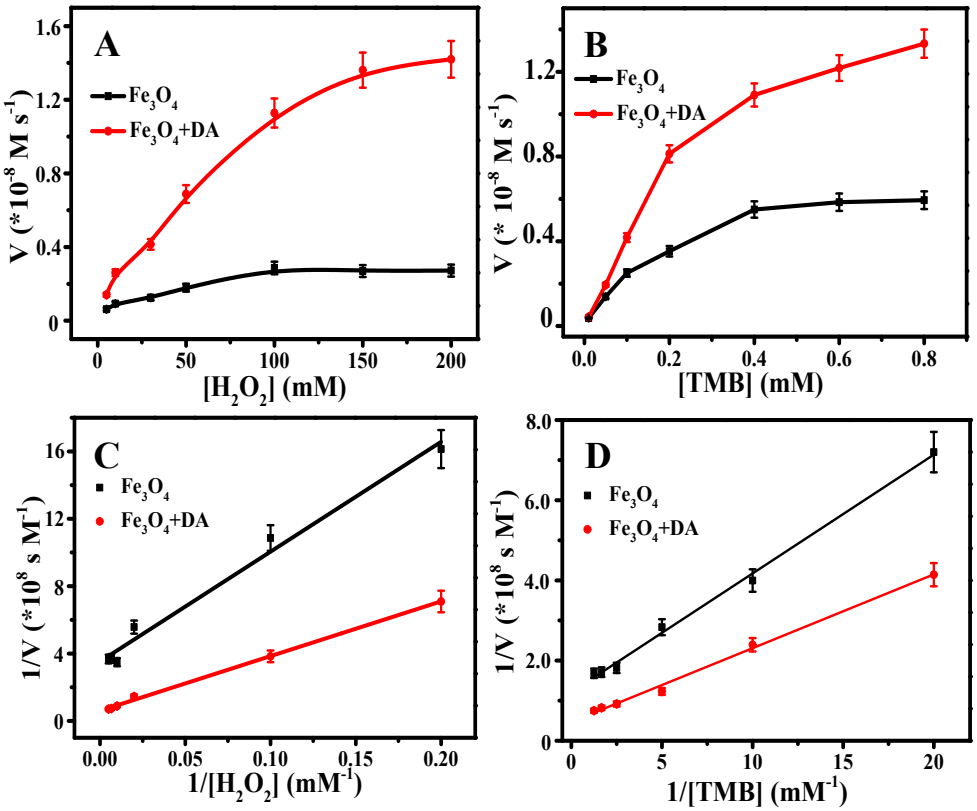

Fig. S1 Colorimetric dynamic studies on the catalysis activities of Fe_3O_4 NPs (0.0050 mg/mL) in the absence and presence of DA (1.0×10^{-5} M) using (**A**) various H_2O_2 concentrations and (**B**) various TMB concentrations, with (**C**) and (**D**) the corresponding double-reciprocal plots. The measurements were conducted alternatively at a fixed concentration of one substrate (5×10^{-4} M TMB or 1×10^{-2} M H_2O_2) versus varying concentrations of the second substrate, with the details shown in the Experimental.

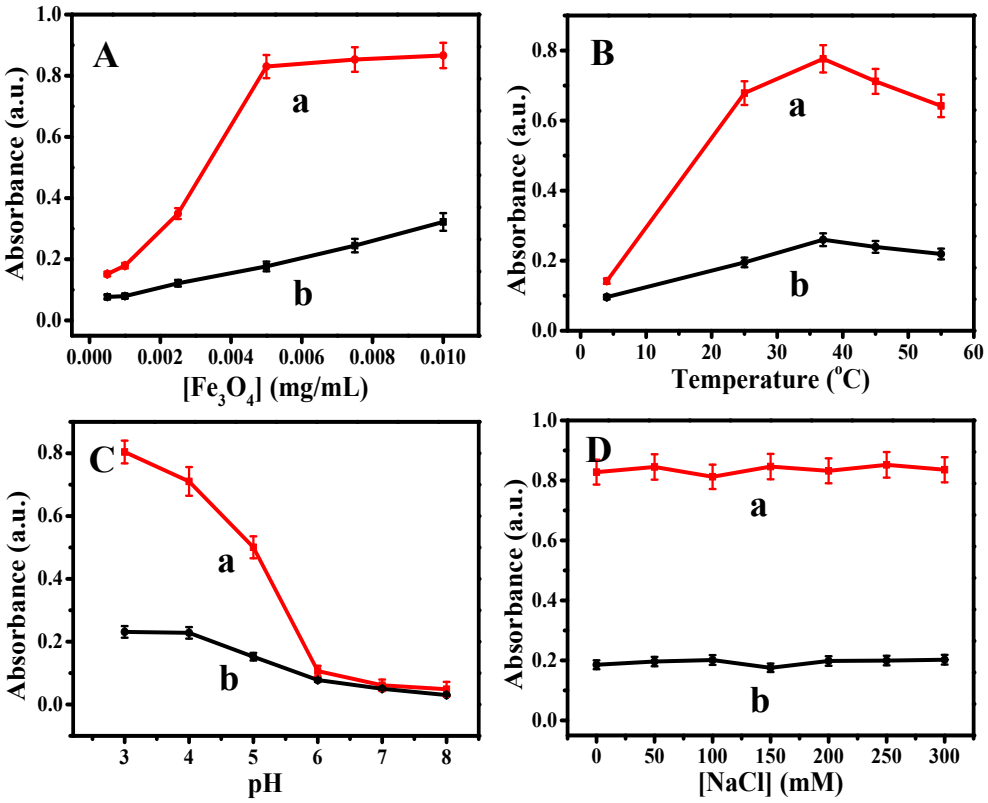

Fig. S2 Comparable catalysis conditions of Fe_3O_4 NPs (0.0050 mg/mL) in the (a) presence and (b) absence of DA (1.0×10^{-5} M) for TMB-H_2O_2 reactions under different (**A**) Fe_3O_4 NPs concentrations, (**B**) temperature, (**C**) pH values, and (**D**) ionic strengths in NaCl concentrations.

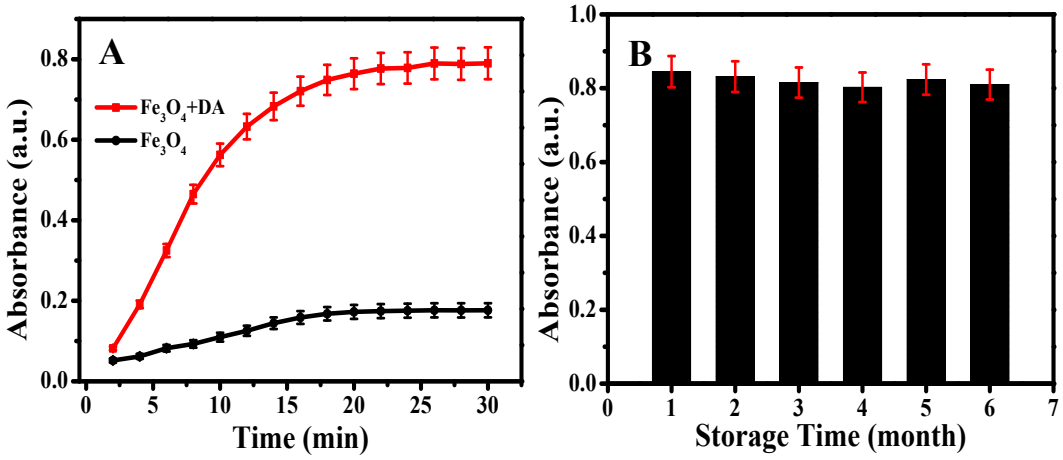

Fig. S3 (**A**) The time-depending catalysis of Fe_3O_4 NPs (0.0050 mg/mL) with and without DA (1×10^{-5} M) for TMB-H_2O_2 reactions. (**B**) The environmental stability of Fe_3O_4 NPs stored in room temperature over different time intervals, where the catalytic activities of Fe_3O_4 NPs with DA were monitored by catalyzing the TMB-H_2O_2 reactions.

Table S1 Comparable results of catalysis parameters of dynamic studies on the catalysis performances of Fe_3O_4 NPs with and without DA in catalyzing the TMB-H_2O_2 reactions.

Catalysis materials	**Substrates**	**K_m (mM)**	**V_m ($\times 10^{-8}$ M S^{-1})**	**K_{cat} (S^{-1})**
Fe_3O_4	TMB	0.3923	2.13	1.495×10^5
	H_2O_2	18.48	0.2834	0.199×10^5
Fe_3O_4 with DA	TMB	0.2445	0.8255	0.579×10^5
	H_2O_2	54.04	1.66	1.165×10^5