Sensors and Actuators B 215 (2015) 86–92

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

# Carbon coated magnetite nanoparticles with improved water-dispersion and peroxidase-like activity for colorimetric sensing of glucose

Qian Li, Gonge Tang, Xiewei Xiong, Yili Cao, Lili Chen, Fugang Xu, Hongliang Tan*

*Key Laboratory of Functional Small Organic Molecule, Ministry of Education, Key Laboratory of Chemical Biology of Jiangxi Province, College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang 330022, PR China*

ARTICLE INFO

*Article history:*
Received 15 December 2014
Received in revised form 3 March 2015
Accepted 18 March 2015
Available online 28 March 2015

*Keywords:*
Core–shell structure
Magnetite nanoparticles
Peroxidase-like activity
Synergetic effect
Glucose

ABSTRACT

Nanomaterials-based enzymatic mimics have recently received considerable attention due to their superior properties over natural enzymes. Particularly, great efforts have been made to the fabrication of enzymatic mimics based on carbon matrix-based hybrid nanocomposites because of their improved dispersion, stability and catalytic activity compared with naomaterials alone. In this work, a new peroxidase mimic based on carbon coated magnetite nanoparticle ($Fe_3O_4$@C) with improved water-dispersion and peroxidase-like activity was presented for the first time. Unlike conventional hybrid nanocomposites, $Fe_3O_4$@C is a typical core–shell structure in the nanoscale regime and possesses the properties of easy preparation, super water-dispersion and excellent stability. Owing to the presence of carbon shell, $Fe_3O_4$@C showed an enhanced peroxidase-like activity as compared with its two components. As a peroxidase mimic, the catalytic kinetic of $Fe_3O_4$@C follows Michaelis–Menten behaviors and shows stronger affinity to peroxidase substrates than horseradish peroxidase. On the basis of these findings, a simple colorimetric method for glucose detection was developed by combining the catalytic reactions of $Fe_3O_4$@C and glucose oxidase. Additionally, the proposed method was successfully applied to determine the levels of glucose in serum and urine samples and showed a satisfactory result.



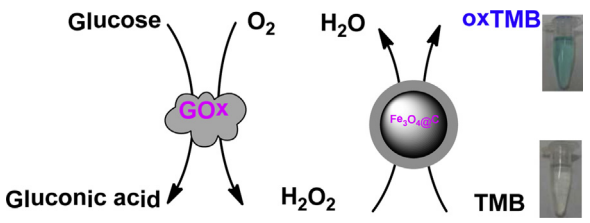
## 1. Introduction

Glucose is an important body's fuel, which can be converted into energy through complicated metabolism pathways. However, abnormal glucose regulation is linked to diabetes, which can cause serious complications including lower limb amputations, blindness and cardiovascular disease, and consequently threats human health seriously [1]. Therefore, it is a very important task to tight monitor blood glucose levels for reducing the risk of diabetes-associated complicates. In the last decades, numerous methods for the detection of glucose have already been developed, of which enzyme-like nanomaterials-based colorimetric methods are particularly interested due to their low cost, simplicity, and practicality [2–4]. In addition, colorimetric assays can be easily performed without the requirement of expensive or sophisticated instruments. In compared with natural enzymes, enzyme-like namomaterials exhibit the advantages of easy preparation, great resistance to harsh chemical environment and tunable catalytic activity [5]. As such, a variety of nanomaterials including $Fe_3O_4$ nanoparticles (NPs) [2], carbon dots [6], carbon nanotubes [7], graphene dots [8], and $WS_2$ nanosheets [9] have been evaluated as enzymatic mimics for the detection of glucose. Nevertheless, laborious procedures are often required for the preparation of these nanomaterials. Moreover, most of these nanomaterials are unstable and prone to be aggregated and settled in aqueous solutions, which may result in the decrease of their catalytic activity due to the reduction of effective surface area [10]. It is therefore crucial to develop enzyme-like nanomaterials with simple preparation procedures, good stability, excellent dispersion, and high catalytic activity.

An often-used way to prevent the aggregation of nanomaterials is the introduction of stabilizer on the surface of nanomaterials. However, it was found that the existence of surface stabilizer could suppress the catalytic activity of nanomaterials. Recently, hybrid nanocomposites constructed by anchoring of nanomaterials on carbon matrixes with large surface area has been intensively explored because of the improvement of the stability and dispersion of nanomaterials. Owing to enlareged effective surface area and the synergetic effect of nanomaterials and supports, such hybrid

* Corresponding author. Tel.: +86 791 88120861; fax: +86 791 88120861.
*E-mail address:* hltan@jxnu.edu.cn (H. Tan).

http://dx.doi.org/10.1016/j.snb.2015.03.029
0925-4005/




**Scheme 1.** Illustration of colorimetric detection of glucose by combining GO*x* and $Fe_3O_4$@C-catalyzed reactions.

nanocomposites usually exhibit enhanced catalytic activity and/or some new properties that cannot be accessed by either component alone [10–12]. Nevertheless, the preparation procedures of water-soluble carbon matrixes and loading steps of nanomaterials are complicated and time-consuming. Additionally, the hydrophobicity and large size (usually several micrometers) of carbon matrixes may also limit the applications of hybrid composites as catalysts [12]. Therefore, it still remains a great challenge to develop colorimetric sensors based on water-dispersive hybrid composites in the nanoscale regime. Very recently, a carbon matrix-based magnetic hybrid nanoparticle ($Fe_3O_4$@C) comprised of magnetite nanoparticle ($Fe_3O_4$) as core and porous carbon (C) as shell was reported [13]. Different from conventional hybrid nanocomposites, $Fe_3O_4$@C is a typical core–shell structure in the nanoscale regime, which can be obtained only by a facile one-step solvothermal method. Moreover, $Fe_3O_4$@C exhibited excellent dispersion in several types of solutions. However, most studies of $Fe_3O_4$@C focus on the exceptional porosity and absorbents properties of carbon shell, less work concerns with the using $Fe_3O_4$@C as an enzymatic mimic for bioanalysis [14]. Particularly, the contributions of carbon shell on the peroxidase-like activity of $Fe_3O_4$@C and the catalytic mechanism of peroxidase-like activity of $Fe_3O_4$@C remain to be exploited. To the best of our knowledge, furthermore, there have been few attempts to investigate the catalytic properties of peroxidase mimics with core–shell structure.

In this work, we attempt to explore the role of carbon shell in the improvement of water-dispersion and peroxidase-like activity of $Fe_3O_4$@C. Unlike previous report [14], we found that there is a synergetic interaction between $Fe_3O_4$ core and carbon shell. In compared with $Fe_3O_4$ NPs and carbon shell alone, enhanced catalytic activity can be achieved from $Fe_3O_4$@C, which is resulted from the synergetic effects of magnetic core and carbon shell rather than a simple addition of the activities of these two components. The catalytic kinetic behaviors and catalytic mechanism of $Fe_3O_4$@C as a peroxidase mimic were further investigated. On the basis of these findings, the application of $Fe_3O_4$@C as peroxidase mimic for colorimetric sensing of glucose was explored (Scheme 1). The presented colorimetric method for glucose displayed high sensitivity and selectivity, and was successfully applied for glucose detection in serum and urine samples.

## 2. Experimental

### 2.1. Chemicals

All chemicals were obtained from commercial source and used without further purification. Ferrocene, hydrogen peroxide ($H_2O_2$, 30%), acetone, glucose, leucine (Leu), aspartic acid (Asp), alanine (Ala), phenylalanine (Phe), tyrosine (Try), histidine (His), mannose (Man), galactose (Gal), fructose (Fru), sucrose (Suc), and metal salts ($Cu(NO_3)_2$, NaCl, KCl, $CaCl_2$, $NaNO_3$, $Na_2SO_4$) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China); 3,3′,5,5′-tetramethylbenzidine (TMB) was obtained from Aladdin (Shanghai, China); horseradish peroxidase (HRP) and glucose oxidase (GO*x*) were obtained from Sangon Biotech (Shanghai, China). Ultrapure water (18 MΩ cm) was used for the preparation of all aqueous solutions. Unless otherwise stated, all chemicals are of analytical reagent grade.

### 2.2. Instruments and characterization

The JEM-2100 transmission electron microscopy (TEM, JEOL, Japan) and SU8020 field-emission scanning electron microscopy (FE-SEM, Hitachi, Japan) equipped with an energy dispersive spectra (EDS) detector were used for examining the morphology of $Fe_3O_4$@C. The UV–vis absorption spectra and fluorescence spectra were recorded by using UV-3900H spectrophotometer (Hitachi, Japan) and F-7000 fluorescence spectrophotometer (Hitachi, Japan), respectively. Avatar 360 FTIR spectrometer (Nicolet, USA) was used to record the Fourier transform infrared (FTIR) spectra with the KBr pellet technique. Thermogravimetric analysis (TGA) was conducted under a $N_2$ flow with a heating rate of 5 °C $min^{-1}$, using an SDT 2960 instrument.

### 2.3. Preparation of $Fe_3O_4$@C

According to previous report [13], $Fe_3O_4$@C was prepared by using a solvothermal method. Typically, a ferrocene solution was first prepared by dissolving 0.3 g of ferrocene in 30 mL of acetone. After intense sonication for 30 min, 1 mL of $H_2O_2$ was added dropwise into the above ferrocene solution for reacting 15 min under stirring. Then, the result solution was transferred to Teflon lined stainless autoclave with a total volume of 50 mL. The solvothermal reaction was carried out for 24 h at 210 °C. The products were collected under a magnetic field after cooling to room temperature and washed several times with absolute ethanol to remove unreacted reactants. Finally, the $Fe_3O_4$@C nanoparticles were dispersed in ultrapure water.

### 2.4. Peroxidase-like activity of $Fe_3O_4$@C

The peroxidase-like activity of $Fe_3O_4$@C was examined by adding 30 μg of $Fe_3O_4$@C to the NaAc buffer (10 mM, pH 4.5) contained 500 μM of $H_2O_2$ and 100 μM of TMB for reacting 10 min at 50 °C. NaAc buffer (10 mM, pH 4.5) was used to make up the final volume to 500 μL. The $Fe_3O_4$@C-based catalytic reaction was monitored by a spectrophotometer. The concentrations of oxidized TMB were quantified by UV–vis absorption at 652 nm by using a molar extinction coefficient of $\varepsilon = 3.9 \times 10^4\ M^{-1}\ cm^{-1}$ [15]. The kinetic behaviors of $Fe_3O_4$@C were studied by monitoring the absorbance with a 3 min interval after changing the concentration of TMB and $H_2O_2$. The Michaelis–Menten constant was calculated by using Lineweaver–Burk plots of the double reciprocal of the Michaelis–Menten equation, $1/\nu = K_m/V_m\ (1/[S] + 1/K_m)$, where $\nu$ is the initial velocity, $V_m$ represents the maximal reaction velocity [S] corresponds to the concentration of substrate and $K_m$ is the Michaelis constant. The determination of each sample was repeated three times. All error bars represent standard deviations from three repeated experiments. The reaction conditions for studying of peroxidase-like activity and the kinetic behaviors of carbon shell and $Fe_3O_4$ NPs are same as $Fe_3O_4$@C.

### 2.5. Detection of glucose

The glucose was detected by using following steps: 10 μL of GO*x* (1 mg/mL) was first mixed with glucose with various concentrations from 0 to 500 μM in 300 μL of PBS buffer (10 mM, pH 7.0). The mixture was reacted for 10 min at 37 °C. Then, the mixture was added to NaAc buffer (10 mM, pH 4.5) contained 30 μg of $Fe_3O_4$@C and 100 μM of TMB. After reacting for 10 min at 50 °C,