Synergistic peroxidase-like activity of CeO_2-coated hollow Fe_3O_4 nanocomposites as an enzymatic mimic for low detection limit of glucose

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Fe_3O_4@CeO_2 yolk–shell nanocomposites (NCs) were synthesized by growing CeO_2 onto Fe_3O_4 hollow nanoparticles (NPs) as an artificial peroxidase for the purpose of elevated activity. The resultant NCs exhibited strong magnetic response with narrow size distribution and high surface area. Owing to the individual peroxidase-like catalytic activity of CeO_2 onto Fe_3O_4 nanoparticles (NPs), Fe_3O_4@CeO_2 NCs was investigated to achieve synergistic catalytic activity. As a result, the as-prepared Fe_3O_4@CeO_2 NCs exhibited impressive peroxidase-like catalysis activity for 3, 3′, 5, 5′-tetramethylbenzidine (TMB). Moreover, comparing with natural enzymes, hollow Fe_3O_4 NPs and CeO_2 NPs, the Fe_3O_4@CeO_2 NCs displayed a lower Michaelis constant (K_m) value or stronger affinity to H_2O_2 and TMB. A limit of detection of 8.5 µmol/L for H_2O_2 and 21 µmol/L for glucose was attained with acceptable stability and reproducibility. The activation mechanism was also studies using competitive radical tests and Electron spin resonance (ESR) analysis.

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1. Introduction

Enzymes as an extremely efficient biological catalyst have attracted great interests because of these advantages, such as high substrate specificity, activities, and yields under mild reaction conditions so that broadened their application fields in pharmaceutical processes, agrochemical production, food industry and biosensing [1]. However, the disadvantages of natural enzymes lie in easy loss of activity, low stability and high cost, thus seriously restrict their practical applications [2]. Recently, artificial enzyme mimics based on inorganic nanoparticles (NPs) have been arising intense interest due to their impressive advantages, including easy preparation, low cost, high stability and tunable catalytic activities [3]. The typical examples include MFe_2O_4 (M=Co, Zn, Mn) [4–6], Ceria NPs [7], graphene oxide (GO) [8], Fe_3O_4 magnetic NPs [9,10], gold NPs [11], CuO [12] and so on. Ceria has been comprehensively explored and applied in the field of catalysis, water treatment, fuel cells, and gas sensor due to its single crystal structure, good electrical and optical properties [13]. In addition, ceria NPs are a good candidate for

catalysis materials owing to its oxygen storage capacity (OSC) associated with the ability to undergo a facile conversion between Ce (III) and Ce (IV) [14]. However, these inorganic NPs may somewhat achieve limited success in terms of catalysis efficiency, aqueous dispersion, separation and recovery. Fe_3O_4 magnetic NPs possess this kind of enzyme activity [15] and offer the advantages of convenient enrichment and subsequent easy separation or recovery under external magnetic field. The intrinsic instability or being easily oxidized in air might still restrain applications [16].

Yolk–shell structured materials with discrete functions are able to extend their application [17,18]. This kind of structured materials represent an unique class of complex hybrids, preventing the undesirable coalescence, sintering, aggregation or corrosion/dissolution of entrapped catalytic species [19]. Up to now, the well-defined structures have been usually prepared in complicated procedures and severe reaction conditions. Therefore, a simple and reliable synthesis research is desired to extend its practical applications.

Herein, Fe_3O_4@CeO_2 nanocomposites (NCs) were successfully synthesized via a simple hydrothermal chemical reaction route. The as-prepared Fe_3O_4@CeO_2 NCs were then used to catalyze the oxidation of a peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to the oxidized colored product, which provided a colorimetric detection of H_2O_2 concentration. Additionally, the detection of H_2O_2 can be readily extended to figure out the

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concentration of glucose in terms of the fact that H_2O_2 was evolved in the catalytic reaction of glucose with glucose oxidase (GOx). An extremely low detection of glucose was thus achieved benefiting from the elevated catalytic activity of the $Fe_3O_4@CeO_2$ NCs in contrast to Fe_3O_4 NPs and CeO_2 NPs alone. The result will allow practical and wide applications in clinics.

2. Experimental section

2.1. Materials and reagents

Cerium (III) nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$, 99.99%, Sigma-Aldrich), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), hydrogen peroxide (H_2O_2, 30%), ethylene glycol ($C_2H_6O_2$), 5,5-dimethyl-pyrroline-oxide (DMPO), polyvinyl pyrrolidone (PVP, $(C_6H_9NO)_n$), N,N- dimethylformamide (DMF, C_3H_7NO), ammonium acetate ($C_2H_7NO_2$, 98%), urea (CH_4N_2O, 99%), N,N,N,N-tetramethylethylenediamine (TMEDA, $C_6H_{16}N_2$, 99%), glucose ($C_6H_{12}O_6 \cdot H_2O$), glucose oxidase (GOx from Aspergillus niger, Type VII, 100–250 units/kg) and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents in this study were in analytic grade and used without any further treatment. Deionized water was used throughout this experiment.

2.2. Instruments and characterization

Transmission electron microscopy (TEM, FEI, Tecnai G20) were used to observe the morphology of the samples. The crystal structure was examined by X-ray diffraction (XRD, Germany Bruker D8 advance X-Ray diffractometer with Cu Kα radiation). Fourier transfer infrared (FT-IR) spectra of the samples were obtained by a PerkinElmer 580B using the KBr pellet technique. The magnetic properties were measured by a vibrating sample magnetometer (VSM, Quantum design, USA physical properties measurement system (PPMS-9T) with VSM option) at room temperature. Electron spin resonance (ESR) spectra were acquired using a Bruker e-scan EPR spectrometer (Burker, Karlsruhe, Germany). The surface area was estimated using the Brunauer–Emmett–Teller (BET) equation and N_2 adsorption-desorption experiments were performed on a Micromeritics ASAP 2460 analyzer.

2.3. Synthesis of Fe3O4 hollow NPs

The Fe_3O_4 hollow NPs were prepared through a modified solvothermal reaction [20]. Typically, 0.8 g of $FeCl_3 \cdot 6H_2O$ and 2.0 g of NH_4Ac were dissolved in 40 mL of ethylene glycol with vigorous stirring, and then 1.0 mL of TMEDA was added. The mixture was stirred vigorously for 2 h to form homogeneous yellow solution, and then transferred into a Teflon-lined stainless-steel autoclave (50 mL capacity). The autoclave was heated to 200 °C and maintained for 10 h. After cooling to room temperature, the dark product was collected and rinsed with ethanol several times, and dried in a vacuum oven at 60 °C overnight for further experiments.

2.4. Synthesis of CeO2 NPs

0.12 g of $Ce(NO_3)_3 \cdot 6H_2O$ and 0.18 g PVP were stirred in 19 mL of deionized water until colorless transparent solution was obtained. Then 1 mL of DMF and 0.1 mL H_2O_2 were added. The obtained solution was transferred to a stainless-steel sealed autoclave (50 mL capacity) and maintained at 180 °C for 20 h. The grey product was collected by centrifuge and dried in a vacuum oven at 60 °C overnight.

2.5. Synthesis of Fe3O4@CeO2 yolk–shell NCs

50 mg of Fe_3O_4 hollow NPs was dispersed in 80 mL of 0.4 mol/L glucose solution under ultrasonication for 30 min. Then 0.4 g of $Ce(NO_3)_3 \cdot 6H_2O$ and 0.6 g of urea were added to the above mixture. After 30 min sonication, the homogeneous mixture was transferred into a 100 mL Teflon-lined autoclave and heated at 120 °C for 8 h. After cooled to room temperature, the dark precipitate was collected and washed with ethanol several times with magnetic separation. Finally, the as-prepared $Fe_3O_4@CeO_2$ yolk–shell NCs dried at 60 °C for 20 h in a vacuum oven.

2.6. Peroxidase-like catalytic activity of Fe3O4@CeO2 NCs

To investigate the peroxidase-like catalytic activity of the $Fe_3O_4@CeO_2$ yolk–shell NCs, the catalytic oxidation of TMB was measured in the presence of H_2O_2. In a typical procedure, 0.72 mg of $Fe_3O_4@CeO_2$ NCs was dispersed in 4 mL of 0.02 mol/L acetate buffer and sonicated for 10 min. Then 300 μL of 50 mmol/L DMF solution of TMB and 31 μL of 100 mmol/L H_2O_2 were added to the above solution. The absorbance of oxidized TMB evolved with time was monitored at 652 nm unless otherwise stated.

Effect of pH on the catalytic activity of $Fe_3O_4@CeO_2$ NCs was investigated by catalyzing the substrate at 40 °C in the pH range of 1.0–7.0. Effect of temperature was also studied at different temperatures (20–60 °C). The relative activity (%) was adopted and defined as the ratio of the activity over the maximum activity in each group of experiments.

The kinetic parameters of $Fe_3O_4@CeO_2$ NCs were evaluated at varied concentrations of H_2O_2 (0.5–4.0 mmol/L) or TMB (0.05–1.0 mmol/L) in buffer solution (pH 3.5). The Michaelis–Menten constant was calculated from the Lineweaver–Burk plot: $1/v = (1/v_{max}) (1 + K_m /[S])$, where v is the catalytic velocity, v_{max} is the maximal catalytic velocity, $[S]$ is the concentration of the substrate, and K_m demonstrates the Michaelis constant.

2.7. Lower limit of detection of glucose by Fe3O4@CeO2 NCs

First, 5 μL of GOx (1 mg/mL) was added into 240 μL of phosphate buffer solution (10 mmol/L, pH 7.0) containing the varying concentrations of glucose (0–2.0 mmol/L) and incubated at 37 °C for 30 min. Then the mixture was transferred to 2.7 mL of acetate buffer (0.2 mol/L, pH 4.0) containing 0.36 mg of $Fe_3O_4@CeO_2$ NCs and 8 mmol/L of TMB and then incubated at 45 °C for 10 min. The supernatant's absorbance was measured. The limit of detection (LOD) was calculated from the typical definition: $S/N \geq 3$, where N is the standard deviation of blank sample and S is the signal of sample.

3. Results and discussion

3.1. Characteristics of Fe3O4 hollow NPs, CeO2 NPs and Fe3O4@CeO2 NCs

The fabrication process of $Fe_3O_4@CeO_2$ NCs is displayed in Scheme 1. At first, the Fe_3O_4 hollow NPs were prepared by a solvothermal process. Then the yolk–shell $Fe_3O_4@CeO_2$ NCs were acquired using a one-pot coating process. CeO_2 NPs were prepared for the comparative study. Their morphologies were observed with TEM. The as-prepared Fe_3O_4 NPs showed regular spherical shape with a average diameter of 590 nm (Fig. 1a). Specifically, an obvious hollow structure was observed in Fe_3O_4 NPs, and this hollow structure was further identified by high magnified TEM images (Fig. 1d). CeO_2 NPs were prepared for the comparative study and shown in Fig. 1b and e. These images revealed that the mean size was about 54 nm. $Fe_3O_4@CeO_2$ NCs had very narrow diameter