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Coral-like CeO_2/NiO nanocomposites with efficient enzyme-mimetic activity for biosensing application

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ABSTRACT

Development of nanomaterials-based enzymatic mimics has gained considerable attention in recent years, because of their low cost, high stability and efficiently catalytic ability. Here, CeO_2 was successfully incorporated into the coral-like NiO nanostructures assembled by nanoflakes with high surface area, forming the coral-like CeO_2/NiO nanocomposites. The morphology and composition of CeO_2/NiO nanocomposites were characterized by XRD, SEM, element mapping and XPS. The results of characterization showed that cerium was highly dispersed in the coral-like NiO nanostructures. The peroxidase-like activity of CeO_2/NiO nanocomposites was investigated, and they exhibited enhanced peroxidase-like activity in comparison to that of pure NiO or CeO_2. The catalytic activity was dependent on the cerium content, and the optimal content was 2.5%. The enhanced catalytic activity of CeO_2/NiO nanocomposites arised from their high ability of electron transfer because of cerium incorporation. The catalytic performance of CeO_2/NiO nanocomposites was evaluated by steady-state kinetic, which showed that the CeO_2/NiO nanocomposites exhibited higher affinity for the substrates and similar catalytic efficiency compared with natural peroxidase. Based on the efficient peroxidase-like activity, CeO_2/NiO was used for H_2O_2 determination. The constructed colorimetric H_2O_2 sensor had fast response for only 5 min, a wide linear range from 0.05 to 40 mM and a low detection limit with 0.88 μM. The CeO_2/NiO nanocomposites were expected to have potential applications in clinical diagnosis and biotechnology as enzymatic mimics.

1. Introduction

Peroxidase is a metalloenzyme catalyzing the oxidation of some substrates by hydrogen peroxide. It is commonly used in techniques such as ELISA and immunohistochemistry because of its monomeric nature and the ease with which it produces colored products, allowing to determine the presence of a molecular target [1]. In addition to biomedical applications, peroxidase is suitable for the removal of hydroxylated aromatic compounds (HACs), which are considered to be the primary pollutants in many industrial wastewaters [2]. In recent years, some inorganic nanomaterials were found to exhibit the peroxidase-like activity, such as carbon-, metal-, and metal oxide-based nanomaterials [3–7]. Compared with natural peroxidase, these nanomaterials-based peroxidase mimics showed low cost, high stability under some extreme conditions, and easy regulation of the catalytic activity by changing their composition and morphology. Due to the unique characteristics of the nanomaterials and the enormous progress in nanotechnology, considerable advancements have been made in the nanomaterials-based mimetic peroxidase so far [8].

In order to achieve high catalytic activity of nanomaterials-based peroxidase mimics, fabrication of composite nanomaterials with well-defined composition and morphology offered the great opportunity [9,10]. The synergetic effects of composite nanomaterials could make their catalytic performance different from any one of the components. The composition and morphology of metal oxide nanomaterials became controllable, and the lattice matching between metal oxides could be easily realized, therefore the metal oxide was a good support matrixes. Some nanocomposites constituting nanoparticles incorporated with the nanoscale metal oxide matrixes with high specific surface area have been designed and proposed as efficient peroxidase mimics, achieving enhanced catalytic performances. For instance, Au-Pt/SiO_2 nanocomposites [11], Palladium nanoparticles/$CoFe_2O_4$ nanotubes [12], CeO_2/TiO_2 nanotubes [13] were exploited.

As an important p-type metal oxide semiconductor, NiO nanomaterials received considerable interest of researchers, and were used in many fields including catalysis [14], supercapacitors [15], lithium ion batteries [16] and adsorbents in water treatment [17]. Recently,

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mesoporous nickel oxide nanomaterials were also demonstrated to be artificial peroxidase mimics. In order to enhance its catalytic performance, the combination of other metal oxides and the strong interaction of metal oxides might enable the NiO-based nanocomposites an efficient peroxidase mimic. As one of the most interesting rare earth oxides in catalysis, CeO_2 was an efficient catalyst itself or structural and electronic promoter in many chemical processes [18,19]. Particularly, CeO_2 could mimick the catalytic ability of redox enzymes, which arised from the fast $Ce^{4+} \leftrightarrow Ce^{3+}$ redox switch [20,21]. Therefore, the introduction of CeO_2 on NiO nanomaterials support with high specific surface area might supply a feasible strategy to increase the catalytic activity of NiO.

In the present work, CeO_2 was successfully introduced into the coral-like NiO nanostructures assembled by nanoflakes. The structural characteristics and peroxidase-like activities of CeO_2/NiO nanocomposites were evaluated. And their catalytic kinetics and mechanisms were further investigated in detail. Additionally, with the enhanced peroxidase-like activity of CeO_2/NiO nanocomposite, a new facile colorimetric method with wide linear range, low detection and fast response for H_2O_2 determination was developed.

2. Experiments

2.1. Materials

$Ce(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, urea, $Na_2HPO_4 \cdot 2H_2O$, citric acid and H_2O_2 were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). 3,3′,5,5′-tetramethylbenzidine (TMB) and horseradish peroxidase (HRP) were obtained from Sigma-Aldrich (St. Louis, USA).

2.2. Synthesis of CeO_2/NiO nanocomposites

For the synthesis of CeO_2/NiO nanocomposites, 5 mmol $Ni(NO_3)_2 \cdot 6H_2O$ and different amount of $Ce(NO_3)_2 \cdot 6H_2O$ with mole ratios (99:1, 98:2, 96:4, 94:6 and 92:8), 5 mmol urea were dissolved in 200 mL water, stirred to form a transparent solution. The above solution was transferred to autoclave and then heated at 120 °C for 6 h. The synthesized products were obtained by centrifugation, washed with water and ethanol three times respectively, and dried at 60 °C overnight. Finally, the CeO_2/NiO nanocomposites were obtained by calcination in air at 300 °C for 2 h under ambient conditions, and denoted as CeO_2/NiO-1, CeO_2/NiO-2, CeO_2/NiO-4, CeO_2/NiO-6, and CeO_2/NiO-8, respectively. In the absence of $Ce(NO_3)_2 \cdot 6H_2O$ or $Ni(NO_3)_2 \cdot 6H_2O$, the pure NiO or CeO_2 products was synthesized with the above similar procedure.

2.3. Characterization

X-ray diffraction (XRD) patterns were measured by a D8 ADVANCE X-ray diffractometer (Bruker, Germany) using Cu Kα radiation ($\lambda = 1.5418$ A). The morphology were performed by a SU-8000 scanning electron microscopy (Hitachi, Japan) and a Tecnai G2 F30 transmission electron microscope (FEI, USA). N_2 adsorption–desorption isotherms were obtained with an ASAP 2020 Physisorption Analyzer (Micromeritics, USA), and the specific surface areas were calculated by the Brunauer–Emmett–Teller (BET) method. X-ray photoelectron spectroscopy (XPS) was carried out on an AXIS Ultra DLD X-ray photoelectron spectroscopy (Shimadzu, Japan). The cerium content was measured by an Optima 8000 ICP-OES inductively coupled plasma atomic emission spectroscopy (ICP-AES) (PerkinElmer, USA). The detailed procedure was as follows. 100 mg CeO_2/NiO nanocomposites and 20 mL of HNO_3: $HClO_4$ (5: 1 v/v) were placed in a beaker and heated slowly with a hot-plate. The above solution was evaporated almost completely, transferred to a 100 mL flask with diluted HNO_3 (1% v/v), and finally measured by ICP-AES with the spectral line of cerium at 413.765 nm.

2.4. Peroxidase-like activity

The peroxidase-like activities of the CeO_2/NiO nanocomposites were measured in phosphate-citric buffer (3 mL, 100 mM, pH 4.5) in presence of bisubstrates (H_2O_2 and TMB) with addition of the CeO_2/NiO nanomateirlas as the catalysts. The reaction systems were monitored with wavelength-scan mode or time-drive mode at 652 nm using a Cary 300 UV–Vis spectrophotometer (Varian, USA). The apparent steady-state reaction rates were obtained by the molar absorption coefficient of 39,000 M^{-1} cm^{-1} at 652 nm for TMB-derived oxidation products and the initial linear range of the catalytic kinetic curves [6]. And these obtained reaction rates were fitted to the Michaelis–Menten equation to calculate the kinetic parameters:

$$v = \frac{V_{\max} \cdot [S]}{K_m + [S]} \tag{1}$$

where v was the reaction velocity, $V_{\max}$ was the maximal reaction velocity, $[S]$ was the concentration of substrate (H_2O_2 or TMB) and K_m was the Michaelis constant.

2.5. Electrochemical studies

The glass carbon electrode (GCE, 3.0 mm in diameter) were firstly polished with metallographic sandpaper and alumina slurry, and then cleaned ultrasonically in diluted nitric acid, ethanol and distilled water successively. NiO or CeO_2/NiO nanocomposites were dispersed into ethanol by ultrasonic dispersion to obtain the suspension solution (12 mg mL^{-1}). And 4 μL suspension was then dropped on the pretreated GCE and allowed to dry. Then, 2 μL nafion solution (0.5 wt%) was dropped on the layer of NiO or CeO_2/NiO nanocomposites and dried. Cyclic voltammetry (CV) measurements were performed on Epsilon Electrochemical Workstation (BAS, USA). A three-electrode system comprising a platinum wire counter electrode as auxiliary, a Ag/AgCl electrode as reference and the NiO or CeO_2/NiO nanocomposites modified GCE as working electrodes was used for the above electrochemical experiments with 5 mM H_2O_2 in 100 mM phosphate–citric containing 100 mM KCl buffer (pH 4.5).

Electrochemical impedance spectroscopy (EIS) was measured using an Autolab (Netherlands) in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]$ which served as a redox probe, in the frequency range from 100 KHz to 0.1 Hz at an open circuit potential, with the amplitude of sine voltage of 10 mV. A three-electrode system mentioned above was used for the ESI experiments.

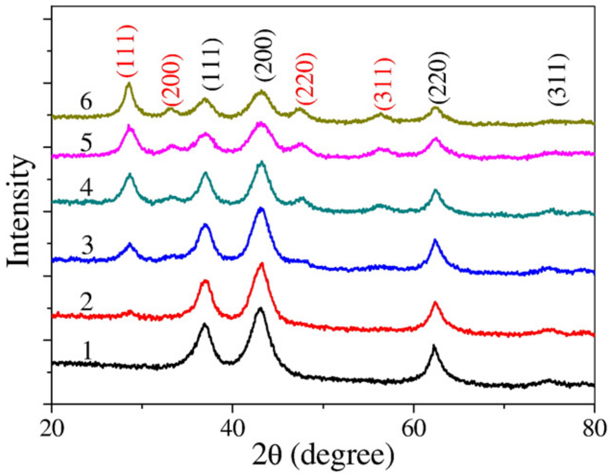

Fig. 1. XRD patterns of NiO and CeO_2-NiO nanocomposites. 1, NiO; 2, CeO_2/NiO-1; 3, CeO_2/NiO-2; 4, CeO_2/NiO-4; 5, CeO_2/NiO-6 and 6, CeO_2/NiO-8.