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Electrogenerated chemiluminescence of ZnO nanorods and its sensitive detection of cytochrome C

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ABSTRACT

Various kinds of ZnO nanoparticles have been successfully used in gas sensing applications, however, these nanomaterials have been rarely investigated in electrogenerated chemiluminescence (ECL). In the present work, ZnO nanorods (ZnONRs) were synthesized by hydrothermal method, and characterized by field emission scanning electron microscopy (FE-SEM) and X-ray diffraction (XRD). ECL behaviors of ZnONRs were investigated in neutral aqueous condition in the presence of $K_2S_2O_8$. Cyclic voltammetry (CV) results revealed that ZnONRs can react with $K_2S_2O_8$ to generate strong light emission, revealing that $K_2S_2O_8$ can act as coreactant of ZnONRs ECL. ZnONRs synthesized under different pH conditions exhibited different ECL intensities, and the most intense ECL signal was obtained at pH 7.0. Cytochrome C could compete with ZnONRs to react with $K_2S_2O_8$, and exhibited apparent inhibiting effect on ZnONRs ECL, which can be sensitively detected in the range of 1.0×10^{-11}–5.0×10^{-9} mol L^{-1}, with a detection limit of 4.7×10^{-12} mol L^{-1} (3σ). The present ECL system exhibited high sensitivity and good stability, which is suitable for the fabrication of novel ECL sensors.

1. Introduction

In recent years, semiconductor nanoparticles have attracted extensive attention because of their size-dependent electronic and optical properties. Electrogenerated chemiluminescence (ECL) has been considered as a useful technique for the analytical application of semiconductor nanoparticles [1–5]. The semiconductor nanoparticles can be electrochemical excited to generate the reduced or the oxidized states of nanoparticles, which can react with some coreactants to produce ECL signal. The electron-transfer reaction between the electrochemically excited nanoparticles and coreactants implies that nanoparticles have great potential in developing novel ECL sensors. Up to date, various ECL systems have been established based on semiconductor nanoparticles, which greatly spurred the biosensing application of ECL technique [6]. However, most of those ECL systems focused on the small size nanoparticles (e.g. quantum dots), and the ECL of larger size semiconductor nanoparticles has been rarely reported.

Among the wide variety of metal-oxide semiconductor nanoparticles, ZnO nanoparticles (ZnONPs) provoked the interesting research efforts in the past few decades due to its remarkable characteristics, such as functional catalytic and optical properties, high chemical stability, electrochemical activity, biocompatibility, and fast electron transfer kinetic functions [7–16]. According to these unique properties, ZnONPs are considered as an excellent platform for the construction of electrochemical and ECL biosensors [16–20]. Several groups reported that ZnONPs exhibited excellent catalytic effect on ECL investigation. For example, Haghighi et al. reported that ZnO/MWCNTs hybrids exhibited excellent electrocatalytic activity towards luminol ECL, which can be used to fabricate ECL biosensor for lactate [16]. Cheng et al. reported that ZnONPs can catalyze luminol-H_2O_2 ECL system, and can be used to fabricate a sandwiched luminol ECL immunosensor [21]. Although ZnONPs exhibited good catalytical effect on the traditional luminescent system, its own ECL behavior has not been reported.

Cytochrome C (Cyt C) is one of heme protein involved in mitochondrial electron transfer. Therefore, it is important to develop the sensitive detection method for Cyt C in bioanalysis. Up to date, several techniques including electrochemistry, fluorescence, and ECL have been employed in the detection Cyt C. Among these methods, ECL has special advantages over other techniques, such as high sensitivity and simple instrument [22–25]. Herein, ECL behaviors of ZnO nanorods (ZnONRs) were investigated with $K_2S_2O_8$ as coreactant. Strong cathodic ECL signal was obtained in neutral condition. Cyt C exhibited apparent inhibiting effect on ZnONRs ECL, and can be sensitively detected.

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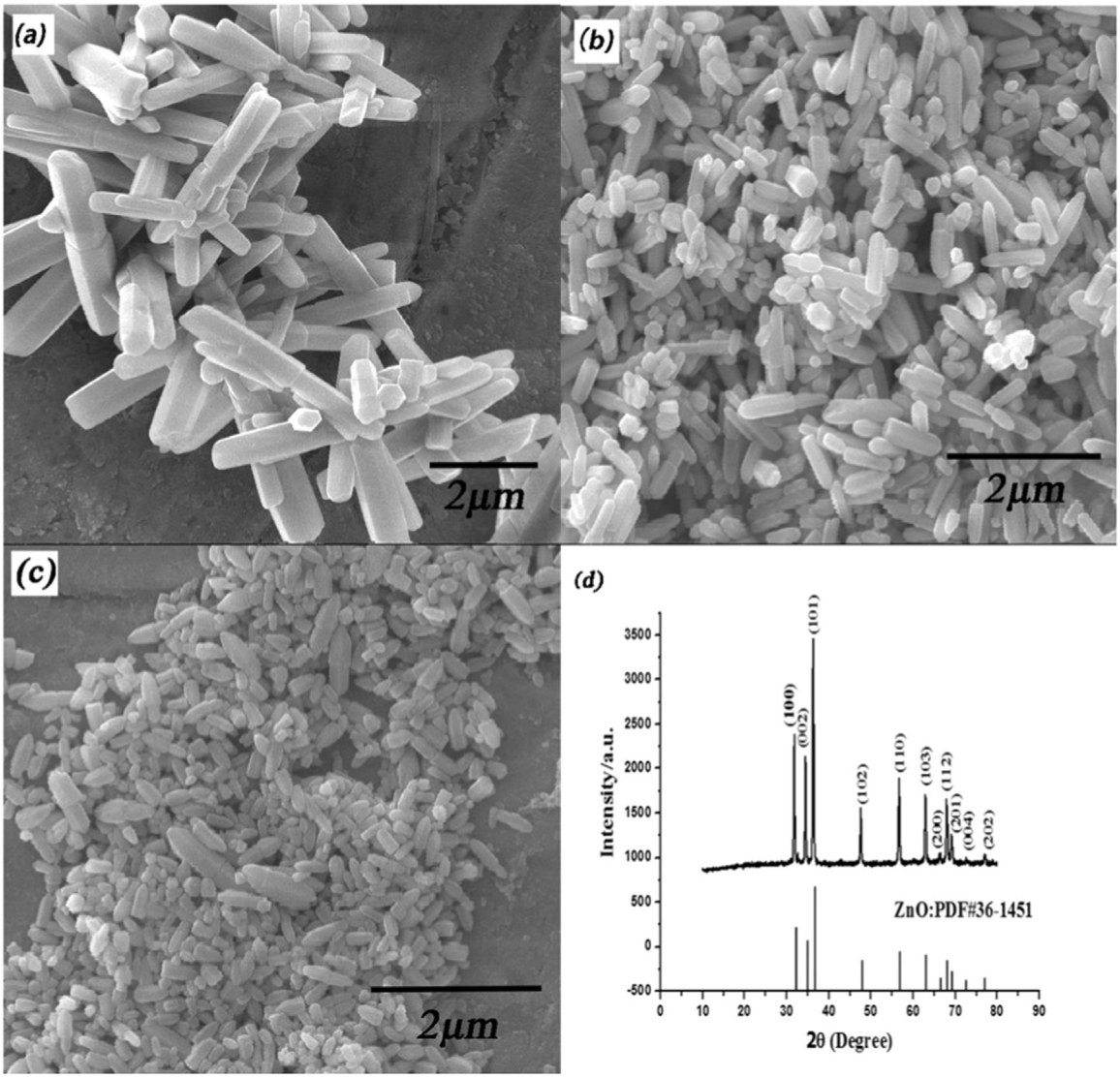

Fig. 1. SEM images of ZnONRs synthesized at pH 6.0 (a), 7.0 (b), 8.0 (c) and XRD of ZnONRs (d).

2. Experimental

2.1. Chemicals

Zinc nitrate hexahydrate, sodium hydroxide, potassium peroxydisulfate, potassium hexacyanoferrate, potassium ferrocyanide, glucose, and L-cysteine hydrochloride monohydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. Cytochrome C (from bovine heart, ≥ 95%) and ascorbic acid were purchased from Aladdin. All other regeants are of analytical grade. Double-distilled water was used throughout.

2.2. Synthesis of ZnO nanorods

The zinc oxide nanorods (ZnONRs) were synthesized according to the reported method with minor modification [21]. In a typical experiment, 2.5 g $Zn(NO_3)_2{\cdot}6H_2O$ were dissolved in double distilled water and then 1 mol L^{-1} NaOH was slowly dripped into solution to adjust the pH (6.0, 7.0, 8.0), and 40 mL mixture was obtained which was stirred for 30 min at the same time, and sequentially was dispersed in a ultrasonic cleaner for 20 min. Then the mixture was sealed into a Teflon equipped stainless steel autoclave, which was placed in a drying oven followed by hydrothermal treatment at 180 °C for 12 h. The product was filtered and washed with ethanol solution and double distilled water, which were finally dried at 60 °C for 24 h.

2.3. Preparation of ZnONRs modified electrode

5 wt% Nafion was diluted with ethanol to 0.5 wt%. 10 mg of ZnONRs was dispersed in 1 mL double distilled water with ultrasonication for 30 min to obtain homogeneous, well-distributed suspension of ZnONRs. A glassy carbon electrode (GCE, 3 mm in diameter) was mechanically polished with alumina pastes of 0.3 μm, and cleaned thoroughly in an ultrasonic cleaner with alcohol and water sequentially. Then, the electrode was cycled in 1 mM $K_3Fe(CN)_6$ solution between − 0.20 and 0.60 V (vs SCE) at a potential scan rate of 100 mV s^{-1} until a pair of reversible peaks was obtained, indicating that the electrode surface was cleaned. After it was dried with blowing N_2, 10 μL of ZnONRs suspension was spread on the working electrode and dried at the room temperature to fabricate ZnONRs modified GCE (denoted as ZnONRs/GCE). Finally, 10 μL of 0.5 wt% Nafion solution was casted on the ZnONRs film to improve the stability of the modified electrode.

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