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ORIGINAL ARTICLE

Cyclic voltammetry, square wave voltammetry, electrochemical impedance spectroscopy and colorimetric method for hydrogen peroxide detection based on chitosan/silver nanocomposite

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Abstract In this paper, we demonstrate a promising method to fabricate a non-enzymatic stable, highly sensitive and selective hydrogen peroxide sensor based on a chitosan/silver nanoparticles (CS/AgNPs) hybrid. Using this composite, we elaborated both electrochemical and colorimetric sensors for hydrogen peroxide detection. The colorimetric sensor is based on a homogenous reaction which fades the color of CS/AgNPs solutions from red-orange to colorless depending on hydrogen peroxide concentration. For the electrochemical sensor, CS/AgNPs were immobilized on glassy carbon electrodes and hydrogen peroxide was measured using cyclic voltammetry, square wave voltammetry and electrochemical impedance spectroscopy. The response time is less than 10 s and the detection limit is 5 μ M.

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1. Introduction

Hydrogen peroxide (H_2O_2) detection is of great significance because of wide industrial applications and vital role in many different fields such as environmental, food, pharmaceutical and clinical analysis. Moreover, H_2O_2 is involved in several biological events and intracellular pathways. It is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states linked to several diseases. Therefore, rapid and accurate determination of hydrogen peroxide is of crucial practical importance in various fields such as food, clinical and environmental analysis (Fang et al., 2014;

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Tao et al., 2014; Yu et al., 2013; Kumar et al., 2014; Wang et al., 2013; Xia et al., 2014). Currently, several analytical techniques for determination of H_2O_2 have been reported, including fluorimetry (Vasicek et al., 2011; Palamakumbura and Trackman, 2002), chemiluminescence (Falcó et al., 2001; Fletcher et al., 2001), spectrophotometry (Sunil and Narayana, 2008; Mahmoodi et al., 2005) and various electrochemical methods (Chen et al., 2012; Zhu et al., 2007). Compared to other available methods, electrochemical sensing offers a fast and cost-effective approach for sensitive determination of H_2O_2 (Jakubec et al., 2015; Tian et al., 2014).

Hydrogen peroxide sensors can be achieved by either enzyme-based or enzyme-less approaches. The former includes immobilization of enzymes (such as horseradish peroxidase, HRP) in a host material or on a surface. However, enzymes are generally less active when immobilized, and their turnover depends on a number of parameters such as pH, temperature or the presence of toxic chemicals or even ions (Khan and Bandyopadhyaya, 2014). Enzyme-less sensors can well avoid such disadvantages, more particularly instability and poor reproducibility, providing an effective way for improving electrocatalytic detection of H_2O_2 (Tian et al., 2014).

Non-enzymatic H_2O_2 sensors are usually achieved based on transfer metal oxides, such as Cu_2O (Liu et al., 2013), CuO (Gao and Liu, 2015; Song et al., 2010), MnO_2 (He et al., 2014), $CoOOH$ (Lee et al., 2013), titanium silicalite-1 zeolite microparticles (TSZMs) Liu et al., 2011 or using noble metal nanoparticles (NPs), such as Au Zhang et al. (2013a,b), Ag (Zhao et al., 2013, 2009; Habibi and Jahanbakhshi, 2014) or Pt (Heli et al., 2014) which have received increasing attention due to their high electrocatalytic activities (Chen et al., 2012; Zhou et al., 2012).

Among these noble metal NPs, silver nanoparticles (AgNPs) have aroused growing interest in applications because of their unique properties of biocompatibility, low toxicity and sustainable electrocatalytic activity (Tian et al., 2014). However, strong van der Waals force between AgNPs causes severe aggregations, resulting in a sharp loss in available electrochemical activity and detection sensitivity. In response to the aggregation problem, AgNPs are usually immobilized on various organic/inorganic support materials (Wang et al., 2013; Zhao et al., 2013, 2009; Habibi and Jahanbakhshi, 2014; Zhong et al., 2013), which are confirmed to be an effective strategy in protecting these metal NPs against agglomeration and improving their electrocatalytic activity and stability, such as AgNPs/mesoporous carbon (Habibi and Jahanbakhshi, 2014), AgNPs/graphene (or graphene oxide) Wang et al., 2013; Tian et al., 2014; Zhao et al., 2013; Zhong et al., 2013, AgNPs/carbon nanotube (Zhao et al., 2009) or AgNPs/chitosan (Tran et al., 2016). For this purpose, chitosan was chosen because it was already described as an efficient green reducing reagent and stabilizer for synthesis of AgNPs (Tran et al., 2010). It also plays a role in the AgNPs immobilization on the electrode surface (Tran et al., 2016). Moreover, chitosan is a bio-polymer which presents good compatibility with biomolecules, and contains many amino groups which could eventually be used for anchoring of biomolecules (Tran et al., 2011).

In this work, we propose a simple approach for fabrication of a sensitive and selective H_2O_2 sensor, which can be used for several electrochemical detections (electrochemical impedance spectroscopy, square wave voltammetry or cyclic voltammetry) or for optical detection (spectrophotometry), thanks to the electrocatalytic property of a chitosan/AgNPs hybrid materials which efficiently and selectively catalyze H_2O_2 reduction.

2. Experimentals

2.1. Chemicals and reagents

Chitosan (CS, MW = 400,000 g mol⁻¹, degree of acetylation DA = 70%) was prepared by deacetylation of chitin (Tran

et al., 2010). H_2O_2 , phosphate buffered saline (PBS), glucose, ascorbic acid and galactose were purchased from Sigma Aldrich. All chemicals were of analytical grade. Glassy carbon electrodes (GCE, 3 mm diameter, $S = 0.07 \text{ cm}^{-2}$) were purchased from BAS Inc (Japan).

2.2. Synthesis of CS/AgNPs

CS/AgNPs were synthesized following (Tran et al., 2010) with minor modifications. 25 mL of a fresh solution of 0.1 M $AgNO_3$ was added into 100 mL of 1% w/w CS dissolved in 1% w/w acetic acid/water mixture. This colorless solution was heated to 90 °C for 6 h to obtain an orange-yellow CS/AgNPs colloid, then cooled down to room temperature and centrifuged at 10,000 rpm for 30 min to eliminate the biggest CS/AgNPs particles. The supernatant constitutes the CS/AgNPs stock solution with a AgNPs concentration of 2.16 g L⁻¹. Before use, 10 mL of stock solution was diluted in 90 mL of deionized (DI) water.

2.3. Immobilization of CS/AgNPs on GCE

Glassy carbon electrodes (GCEs) were first polished on wet silicon carbide paper using 1 μm and 0.05 μm Al_2O_3 powders sequentially, and then washed with DI water followed by ethanol for 2 min. GCEs were afterward modified with chitosan/silver nanoparticles (CS/AgNPs) hybrid by drop-casting: a 5 μL drop of diluted CS/AgNPs solution was casted onto the GCE surface and allowed to dry at ambient temperature.

2.4. Electrochemical measurements

All electrochemical experiments were performed using a PGSTAT30 potentiostat (Metrohm Autolab B.V.) coupled with GPES software. A conventional three electrode cell configuration was employed. CS/AgNPs-modified GCEs were used as working electrodes, while a saturated Ag/AgCl electrode (Radiometer Analytical SAS, France) and a platinum wire served as reference and counter electrodes, respectively. Prior to electrochemical measurements, solutions were deoxygenated by purging with pure argon. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used for electrochemical measurements. For CV, the potential was scanned from -0.1 V to -1 V (vs. SCE) at a scan rate of 50 mV s⁻¹ in 0.1 M degassed PBS. For SWV, the following parameters were used: pulse height 50 mV, pulse width 50 ms, scan increment 2 mV, and frequency 12.5 Hz.

EIS was performed using an Autolab PGSTAT30 equipped with the FRA module. Impedance spectra were recorded in 0.1 M PBS buffer at room temperature at a fixed potential within a frequency range from 10 kHz to 100 mHz with a perturbation amplitude of 10 mV. As a pretreatment before each experiment, a constant potential corresponding to the one used for EIS was imposed, for 120 s.

All electrochemical experiments were performed at 25 °C under stirring. Electrochemical signals (CV, SWV, EIS) of CS/AgNPs-modified GC electrodes were first recorded in pure PBS, and then H_2O_2 was added and let to react for 10 s before another electrochemical measurement was done.

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