Talanta 137 (2015) 18–24

Contents lists available at ScienceDirect

Talanta

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

# Anamperometric superoxide anion radicalbiosensor based on SOD/PtPd-PDARGO modified electrode

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A R T I C L E I N F O

*Article history:*
Received 24 October 2014
Received in revised form
4 January 2015
Accepted 6 January 2015
Available online 13 January 2015

*Keywords:*
Superoxide anion radical
PtPd NPs
PDARGO
Superoxide dismutase

A B S T R A C T

In the present work, a high-performance enzyme-based electrochemical sensor for the detection of superoxide anion radical ($O_2^{\bullet -}$) is reported. Firstly, we employed a facile approach to synthesize PtPd nanoparticles (PtPd NPs) on chemically reduced graphene oxide (RGO) coated with polydopamine (PDA). The prepared PtPd-PDARGO composite was well characterized by transmission electron microscopy, scanning electron microscopy, Fourier transform infrared spectra, X-ray diffraction, X-ray photoelectron spectroscopy and electrochemical methods. Then the assembled composite was used as a desired electrochemcial interface for superoxide dismutase (SOD) immobilization. Owing to the PDA layer as well as the synergistic effect of PtPd NPs, the fabricated SOD/PtPd-PDARGO sensor exhibited an outstanding sensitivity of 909.7 μA $mM^{-1}$ $cm^{-2}$ upon $O_2^{\bullet -}$ in a linear range from 0.016 mM to 0.24 mM ($R^2=0.992$), with a low detection limit of 2 μM (S/N=3) and excellent selectivity, good reproducibility as well as favorable long-term stability.

## 1. Introduction

Oxygen-derived free radicals, known as reactive oxygen species (ROS), are closely implicated in many pathological and physiological processes [1]. Among various ROS, superoxide anion radical ($O_2^{\bullet -}$) is considered as a defense against viral or bacterial attack as well as a damage towards proteins, DNA and lipidosomes in human body [2,3]. Therefore, quantitative analysis of $O_2^{\bullet -}$ turns to be of great importance to reveal the relevance of many diseases caused by excessive generation of $O_2^{\bullet -}$. To date, a variety of methods have been developed to monitor $O_2^{\bullet -}$, including electron spin resonance [4], chemiluminescence [5], spectrophotometric [6], triple quadrupole mass spectrometry [7] and colorimetry [8]. However, these methods cost much and usually occupy too much space. Recently, enzyme-based electrochemical sensors [9–14] appear to be a hotspot for determining $O_2^{\bullet -}$, because they can provide desired analytical performance with high reliability, sensitivity and selectivity as well as the preparation simplicity and capability in terms of direct and real-time detection. With the ability to catalyze the dismutation of $O_2^{\bullet -}$ to $O_2$ and $H_2O_2$ (employed as a biomarker) through a cyclic oxidation-reduction electron-transfer mechanism [15], copper, zinc-superoxide dismutase(Cu, Zn-SOD) is the most widely used enzyme to be immobilized onto the electrode surface to fabricate the electrochemical $O_2^{\bullet -}$ biosensor [16].

Noble metals, such as Pt, Pd and Au, have become the ideal material to construct an electrochemical sensor, for its favorable electrochemical stability and prominent catalytic activity [17]. However, monometal materials are known to be easily poisoned by absorbed intermediates, which would lead to a certain amount of loss in sensitivity and stability [18]. Moreover, many redox-active species including ascorbic acid, dopamine and uric acid in the physiological condition could significantly affect the detection performance of those monometal-based sensors [19,20]. Thus, intense research interest has been focused on eliminating these drawbacks [21,22]. Modifying noble metals with ad-metals to prepare bimetallic nanoparticles seems to be a promising solution [23]. Researches imply that bimetallic nanoparticles including PtPd [24,25], PtAu [26,27] have showed their capability of providing enhanced electrocatalytic as well as improved anti-interferential performance due to the beneficial synergistic effects.

In recent decades, graphene, a $sp^2$-bonded carbon sheet with a thickness of a single atom, has received great attention due to its excellent mechanical, thermal, optical and electrical properties [28]. These properties make it as an appealing candidate for fabricating various functional devices, such as electrodes, sensors, photovoltaics and photodetectors. However, the chemically reduced graphene oxide

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http://dx.doi.org/10.1016/j.talanta.2015.01.009
0039-9140/

(RGO) tends to form irreversible agglomerates or even restack to graphite through strong π–π stacking and vander Waals interaction, which limits its application [29]. Therefore, numerous energy has been devoted into graphene modification to meet the requirements for the enhanced performance of biosensors [30].

Recently, a thin surface adherent and multi-functional polydopamine (PDA) layer has been prepared on awide variety of substrate through simple dip-coating with dopamine solution to cause the self-polymerization of dopamine [31]. This approach could be one of the solutions to reduce the agglomeration of graphite. Inspired by this idea, we prepared a superoxide anion radical sensor based on well-dispersed PtPd bimetallic nanoparticles (PtPd NPs) supported on the PDA modified reduced graphene oxide (RGO) nanocomposite (denoted as PtPd-PDARGO). The polymerization of dopamine offers the advantage of a one-step surface functionalization and allows the introduction of adhesive materials such as noble metals and metal oxide [32]. PtPd NPs were grown on PDARGO by a simple chemical reduction method. Compared to the bare GO and monometal modified materials, the PtPd-PDARGO composite, for the application of PDA to modify GO and synergistic effect of Pt and Pd, greatly improve the electrochemical proprieties and sensing ability. The fabricated enzyme sensor seems to be an ideal platform to detect $O_2^{\bullet-}$ with high sensitivity, short response time and excellent selectivity.

## 2. Experimental

### 2.1. Materials and reagents

Graphite powder (99.85%) was purchased from Shanghai Huayi Co. Ltd. Dopamine hydrochloride (DA) was obtained from Sigma. $H_2PtCl_6 \cdot 6H_2O$, $(NH_4)_2PdCl_4$, $KO_2$, DMSO, 18-Crown-6, uric acid (UA), 4-acetaminophen (AP) and glucose were bought from Aladdin Industrial Inc. Tri(hydroxymethyl) aminomethane (Tri), $NaBH_4$, ascorbic acid (AA) were from Sinopharm Chemical Reagent Co. Ultrapure water (18.2 MΩ cm, Laboratory Water Purification Systems) was used in all experiments. All other chemicals were of analytical grade and used directly without further purification.

### 2.2. Preparation of PDA modified reduced graphene oxide

The PDARGO composites were prepared according to previous literature with slight modification [32]. GO was prepared from commercial graphite powder using a modified Hummers method [33]. Then, PDARGO was prepared as follows. 20 mg GO was dispersed into water by ultrasonication for 30 min, followed by the addition of 20 mg dopamine. Subsequently the pH of the mixture was adjusted to 8.5 by using Tris–HCl solution, and the reaction mixture was stirred vigorously at room temperature for 24 h, where the dark brown suspension turned into a black solution. The black powders were recovered by lyophilization.

### 2.3. Preparation of PtPd NPs on PDA modified reduced graphene oxide

PtPd-PDARGO was prepared using $NaBH_4$ as the reduction agent. 10 mg PDARGO was dispersed into water by ultrasonication for 30 min, then $H_2PtCl_6$ and $(NH_4)_2PdCl_4$ (molar ratio=1:1) was put into the black suspension under stirring. Later, 12 mL 2 mg/mL freshly prepared $NaBH_4$ was added drop by drop to the suspension, and further reaction for 2 h. The black solids were washed thoroughly with water and obtained through lyophilization. For comparison, PtPd NPs, PtPd-RGO, Pt-PDARGO, Pd-PDARGO composites were prepared in the same condition with controlled revision.

### 2.4. Fabrication of SOD-based sensor

Screen-printed gold film electrodes (SPGE), as the substrate, were prepared based on our previous work [34], and the diameter of the working electrode is designed to be 3 mm. As for the SOD/PtPd-PDARGO sensor, 1 mg/mL PtPd-PDARGO was prepared, then 5 μL of the mixture, 3 μL of Cu, Zn-SOD (3000 U/mL) and 2 μL of 0.05 wt% Nafion were drop-casted onto the surface of the electrode, and dried at room temperature. The electrode was stored at 4 °C when not in use.

### 2.5. Generation of superoxide anion

A stock solution of $KO_2$ was prepared by adding $KO_2$ to DMSO containing 18-crown-6 by ultrasonication, where $KO_2$ dissociated and produced $O_2^{\bullet-}$. According to the molar absorptivity of $O_2^{\bullet-}$anions in DMSO (2006 $M^{-1}$ $cm^{-1}$ at 271 nm), the concentration of $O_2^{\bullet-}$ could be estimated by UV spectrophotometry [10]. The stock solution of $O_2^{\bullet-}$ is determined to be 40 mM.

### 2.6. Characterization

FTIR spectra were conducted at a Nicolet 6700 Spectrometer and transmission spectrum of the samples were obtained by forming thin transparent KBr pellet. X-ray diffraction (XRD) patterns were carried out on a Rigaku D/max-2550 diffractometer with Cu Ka radiation (($\lambda$=0.15408 nm) radiation). X-ray photoelectron spectroscopy (XPS) was performed using a Thermo Fisher ESCALAB 250Xi instrument. Transmission electron microscopy (TEM) images were obtained on a JEOL JEM-1400 microscope. Scanning electron microscopy (SEM) images were collected on a JEOL JSM-6360LV microscope. Ultraviolet-Visible spectroscope (UV–vis, EVOLUTION 220, Thermo Scientific) was used to detect the concentration of $O_2^{\bullet-}$ obtained from the $KO_2$ stock solution.

### 2.7. Electrochemical measurements

All electrochemical measurements were performed on a CHI 660D electrochemical workstation. A three-electrode system was employed throughout the experiments with a modified SPGE as working electrode, a platinum wire as auxiliary electrode, and an Ag/AgCl (3 M KCl) electrode as reference electrode.

## 3. Results and discussion

### 3.1. Synthesis and characterization of the PtPd-PDARGO composites

The PDARGO was prepared by the simultaneous self-polymerization of dopamine and the reduction of GO with dopamine hydrochloride at pH of 8.5 adjusted by Tri-HCl. Then the PDARGO was employed as the substrate to load PtPd NPs through a facile route by using $NaBH_4$ as the reduction agent.

The morphology of PDARGO is presented in Fig. 1(A), a clean sheet with plenty of wrinkles was observed, owing to the thin structure of the sheet. Fig. 1(B) shows the FTIR spectra of GO and PDARGO, compared to PDARGO, the band at ~3200 $cm^{-1}$and ~1720 $cm^{-1}$ attributed to –OH group and –CO group of GO have disappeared, revealing that GO was successfully reduced by dopamine. Moreover, in the spectrum of PDARGO, a strong band at ~3400 $cm^{-1}$ attributed to –NH group indicates that PDA was self-polymerized to modify the surface of RGO [35]. The formation of PDARGO originating from GO was also characterized by XRD. As shown in Fig. 1(C), the sharp diffraction peak of GO ($2\theta$=9.3°) has obviously decreased and a new diffraction peak ($2\theta$=28.4°) has appeared in the PDARGO, which is close to the typical diffraction