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An Electropolymerized Rhodamine B sensing Film-Based Electrochemical Sensor for Nitrite with High Sensitivity and Selectivity

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The fabrication of an electropolymerized Rhodamine B (PRhB) sensing film based electrochemical sensor and its application for electrochemical detection of nitrite are described. The PRhB film modified GC electrode exhibited good catalytic activity towards the electrochemical oxidation of nitrite. The effects of the experimental variables such as the thickness of the film, solution pH values and the working potential were investigated using steady-state amperometry. The present sensor (PRhB-modified electrode) had a fast response towards NO_2^- (less than 10 s), and excellent linear relationships were obtained in the concentration range of 0.5 μM-7.0 mM, with a detection limit of 0.1 μM (S/N = 3). The sensitivity of the sensor was calculated to be 308.1 μA mM^{-1} cm^{-2}. The possible interferences from several common ions were tested. Moreover, the stability and reproducibility of this sensor were evaluated with satisfying results.

Keywords: Rhodamine B; Nitrite; Electropolymerization; Sensor; Amperometry

1. INTRODUCTION

Nitrite is present widely in environmental and food systems as additive and corrosion inhibitor. It is also a ubiquitous compound in physiological systems and has been well-known as a human health-hazard. Once introduced into the bloodstream, it can result in the irreversible conversion of hemogloblin to methemoglobin with oxygen uptake and transportation damaged, which may induce the "blue baby" syndrome [1]. Moreover, via reaction with secondary and tertiary amines under acidic conditions of the stomach, the excess uptake of nitrite would cause gastric cancer [2]. Therefore, the development of methods for quantitative determination of nitrite concentrations in various samples is of considerable significance, and has become the important subject of current analytical chemistry research.

Several techniques have been developed for nitrite determination, including spectrophotometry [3], chemiluminescence [4], chromatography [5] and capillary electrophoresis [6]. These methods offer good limits of detection and wide linear ranges. However, most of these techniques necessitate the use of sophisticated and high cost apparatus and require complicated operation. They are not suitable for on-line or field monitoring. Recent years have seen increasing interest in electrochemical sensors for nitrite due to their remarkable advantages such as high sensitivity, simple instrumentation, low production cost and promising response speed [7-15].

Quite a few reported electrochemical sensors for nitrite are based on the reduction of nitrite, which suffer from poor sensitivity and interference from other coexisting compounds [7-9]. Different from the electrochemical reduction of nitrite, the oxidation of nitrite is not interfered by nitrate ions and molecular oxygen, which are usually the major interferants in reduction determination of nitrite [10]. In additional, the electrochemical reduction of nitrite produces several products depending on the catalyst employed, while nitrate is the only final product of electrochemical oxidation for nitrite [11]. So, electrochemical oxidation of nitrite seems to be favorable for determination of this analyte. However, the oxidation of nitrite needs extremely high potentials, and a poor response can be obtained by using a commonly bare glassy carbon electrode. To overcome these shortages, the chemically modified electrodes have been developed, which can theoretically decrease the over-potential for nitrite oxidation and improve the sensitivity. For example, some electrochemically modified electrodes based on poly-Nile Blue [12], thionine/ACNTs [13], poly(3,4-ethylenedioxythiophene)/multi-wall carbon nanotubes [14], and Nafion/lead-ruthenate pyrochlore [15] have been proved to lower the working oxidation potentials for nitrite determination.

Polymers that possess high electrical conductivity have been of increasing scientific and technological interest since the late 1970s. A special interest is that of polymer films with direct charge transport features and which can therefore act as redox mediators for development of electrochemical sensors [16, 17]. Such films are usually prepared from azines by in situ electropolymerization of their monomers at a bare electrode [18]. Various azines have been reported for this purpose, including azur A [19, 20], methylene blue [21, 22], methylene green [23, 24], neutral red [25, 26], toluidine blue [27, 28], and phenosafranine [29, 30]. Rhodamine derivatives are a group of xanthene dyes that possess excellent spectroscopic properties such as large molar extinction coefficient, high fluorescence quantum yield, visible light excitation as well as long-wavelength emission, and have been used extensively as dyes laser source [31, 32] and fluorescent labeling reagents in bioanalytical chemistry [33, 34]. However, up-to-date, few PRhB based polymer films are reported as redox mediators for developing electrochemical sensors. In this paper, we developed a novel nitrite amperometric sensor based on an in situ electropolymerized PRhB film. The proposed PRhB sensing film exhibited a high electro-catalytic activity toward the oxidation of nitrite. It showed a fast response towards NO_2^- with high sensitivity and selectivity. Furthermore, the stability and reproducibility of this sensor were evaluated with satisfying results.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Rhodamine B was purchased from Shanghai Reagents (Shanghai, China). Phosphate buffer solution (PBS) (0.1 M, pH 3.5) was used as buffer solutions for other electrochemical experiments. Triply de-ionized water was used throughout all experiments. All other chemicals were of analytical grade and used without further purification.

The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter. Cyclic voltammetric and amperometric measurements were carried out on a CHI 760B electrochemical work station (Shanghai, China). A three-electrode cell (10 mL) was employed with the modified GC electrode as the working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum foil electrode as counter electrode. All the potentials given in this paper were referred to the SCE and all experiments were carried out at room temperature.

2.2. Preparation of electrochemical sensor

Prior to modification, the bare GCE was polished successively with 0.3, 0.05 μm α-alumina slurry, rinsed with doubly distilled water, and, finally, cleaned thoroughly in an ultrasonic cleaner with 1:1 nitric acid solution, alcohol, and doubly distilled water, sequentially. The GCE was then submerged in a 0.1 M K_2HPO_4-KH_2PO_4 (pH 5.0) solution containing 0.25 mM Rhodamine B and 0.3 M $NaNO_3$. A PRhB film was electrochemically deposited onto the pretreated electrode by cyclic voltammetry with the applied potential ranging from -1.4 to 1.8 V at a scan rate of 0.05 V s^{-1}. The thickness of the polymer could be easily controlled by the number of scans. The resulting electrode was rinsed with 0.1 M PBS (pH 7.0) and stored in the same solution for further use. The final electrode was denoted as PRhB/GCE.

3. RESULTS AND DISCUSSIONS

3.1. Electropolymerization of Rhodamine B at GC electrode surface

Electroactive polymer-based sensor prepared by in situ electropolymerization of dye monomers at the electrode surface can provide stable redox couples [35], so does Rhodamine B. In present work, the electrochemical polymerization process was carried out by cyclic potential sweep from -1.4 to 1.8 V at a scan rate of 50 mVs^{-1} in PBS (pH 5.0) containing 0.25 mM Rhodamine B and 0.3 M $NaNO_3$ at the bare GC electrode. As seen from Figure.1, the peak currents increase with the successive scans in the potential range from -1.4 to 1.8V, indicating that Rhodamine B is electropolymerizing on the GC electrode.

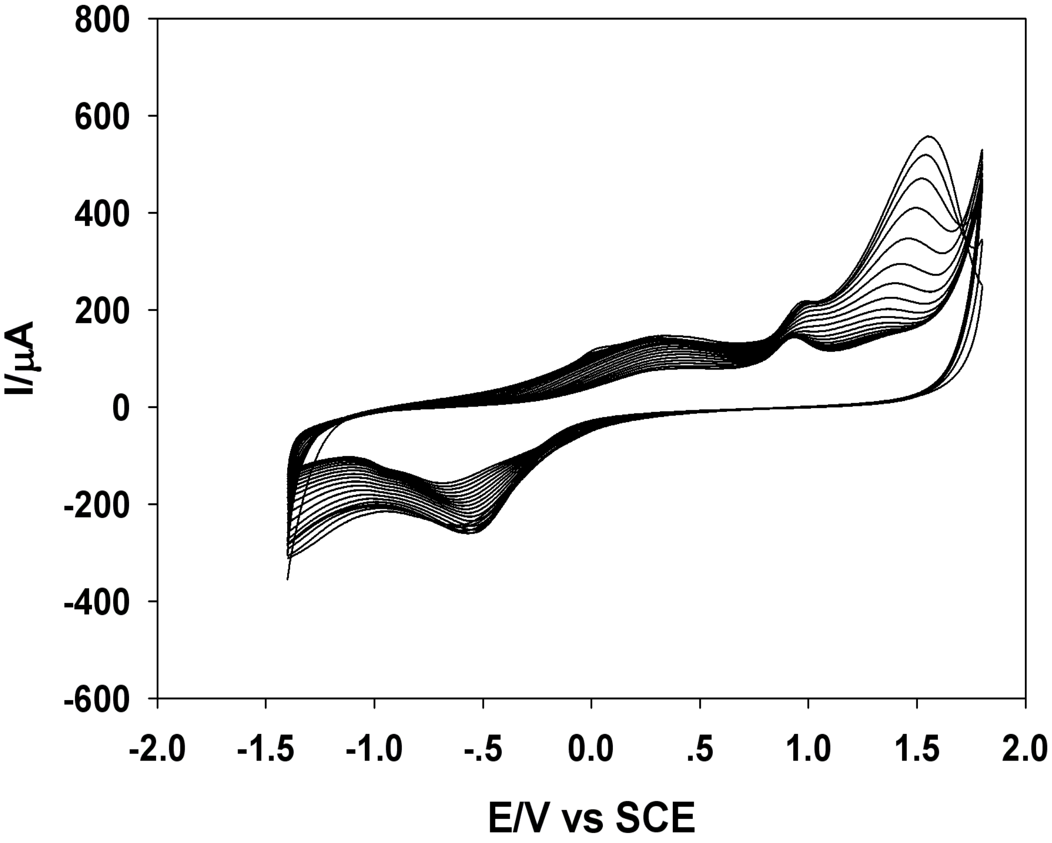

Figure 1. Cyclic voltammograms of 0.25 mM Rhodamine B at the GCE in 0.1 M PB (pH 5.0) containing 0.3 M $NaNO_3$ at a scan rate of 0.05 V s^{-1}.

3.2. Electrochemical responses of nitrite at PRhB film-based sensor

To investigate the electrochemical performance of the PRhB film-based sensor, cyclic voltammetry was employed over a potential range from -0.2 V to +1.2 V. As shown in Figure. 2A, the electrochemical responses of 3 mM $NaNO_2$ was investigated in PBS (pH 3.5) on bare GC electrode and PRhB film-based sensor (GC/PRhB sensor). A well-defined oxidation peak due to the electrochemical responses of $NaNO_2$ is observed at 0.96V when potential initially sweeps from -0.20 V to 1.20 V and no corresponding reduction peak is obtained on the reversal scan (curve b in Figure. 2A). It suggests that the electrochemical process of nitrite oxidation at GC/PRhB sensor is a totally irreversible reaction. Compared with poor voltammetric responses of $NaNO_2$ at about 1.02 V on bare GC electrode (curve c in Figure. 2A), the oxidation of $NaNO_2$ at GC/PRhB electrode was greatly improved. The electrocatalytic process could be expressed as follows:

$PRhB_{OX} + 2e^- + 2H^+ \rightarrow PRhB_{red}$

$PRhB_{red} + NO_2^- + H_2O \rightarrow NO_3^- + 2e^- + 2H^+ + PRhB_{OX}$

The potential scan rate is a key factor affecting the electrode process kinetics. The effects of scan rate (v) on the oxidation of 3 mM $NaNO_2$ in PBS (pH 3.5) were examined by cyclic voltammetry (CV) as shown in Figure. 2B. The anodic peak current (Ip) has a linear relationship with the square root of scan rate in the range of 0.01-0.15 Vs^{-1} (inset of Figure. 2B), It suggests that the nitrite oxidation process on GC/PRhB sensor is controlled by the diffusion step.

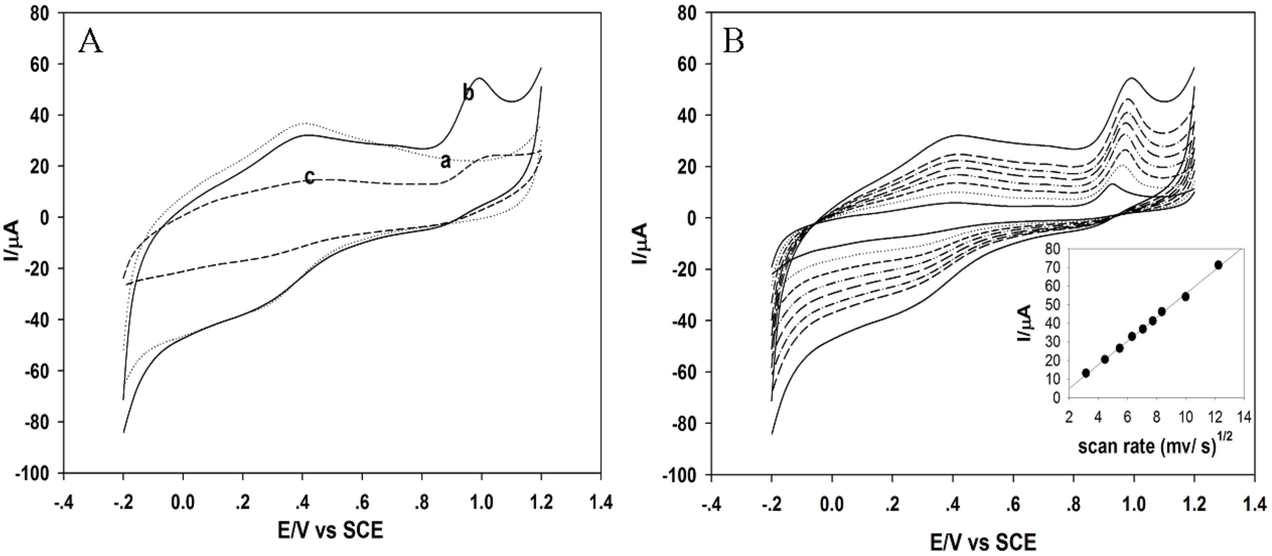

Figure 2. (A). Cyclic voltammograms of PRhB/GCE (a and b) and bare GCE (c) in the presence of 0 mM (a), 3 mM (b), and 3 mM (c) $NaNO_2$ in 0.1 M PBS (pH 3.5) at a scan rate of 0.1 Vs^{-1}; **(B).** Cyclic voltammograms of PRhB/GCE in 0.1 M PBS (pH 3.0) containing 3 mM $NaNO_2$ at different potential scan rates (from inner to outer: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.1, and 0.15 V s^{-1}). The inset shows the dependence of the redox peak currents on the square root of scan rates.

3.3. Optimization of Experimental Parameters

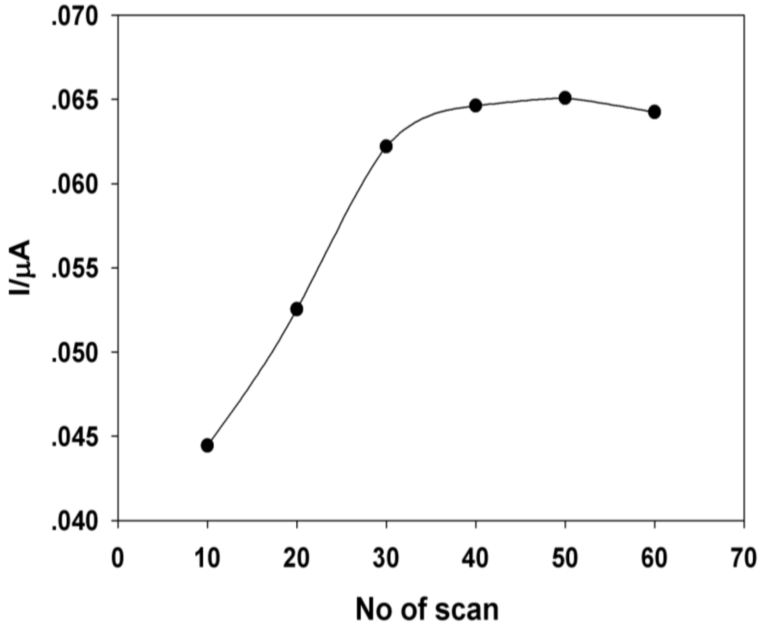

Figure 3. Effect of the scan number in the electropolymerization process on the amperometric response for 0.05 mM $NaNO_2$ in 0.1 M PBS (pH 3.5) at 0.95 V.

The effect of the thickness of the film on the anodic peak current of nitrite was also evaluated. As shown in Figure. 3, with the increase of the scanning number, the current response of the sensor in the nitrite solution increased. However, the response reached a maximum when the scanning number was 30. Further increasing the scanning number only resulted in a decrease of electrochemical response. This was probably because too thin a film possessed few catalytically active sites [36], while too thick a film would block the electron transfer and sites for electrochemical reactions on the surfaces of the electrodes [37], both cases might result in the small current response. Through the experiments, a potential cycling of 30 scans was chosen as the optimal electropolymerization for Rhodamine B.

In order to find the effect of the pH value of the PBS to the detection of nitrite, different pH value of the detection solution was investigated was also investigated and the corresponding result is shown in Figure.4. Apparently, current response of the sensor was greatly influenced by pH value. The current increased slightly as the pH changed from 2.0 to 3.5, following with a largely decrease in the pH range of 3.5-6.0. The maximum response was obtained at pH 3.5. Since the pKa of nitrite is 3.3, most nitrite ions are protonated in the acidic solutions. It is possible that protonation is involved in the catalytic reaction, so the catalytic peak current increases with the decrease of solution pH [38]. Therefore, pH 3.5 was used as the electrolyte in further work.

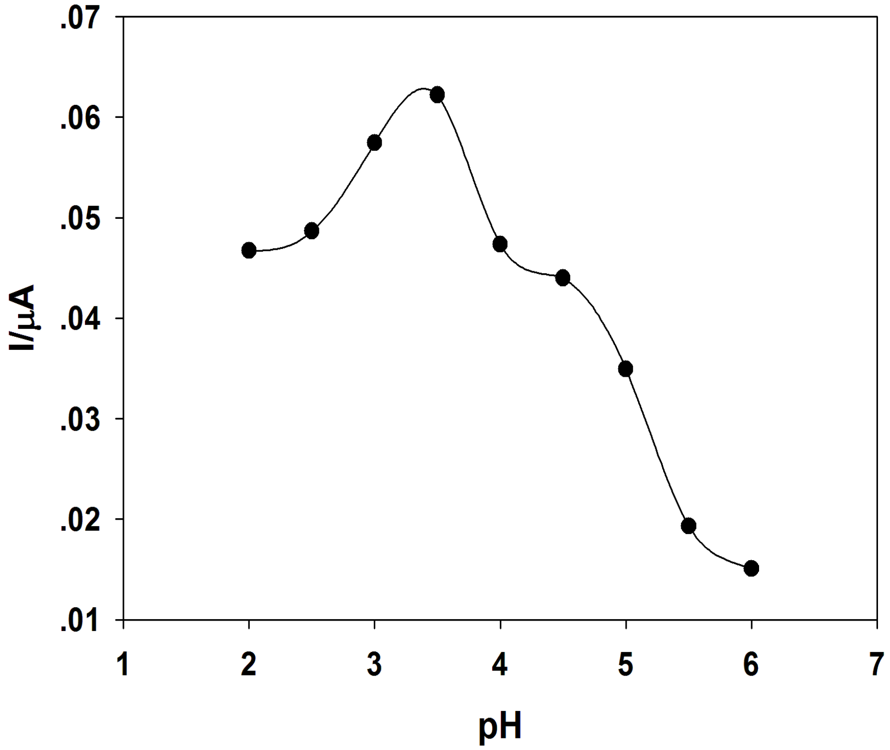

Figure 4. Effect of the pH value of PBS buffer solution to the detection of 0.05 mM $NaNO_2$.

It is well known that the applied potential strongly affects the amperometric response of a biosensor. We have systemically investigated the impact of applied potentials on the amperometric response of the PRhB-modified GC electrode to nitrite. As shown in Figure.5, electrooxidation of nitrite was observed starting at 0.8V, and the steady-state current increased slightly, with the applied potential changing from 0.8 to 1.1V. Using working potentials higher than 0.95 V, a higher signal was obtained, but the background current also increased distinctly. In this experiment, 0.95 V was selected as the working potential.

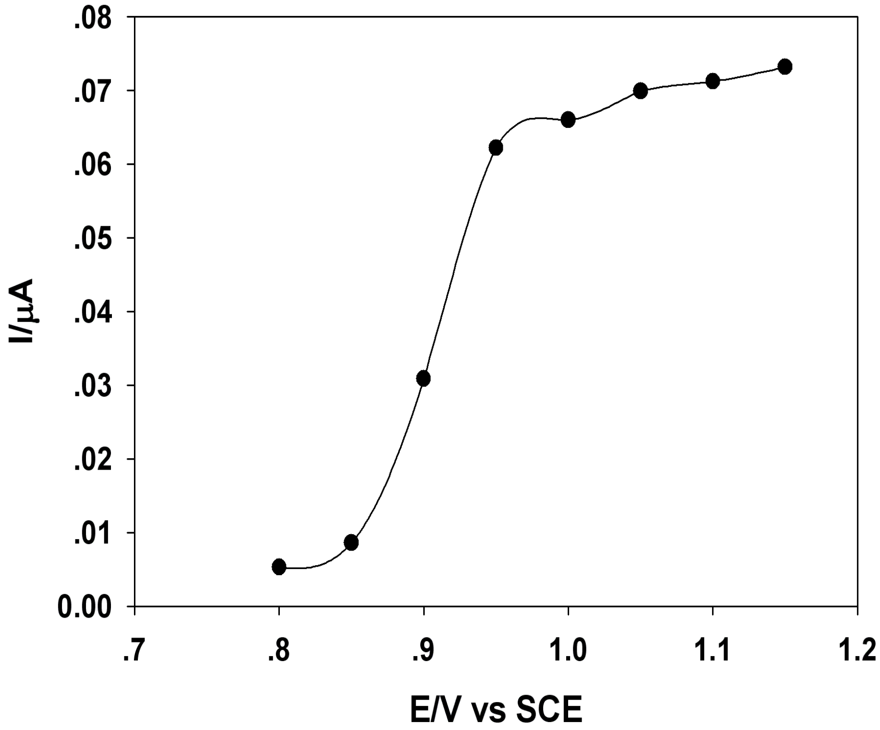

Figure 5. Effect of applied potential on the amperometric response for 0.05 mM $NaNO_2$ in 0.1 M PBS (pH 3.5).

3.4. Amperometric detection nitrite at PRhB/GCE

Figure. 6. displays a typical current–time response for the continuous additions 10 μL of 0.1 M $NaNO_2$ in 10 mL 0.1 M PBS (the change of concentration for $NaNO_2$ being 0.1 mM) at an applied potential of 0.95 V. Upon the additions of nitrite, the oxidation current increased steeply to reach a stable value. The electrode achieved 95% of the steady state current in less than 10 s, indicating clearly that the electrocatalytic response was very fast. The sensitivity of the sensor to nitrite was calculated to be 308.1 μA mM^{-1} cm^{-2}. The current signal is in proportion with the nitrite concentration in the range of 0.5 μM-7.0 mM, with a detection limit of 0.1 μM (S/N = 3). It is much wider than that of nano-Au/poly(3-methylthiophene) composites-modified electrode [39], Nanodiamond powder electrode [40], Cytochrome c-type nitrite reductase/Nafion/methyl viologen modified electrode [41] and Polypyrrole nanowire modified electrodes [42]. The detection limit of 0.1 μM, which is a little lower than that of 0.5 μM at GCE/PVP-Os/TiO_2 [43], 1.2 μM at GCE/β-$(Bu_4N)_7SiW_9O_{37}$ $(CpTi)_3$ doped silica sol [44], and 1.2 μM of Hb based on Aunano/TiO_2 sol-gel [45].

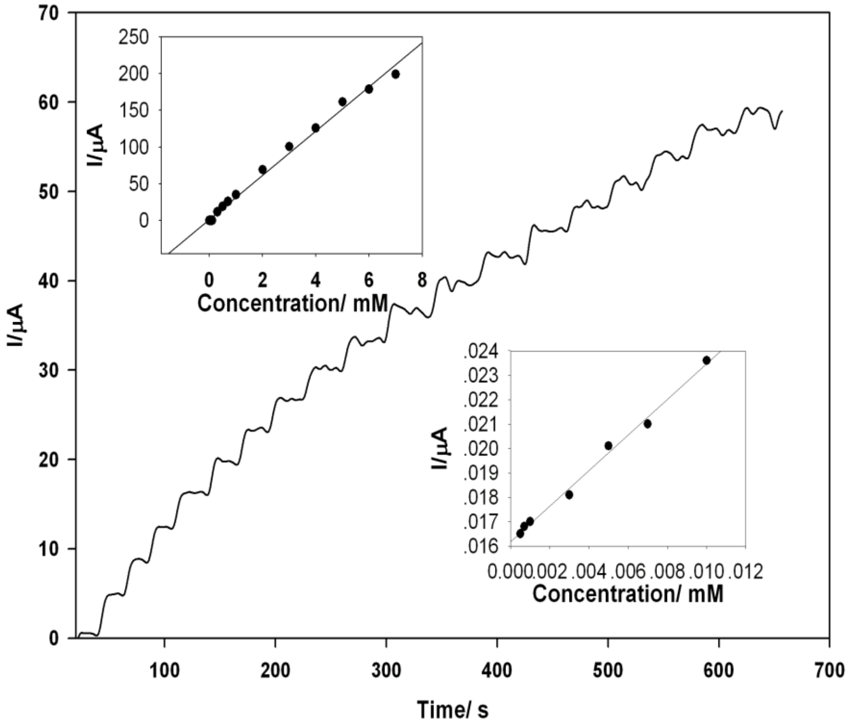

Figure 6. Amperometric response of PRhB/GCE at 0.95 V upon successive additions 10 μL of 0.1 M $NaNO_2$ in 10 mL 0.1 M PBS (pH 3.5) (the change of concentration for $NaNO_2$ being 0.1 mM). (left inset: calibration curve of the sensor for $NaNO_2$ concentrations from 5.0×10^{-7} to 7.0×10^{-3} M,; right inset: calibration curve of the sensor for $NaNO_2$ concentrations from 5.0×10^{-7} to 1.0×10^{-5} M).

3.5. Interferences

Several chemical species were investigated for their levels of interference in the amperometric determination of nitrite. The results showed that 50-fold cholesterol and glucose had no apparent effects on the current responses of 2.0×10^{-6} M nitrite. Most of the ions, such as 50-fold K^+, Na^+, Ag^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, F^-, Cl^-, NO_3^-, SO_4^{2-} , $H_2PO_4^-$, HPO_4^{2-} , and PO_4^{3-} did not interfere with the determination of 2.0×10^{-6} M nitrite. These results showed that the PRhB/GCE possessed high selectivity.

3.6. Reproducibility and stability

After each measurement the sensor was refreshed by successive cyclic voltammetric sweeps in blank solution to get a reproducible electrode surface. The relative standard deviation (R.S.D.) of 3.6% for 10 times parallel detections of 5.0×10^{-5} M nitrite, suggesting good reproducibility of this sensor. The stability of this sensor was determined. The current response had no changes for first 6 days and 95% of the initial response current remained after the sensor kept in air for 11 days.

4. CONCLUSION

A simple and sensitive electrochemical PRhB-based sensor was developed for the determination of nitrite. The PRhB/GCE could greatly enhance the voltammetric and amperometric response of nitrite oxidation. The sensor was specific to nitrite, with other homogeneous species hardly interfering, and its sensitivity, repeatability, and stability were satisfactory.

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