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ORIGINAL PAPER

Development of a novel nitrite amperometric sensor based on poly(toluidine blue) film electrode

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Abstract A novel sensor for the determination of nitrite anion (NO_2^-) was fabricated by electrodeposition of toluidine blue. The sensor exhibited good catalytic activity toward the electrochemical oxidation of nitrite. Amperometry was carried out to determine the concentration of NO_2^-. A linear response in the range from 1.0×10^{-7} to 1.52×10^{-5} M with a substantially low detection limit of 5×10^{-8} M (S/N=3) was obtained. The proposed sensor had a feature of high sensitivity of 4.7×10^5 μA M^{-1} cm^{-2}. The possible interference from several common ions was tested. This sensor was applied to the amperometric determination of nitrite in food samples, and the results were consistent with those obtained with the standard spectrophotometric procedure.

Keywords Toluidine blue · Nitrite · Electropolymerization · Amperometry · Sensor

Introduction

Nitrite is reported to be a human health-hazard chemical, and its toxicity was brought into focus. The passage of nitrite into the bloodstream results in the irreversible conversion of hemoglobin to methemoglobin with oxygen uptake and transportation compromised, which may induce the "blue baby" syndrome [1]. Furthermore, under acidic conditions of the stomach, nitrite is easily converted into carcinogenic nitrosoamines after reaction with secondary and tertiary amines, which may lead to gastric cancer [2]. However, as is well known, nitrite lurks ubiquitously in some foods. For example, due to its antimicrobial action, nitrite is still used as an additive for the preservation of meat produce as commonly as it was centuries ago. Moreover, nitrite can be formed in the production of pickled vegetables because of the biodegradation of nitrate or other nitrogenous substances. Therefore, the quantitative determination of nitrite concentrations is of great importance, especially for the supervision of the quality of food.

The classical method for nitrite determination is the Griess assay [3], which was first developed in 1879 and still widely used. Under acidic conditions, nitrite undergoes a series of reactions to form an azo dye, which is the basis of the spectrophotometric determination [3, 4]. Though it is highly sensitive and specific, the method is not applicable if too much nitrite is present because of its potential side-reactions [5]. In addition, the organic reagents used are often poisonous, and sometimes the formation of the azo dye consumes rather large amounts of time. To seek alternative procedures for nitrite determination, numerous efforts have been devoted and a lot of new methods have been developed, such as chemiluminescence [6], spectrofluorimetry [7], ion chromatography [8], and capillary electrophoresis [9]. Compared to all of these alternatives, electrochemical methods, which offer faster, cheaper, and safer analysis, and which can be performed in real time, have drawn much more attention. Khaled et al. [10] and Cosnier et al. [11] reported potentiometric methods for the determination of nitrite. Studies have been published in the literature dealing with the catalytic reduction of nitrite on electrodes modified with macroporous copper [12], ferric porphyrins [13], and heme protein [14–16]. As a more

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recommendable method, electrochemical determination based on the oxidation of nitrite is immune to interference from nitrate ions and molecular oxygen, which are usually the major limitations in cathodic determination of nitrite [17, 18]. However, the electrochemical behavior of nitrite is very poor and usually involves a large overpotential at the conventional solid electrode. To overcome these difficulties, chemically modified electrodes for the oxidation of nitrite have been extensively explored. Attempts include electrodes modified by thin films of metal porphyrins [19, 20], noble-metal deposits [18, 21], metal phthalocyanines [17, 22], poly pyrochlore doped with Ru^{3+} and Pb^{2+} [23], choline and *L*-glutamic acid mixed monolayers [24], and chitosan-carboxylated multiwall carbon nanotubes [25]. All these electrodes are favorable for nitrite determination with high sensitivity. Some of them give relatively good selectivity and fast responses, and have been successfully used for the determination of nitrite in real samples.

Toluidine blue (TB), a phenothiazine dye, has been extensively studied as a modifier in the construction of chemical and biologic sensors. The researches mainly focus on the electrocatalysis of some bioactive compounds, such as glucose [26, 27], nicotinamide adenine dinucleotide [28], dopamine and ascorbic acid [29], and hydrogen peroxide [30]. Most recently, a sensitive nitric oxide biosensor based on poly(TB) (PTB)/Nafion composite film was presented for the determination of nitric oxide in a biological sample [31]. To our knowledge, no work on nitrite detection by the use of TB-modified electrodes has been reported previously. In this paper, we developed a novel nitrite amperometric sensor based on PTB film. This nitrite sensor has high electrocatalytic activity toward the oxidation of nitrite. The response currents exhibited a linear range from 1.0×10^{-7} to 1.52×10^{-5} M. The detection limit was 5×10^{-8} M (S/N=3) and the sensitivity was calculated to be 4.7×10^{5} μA M^{-1} cm^{-2}. The sensor was applied to the amperometric determination of nitrite in food samples, and the results were consistent with those of the standard spectrophotometric procedure.

Materials and methods

Regents and apparatus

TB (Beijing Reagent Factory, Beijing, China) and other chemicals were all of analytical grade and used without further purification. A 0.1 M stock solution of nitrite was prepared by direct dissolution of $NaNO_2$ (Chemical Co. of Hubei University, Hubei, China) in water and stored in the dark. Phosphate buffers (PB) (0.1 M) with various pH values were prepared by mixing stock standard solutions of KH_2PO_4 (Sinopharm Chemical Reagent, Shanghai, China) and Na_2HPO_4 (Sinopharm Chemical Reagent) and adjusting the pH with H_3PO_4 (Wuhan Reagent, Wuhan, China) and NaOH (Tianjin Longteng Chemical, Tianjin, China). Zinc acetate was purchased from Shanghai Reagent. Doubly distilled water was used throughout.

All electrochemical experiments were performed with a CHI 830 electrochemical workstation (ChenHua Instruments, Shanghai, China) in a conventional three-electrode cell. The working electrode was a bare or modified glassy carbon electrode (GCE) with a geometric area of 0.071 cm^{-2}. The reference and auxiliary electrodes were a saturated calomel electrode (SCE) and platinum wire, respectively. All experiments were performed at room temperature and all the potentials were measured and reported vs the SCE. Spectrophotometric analysis of nitrite was carried out with a UV-Vis spectrophotometer TU-1901 (Beijing Purkinje General Instrument, Beijing, China). pH value was measured with a Delta 320 pH meter (Mettler-Toledo Instrument, Shanghai, China).

Preparation of the PTB-modified electrode

Prior to modification, the bare GCE was polished successively with 1.0, 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 0.1 M PB (pH 6.8) and anodized at a potential of 2.0 V for 25 s. After that, the GCE was transferred into 0.1 M PB (pH 6.8) containing 5×10^{-4} M TB. A TB polymer film was electrochemically deposited onto the pretreated electrode by cyclic voltammetry with the applied potential ranging from −0.8 to 1.1 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 PB (pH 6.8) and stored in the same solution for further use. The final electrode was denoted as PTB/GCE.

Analytical procedure

Assay of standard solution

Cyclic voltammetry was used to study the oxidation behavior of nitrite at the PTB/GCE in 0.1 M PB (pH 3.0). Before nitrite measurement, the sensor was placed in 10 mL PB and the potential scan was cycled between 0 and 1.2 V until a steady cyclic voltammogram was obtained. Amperometry was used to determine the linearity and sensitivity of the nitrite sensor. The amperometric measurement of nitrite was performed at 1.1 V in 10 mL 0.1 M deoxygenated PB (pH 3.0) with stirring. When the amperometric baseline was moving plainly, the standard nitrite solutions

were successively added into the cell. Prior to experiments, the dissolved oxygen was removed from the solution by bubbling high-purity nitrogen for 20 min.

Assay of the nitrite content in food samples

Samples of sausage and pickled vegetables were bought in a local shop, and the pretreatment could be described as the following: first, 5 g of the food sample was beaten into mash and mixed with 12.5 mL saturated borax solution. Then, 300 mL of water of 70 °C was added and the mixture was heated in boiling water for 15 min. To precipitate the proteins, 5 mL of 20% zinc acetate was introduced. After being cooled to room temperature, the mixture was diluted to 500 mL with water and then filtrated. Finally, 100 mL of the filtrate was inspissated to form a 10-mL solution. The resulting sample solution was stored at 4 °C in a refrigerator.

The nitrite content in samples was determined by an amperometric method according to the standard additions method. Standard nitrite solutions were added as internal standards after the injection of the sample solution. Thus, the concentration of nitrite in the real sample could be calculated. Reference determinations were made with the Griess assay.

Results and discussion

Electropolymerization of TB

TB was easily electropolymerized on a GCE under acidic conditions. Figure 1 shows the typical cyclic voltammograms of 5.0×10^{-4} M TB at the GCE in 0.1 M PB (pH 6.8). If the potential scan was confined to the range from −0.8 to 0.6 V, only a pair of peaks at the formal potential of about

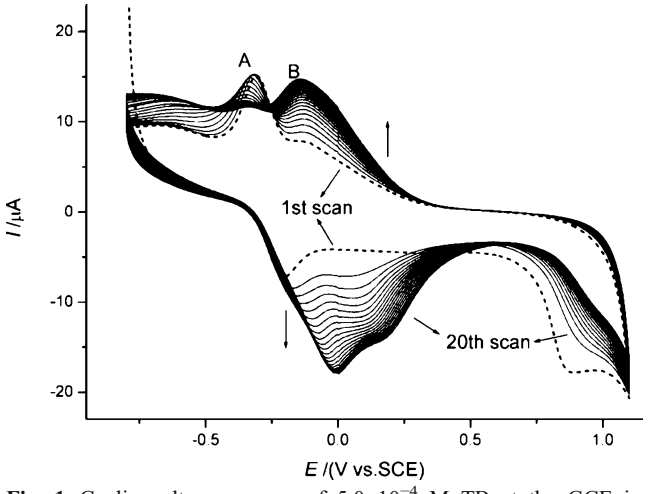


Fig. 1 Cyclic voltammograms of 5.0×10^{-4} M TB at the GCE in 0.1 M PB (pH 6.8) at a scan rate of 0.05 V s^{-1}

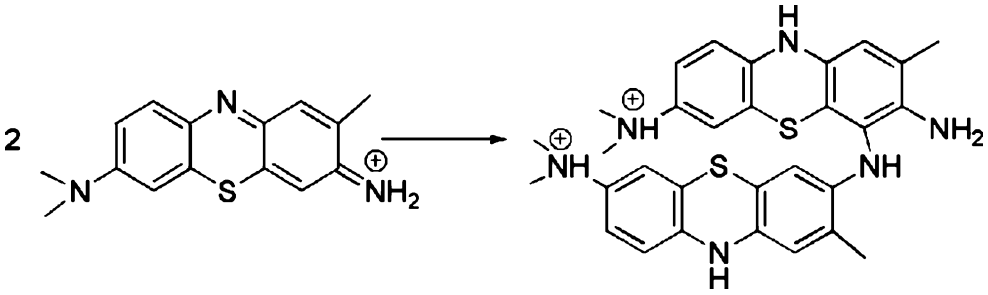
−0.25 V (couple A) could be observed. However, when the potential was swept positively over 0.8 V, the oxidation current increased remarkably, which indicates that TB was polymerized initially on the electrode surface. Meanwhile, a pair of new redox peaks appears at the formal potential of −0.07 V (couple B) after the second scan. According to literature [26, 31], the positive couple B is ascribed to the redox of the resulting polymer and the negative couple A is ascribed to the mono-type redox peaks. Moreover, with further scans, the redox peak currents of couple B and the anodic current of couple A increase steeply, while the cathodic current of couple A decreases gradually until a stable status is reached. When the electrode was then rinsed and transferred into a deaerated PB solution (pH 6.8) containing no TB, couple B still maintained the same form but couple A almost disappeared. These phenomena illuminate that a PTB film was deposited on the electrode surface. An olive-drab film could be seen on the electrode surface when the electrode was taken out, which also gave evidence of the existence of the PTB film. According to literature [26], the total electrode reaction of the polymerization could be described as shown in Scheme 1.

Electrocatalytic oxidation of nitrite at PTB/GCE

Figure 2 shows the cyclic voltammetric responses of bare GCE and PTB/GCE in the presence of 8.0×10^{-5} M $NaNO_2$ at a scan rate of 0.1 V s^{-1}. At the bare GCE, a small and broad anodic peak at the potential of 0.85 V was observed (curve d), while at the PTB/GCE, a significant oxidation peak appeared at about 0.95 V (curve a). Though the potential is more positive than the oxidation peak at the bare GCE, the peak current is of about fivefold enhancement. With an increase of nitrite concentration, the oxidation current increased, accompanied by the evidently negative shift of the peak potential (curve b). These results suggest that PTB film has an excellent electrochemical activity toward the oxidation of nitrite, and that the electrochemical behavior varies slightly with the substrate concentration.

Figure 3a illustrates a series of cyclic voltammograms obtained at different potential scan rates for a typical nitrite concentration. Figure 3b shows the response of the oxidation peak current with the square root of scan rate ($v^{1/2}$) in the range of 0.01~0.15 V s^{-1}. A linear regression equation was obtained as $ip/(\mu A) = 1.559 + 43.25v^{1/2}/(V^{1/2}s^{1/2})$, with a correlation coefficient of 0.9978, which indicates a diffusion-controlled process. The anodic peak potential E_p shifts with the scan rate, indicating the chemical irreversibility of the nitrite electrocatalytic oxidation process. However, the plot of E_p vs the logarithm of the scan rate (log$v$) gives two linear regions with slopes of 0.134 (r=0.9995) and 0.281 (r=0.9998) volts per decade (Fig. 3c), both of which have no resemblance with Tafel slopes. Such results possibly imply

Scheme 1 Total electrode reaction of the polymerization



that electrochemical steps were coupled with chemical reactions [17]. In acidic solutions, PTB is positively charged due to the protonation of the amine group, which is denoted as $PTBH^+$. Obviously, $PTBH^+$ can attract negatively charged nitrite ions in solution, resulting in the accumulation of NO_2^- on the electrode surface. Though the detailed electrochemical mechanism might be rather complicated, the possible electrode reactions can be expressed similarly as follows [25]:

$$PTBH^+ + NO_2^- \rightarrow PTBH^+ \cdot NO_2^-$$

$$PTBH^+ \cdot NO_2^- + H_2O \rightarrow PTBH^+ \cdot NO_3^- + 2H^+ + 2e$$

$$PTBH^+ \cdot NO_3^- \rightarrow PTBH^+ + NO_3^-$$

Optimization of experimental variables for the determination of nitrite

Optimized fabrication of the sensor

The scan potential for the electrochemical polymerization of TB could affect the formation of PTB film. If the scan potential was set at between −0.8 and 1.1 V, an obvious olive-drab film could be observed after 20 cycles. However, if the high potential was set at lower than 0.8 V, there was no obvious redox peak observed, not to mention a megascopic film. Meanwhile, the formation of PTB was closely related with the pH of the supporting electrolytes. In such a system in which pH was close neutral, the reduction potential of mono-type TB was about −0.3 V; thus, it was necessary to set the low potential at not higher than −0.6 V. To form a tight and steady PTB film, a range from −0.8 to 1.1 V was finally selected as the optimal scan potential.

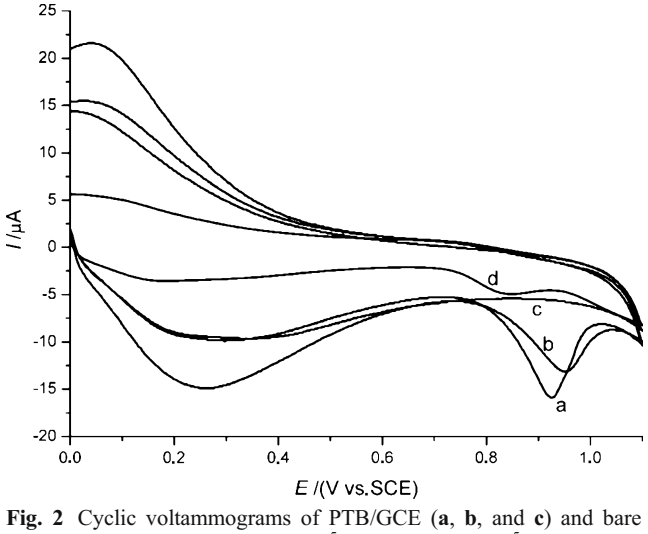


Fig. 2 Cyclic voltammograms of PTB/GCE (**a**, **b**, and **c**) and bare GCE (**d**) in the presence of 8.0×10^{-5} (**a** and **d**), 5.0×10^{-5} (**b**), and 0 M (**c**) $NaNO_2$ in 0.1 M PB (pH 3.0) at a scan rate of 0.1 V s^{-1}

The anodization of bare GCE in neutral medium can result in a negatively charged surface, which benefits the absorption of the protonized PB and makes the polymerization easier. However, a pretreatment of too long a period will lead to a redox activity loss of the electrode due to the formation of an inactive oxycarbide layer. Thus, an appropriate polarization time is crucial. In this system, 25 s was employed as the polarization time because a maximum peak current for nitrite oxidation could be obtained only under such a condition.

The effect of the thickness of the film on the anodic peak current of nitrite was also evaluated. As is well known, the film thickness can be controlled by the number of cyclic scans. 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 20. 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, while too thick a film would block the electron transfer; both cases might result in the small current response. Through the experiments, a potential cycling of 20 scans was chosen as the optimal electropolymerization for TB.

Optimized parameters of nitrite determination

The effect of the solution pH on the current response for 8.0×10^{-5} M nitrite in 0.1 M PB was examined. Acidic conditions are more favorable for a good response and the maximum current is obtained at pH 3.0. Therefore, pH 3.0 was adopted for the nitrite determination.

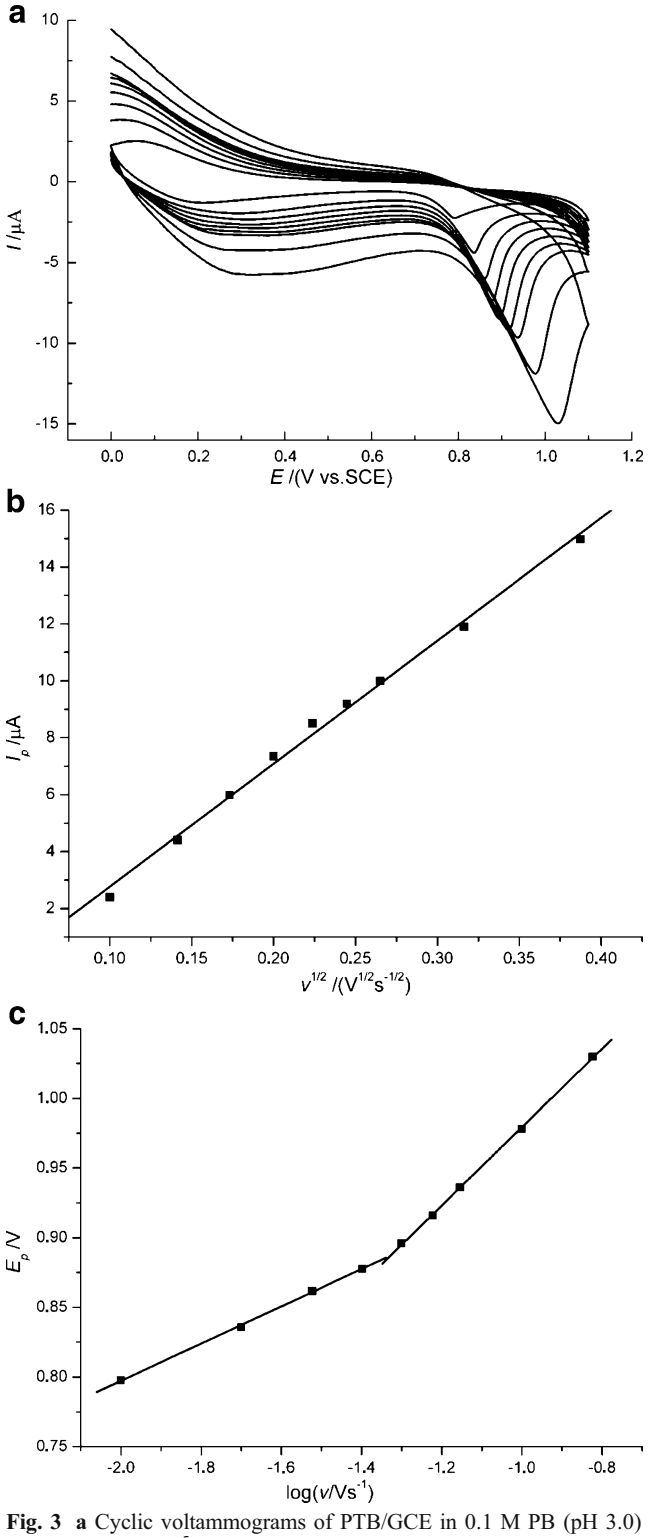


Fig. 3 **a** Cyclic voltammograms of PTB/GCE in 0.1 M PB (pH 3.0) containing 8.0×10^{-5} M $NaNO_2$ at different potential scan rates (from inner to outer: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.1, and 0.15 V s^{-1}). **b** Plot of anodic peak current vs the square root of scan rate $v^{1/2}$. **c** Plot of anodic peak potential vs the logarithm of scan rate log$v$

Figure 4 demonstrates the effect of applied potential on the amperometric response of the sensor. The electrocatalytic oxidation of nitrite could be observed at around 0.8 V, and

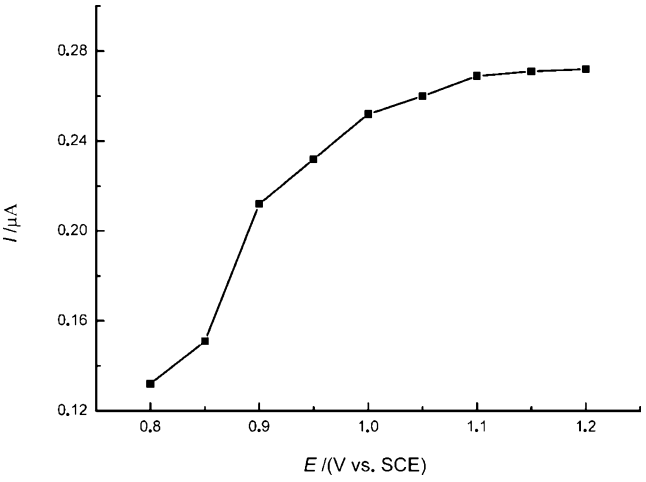


Fig. 4 Effect of applied potential on the amperometric response for 2.0×10^{-6} M $NaNO_2$ in 0.1 M PB (pH 3.0)

when the potential increased from 0.8 to 1.1 V, the steady state current increased due to the increased driving force for the nitrite oxidation at the higher potential. However, when the potential was set higher than 1.1 V, the increase of current was negligible. Thus, 1.1 V appears to be a reasonable working potential for amperometric determination.

Amperometric detection nitrite at PTB/GCE

Figure 5a displays a typical current–time response for the continuous addition of 1×10^{-3} M $NaNO_2$ to pH 3.0 PB at an applied potential of 1.1 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 5 s, indicating clearly that the electrocatalytic response was very fast. The current signal is in proportion with the nitrite concentration in the range of 1.0×10^{-7}–1.52×10^{-5} M, the linear equation was obtained as $ip/(\mu A) = 0.2025 + 0.03342c_{nitrite}/(\mu M)$, r=0.9992 (Fig. 5b). The sensitivity of the sensor to nitrite was calculated to be 4.7×10^5 μA M^{-1} cm^{-2}. At a signal-to-noise ratio of 3, the detection limit of the sensor was found to be 5×10^{-8} M, which was much lower than that in previous work [20, 21].

Stability and reproducibility of the PTB/GCE

The stability of the sensor was examined by amperometric detection and the sensor showed good long-term stability under continuous use. If 20 repetitive measurements were performed in 1 day, at last 97% of the initial response of the sensor was obtained; if the sensor was used ten times each day, a week later, 87% of the initial response was obtained; if the sensor was stored in 0.1 M PB for 7 days, 95% of the initial response was obtained.

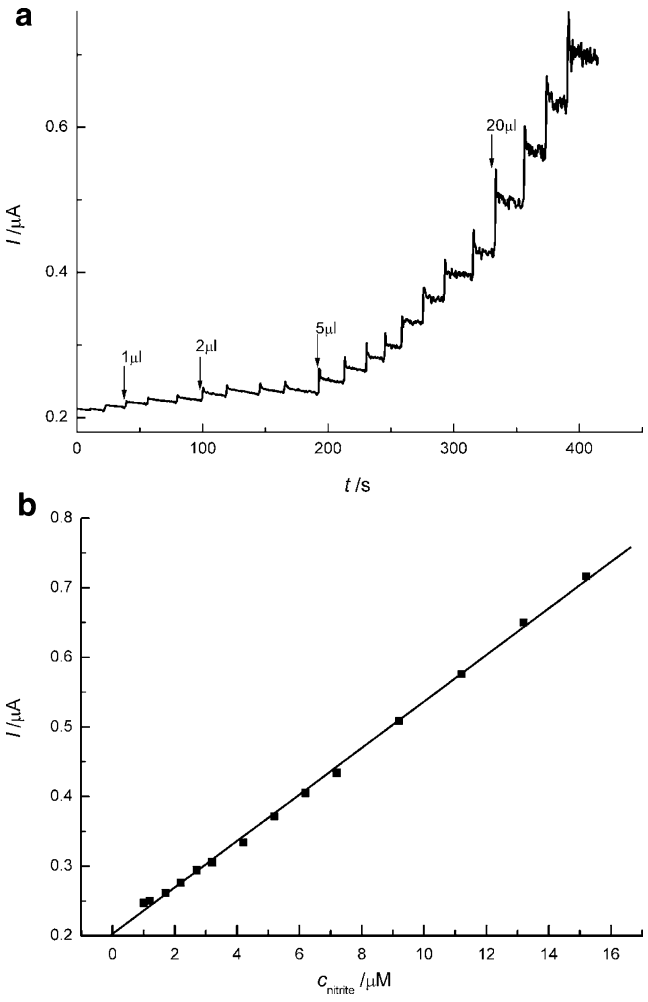


Fig. 5 **a** Amperometric response of PTB/GCE at 1.1 V upon successive additions of 1.0×10^{-3} M $NaNO_2$ to 10 mL 0.1 M PB (pH 3.0). **b** Plot of oxidation current vs nitrite concentration

The relative standard deviation (RSD) for six repeated measurements of 2.0×10^{-6} M nitrite using the same electrode was less than 5%, and the RSD for measurement of 2.0×10^{-6} M nitrite at ten different electrodes was 4.7%, which suggested that the sensor had a good reproducibility for the determination of nitrite.

Interferences

Several chemical species were investigated for their levels of interference in the amperometric determination of nitrite. The results showed that 200-fold riboflavin and cholesterol had no apparent effects on the current responses of 2.0×10^{-6} M nitrite. Most of the ions, such as 500-fold HSO_4^-, SO_4^{2-}, NO_3^-, 200-fold Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Al^{3+}, Cu^{2+}, Pb^{2+}, Bi^{3+}, Cd^{2+}, Hg^{2+}, HCO_3^-, SO_3^{2-}, and Cl^-, did not interfere with the determination of 2.0×10^{-6} M nitrite.

Table 1 Determination of nitrite in food samples

Sample	Detected by Griess assay (mg kg^{-1})	Detected by this method (mg kg^{-1})	Recovery (%)
Sausage 1	14.47	13.89	99.2
Sausage 2	10.78	10.45	98.3
Pickled vegetable 1	8.49	8.03	100.4
Pickled vegetable 2	5.87	5.56	101.8

These results showed that the PTB/GCE possessed high selectivity.

Real sample determination

The enhanced current responses of nitrite oxidation at PTB/GCE in 0.1 M pH 3.0 PB was applied to the determination of nitrite in food samples. The concentration of nitrite was calculated using a standard addition method. The RSD of each sample for five-times parallel detections was less than 5%, and the recovered ratio on the basis of this method was investigated and the value was between 98.3 and 101.8%, as shown in Table 1. These experimental data indicate that the determination of nitrite using PTB-modified GCE was effective and sensitive. Lastly, to estimate the feasibility, precision, and efficiency of this sensor, Griess assay was adopted for the determination of nitrite in the same samples. The good accordance between data from the spectrophotometric method and those from the method we proposed indicates the reliability of the present electroanalytical sensor for nitrite determination in real samples.

Conclusions

TB was electrodeposited onto GCE. The resulting electroactive-polymer-modified electrode could provide more active sites for anodic oxidation of nitrite, and therefore, a novel nitrite sensor was developed. The optimized fabrication and sensing conditions were investigated. The PTB/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. The sensor was applied to the nitrite detection in food samples and the results were consistent with those obtained with the standard spectrophotometric procedure.

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