

Electropolymerization of Indole-3-acetic Acid on Glassy Carbon Electrode for Sensitive Detection of Nitrite

Yu Jun Yang, Xiangli Yin, Zhixin Yu, Yapin Du and Weikun Li

School of Chemistry and Chemical Engineering, Xuchang University, Xuchang 461000, China

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Abstract: Highly uniform and adhesive films of poly(indole-3-acetic acid) (p-IAA) were fabricated on the glassy carbon electrode (GCE) by electropolymerization of indole-3-acetic acid (IAA). The p-IAA modified GCE (p-IAA/GCE) was constructed to develop a promising amperometric sensor for the sensitive determination of nitrite. Field emission scanning electron microscope (SEM) and electrochemical techniques were used for the surface characterization of the modified electrode. It was demonstrated that p-IAA modified GCE (p-IAA/GCE) exhibited remarkable electrocatalytic activity towards the oxidation of nitrite with obvious reduction of overpotential. Under the optimal conditions, the linear range for the detection of nitrite was 5.0×10^{-7} to 5.0×10^{-4} M with a high sensitivity of 0.105 AM^{-1} , and a low detection limit of 1.0×10^{-7} M ($S/N = 3$). The influence of various anions and cations on the nitrite detection has been studied. The proposed method was successfully applied in the detection of nitrite in sausage samples, and the results were consistent with those obtained by UV-vis spectrophotometric methods.

Keywords: Indole-3-acetic acid, Nitrite, Modified electrode, Amperometric determination, Electropolymerization

1. INTRODUCTION

Nitrite is widely used as additive and corrosion inhibitor in food and environmental systems[1], while it is well known that nitrite can also react with amines to form nitrosamines, which are carcinogenic[2]. Because of the potential toxicity of nitrite, its quantitative analysis becomes more and more important in environmental protection and public health. The majority of the methods developed for the determination of nitrite involve spectrophotometry[3], chromatography[4], and electrochemical methods. Among them, determination by electrochemical techniques is easier, faster and cheaper. Electrochemical determination of nitrite was reported in the literature either by reduction or oxidation. Oxidative determination of nitrite is preferred because reduction of nitrite is known to suffer from interferences such as reduction of nitrate and molecular oxygen[5]. And the electrochemical reduction of nitrite gives several products depending on the electrode property and the catalyst employed, while its oxidation is a straight forward reaction, with nitrate being the final product[6]. For the oxidative determination of nitrite, various modified electrodes including Au/choline chloride modified electrode[7], composite electrode of

Au/ZnO/MWCNTs/GC[8], conductive nanodiamond powder electrode[9], electrode modified with electropolymerized film of functionalized thiazole[10] and Au-Fe(III) nanoparticle modified electrode[11] have been reported. Although numerous electrochemical sensors for nitrite have been published in the literature, an electrochemical sensor with high sensitivity and selectivity is still a challenging task for the analytical chemists.

Herein, we are reporting a detection of 500 nM nitrite using the electropolymerized film of indole-3-acetic acid (p-IAA) on glassy carbon electrode (GCE) by amperometric method. The present method is also successfully applied to determine the concentration of nitrite in sausage samples.

2. EXPERIMENTAL METHOD

2.1. Reagents

IAA was purchased from Sinopharm Chemical Reagent Co., Ltd in Shanghai, China. All other chemicals used were of analytical reagents. All the chemicals were used without further purification, and all the solutions were prepared from doubly distilled water. The pH 1.6 PB solution was obtained by dissolving 0.1 mol phosphoric acid in 1 L water.

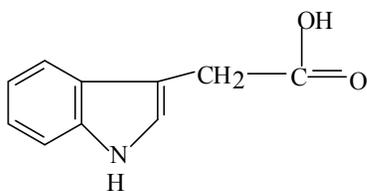


Figure 1. The molecular structure of IAA.

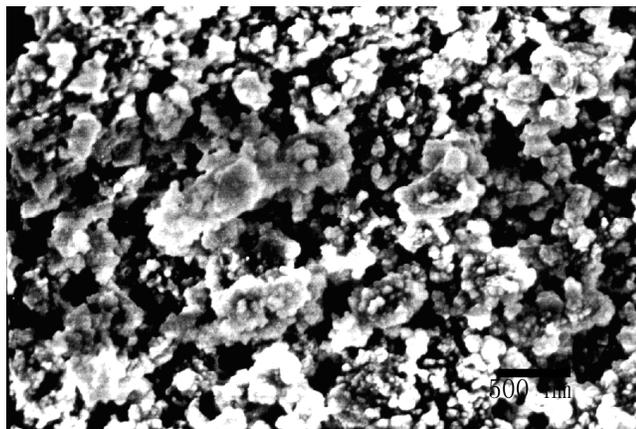


Figure 2. SEM images of p-IAA modified glassy carbon electrode.

2.2. Apparatus

All electrochemical measurements were performed with a model CHI 660b electrochemical workstation (Shanghai Chenhua Co., China). A conventional three-electrode system was employed with a platinum wire as counter electrode; a Ag/AgCl electrode as reference electrode and a p-IAA modified GCE as working electrode. All potentials were quoted with respect to Ag/AgCl electrode. Morphology study of the films on the GCE surface was carried out with a Quanta 200 scanning electron microscope (SEM; FEI Company, Holland).

2.3. Preparation of p-IAA/GCE

GCE of 3 mm in diameter was used. The electrode was polished with 0.05 μm alumina slurry (CH Instrument, Inc., USA) to a mirror finish. Indole-3-acetic acid was electropolymerized on glassy carbon electrode (GCE) *via* galvanostatic method. The p-IAA modified GCE (p-IAA/GCE) was produced by the galvanostatic deposition of p-IAA on GCE at 1 mA in pH 1.6 PB solution containing 1 mg/mL IAA.

3. RESULTS AND DISCUSSIONS

3.1. Electropolymerization of IAA on GCE

The molecule structure of IAA is shown in Fig. 1. Under proper conditions, the nitrogen atom at the pyrrole ring can be oxidized. Waltman et al. proposed that the polymerisation of the indoles occurred at the 1- and 3- positions, resulting in a linear polymer, and that electropolymerisation and film formation on the electrode could only be observed for those monomers which oxidised in a limited range of electrode potentials [12]. Jennings et al. demon-

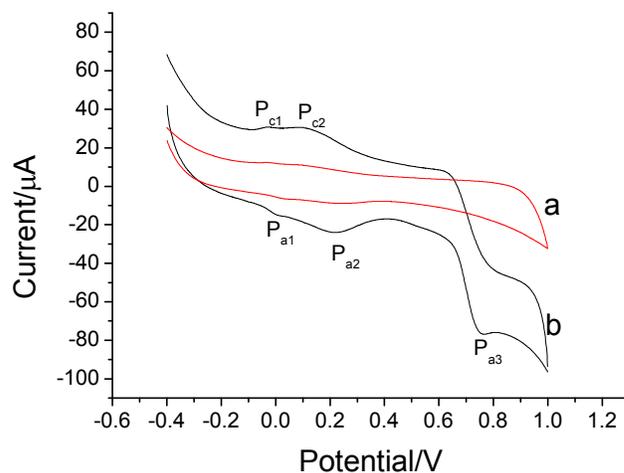


Figure 3. The cyclic voltammetric behaviors of the p-IAA/GCE in the absence (a) and presence (b) of 7.5 mM nitrite in 0.1 M acetate buffer solution (pH 2.6) at 100 mVs^{-1} . The Ag/AgCl electrode is used as a reference electrode.

strated that a wide variety of 5- substituted indoles form electroactive trimers which can further link to give polymers of linked trimers [13]. The electrochemical oxidation of IAA has been investigated in acidic aqueous solution on glassy carbon electrode [14, 15]. The voltammetric oxidation of IAA corresponds to an initial two-electron one-proton reaction to give a cation of 3-methyleneindolenine carboxylic acid. Very rapid decarboxylation of the latter intermediate gives a 3-methyleneindolenine cation which undergoes a complex series of reactions yielding oxidation products [14]. In our previous research, it was discovered that the electro-oxidation of IAA involves the formation of a redox polymer which was deposited onto the electrode surface [15].

The morphology of the p-IAA/GCE surface was characterized by FE-SEM technique. As shown in Fig. 2, the thin film of p-IAA are constituted of a porous structure. This porous structured p-IAA film could extremely enhance the active surface area of GCE and might be very important to promote electron transfer. Consequently, the resulting modified electrode exhibited an excellent electrocatalytic capability towards the oxidation of nitrite.

3.2. Electrocatalytic oxidation of nitrite

The electrocatalytic performance of the p-IAA/GCE toward the oxidation of nitrite was investigated by CV. Fig. 3 shows the CV behaviors of the p-IAA/GCE in the absence (a) and presence (b) of 7.5 mM nitrite in acetate buffer solution. CV of the p-IAA/GCE in 0.1 M PB aqueous solution showed two pairs of redox peaks (Fig. 3, curve a) which are due to the oxidation/reduction process of the redox p-IAA [15]. As can be seen, 7.5 mM nitrite in pH 2.6 acetate buffer showed an oxidation peak at 0.768 V (P_{a3}) at the p-IAA/GCE (Fig. 3, curve b), which was corresponding to the conversion of NO_2^- to NO_3^- through a two-electron oxidation process. At the same time, the two pairs of redox peaks (P_{a1}/P_{c1} and P_{a2}/P_{c2}) increased significantly with the addition of nitrite.

The electrochemical behaviors of nitrite at different electrodes were depicted in Fig. 4. In the presence of nitrite, the CV displays

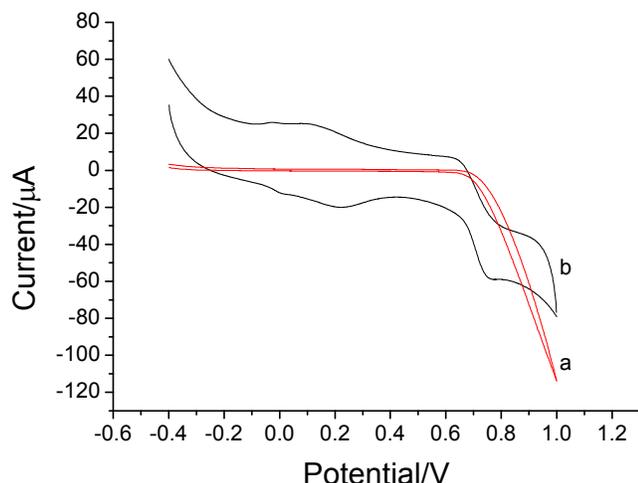


Figure 4. Cyclic voltammograms of different electrodes in the solution of 5 mM nitrite in 0.1 M acetate buffer solution (pH 2.6): (a) bare GCE, (b) p-IAA/GCE. Scan rate: 100 mV/s. The Ag/AgCl electrode is used as a reference electrode.

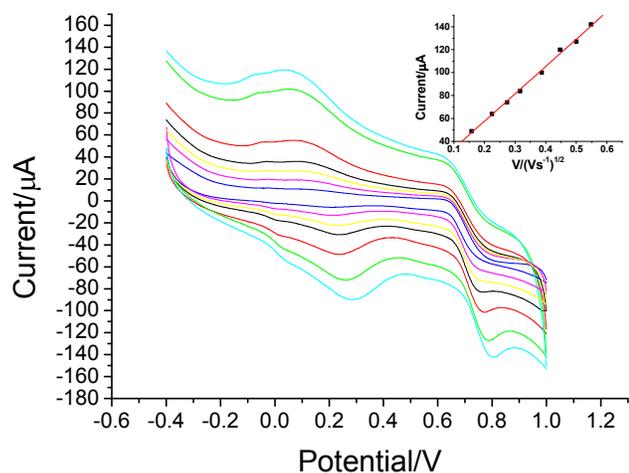


Figure 5. Cyclic voltammograms of p-IAA/GCE in 0.1 M acetate buffer solution (pH 2.6) with 5.0 mmol/L NO₂⁻ at different scan rates (0.025, 0.05, 0.075, 0.1, 0.15, 0.2, 0.25 and 0.3 V/s). The inset shows the linear relationship between the peak current and the square root of the scan rate. The Ag/AgCl electrode is used as a reference electrode.

an oxidative current at potentials between 0.7 V and 1.0 V on bare GCE, while no oxidation peak of nitrite can be observed (Fig. 4, curve a). 5 mM nitrite in pH 2.6 acetate buffer showed an irreversible weak oxidation peak at 0.768 V at the p-IAA/GCE, which was corresponding to the conversion of NO₂⁻ to NO₃⁻ through a two-electron oxidation process. In comparison with that at the bare GCE, a remarkable increase in oxidation current can be observed at the p-IAA/GCE (Fig. 4, curve b). The significant enhancement of peak current indicated an efficient catalytic capability of the p-IAA/GCE towards the oxidation of NO₂⁻. The extraordinary elec-

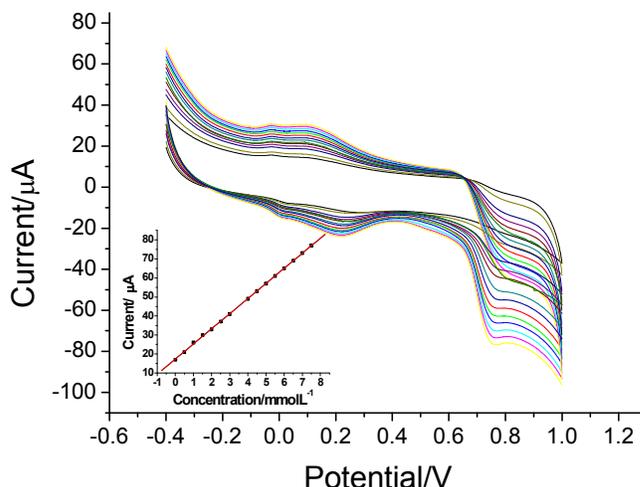


Figure 6. Cyclic voltammograms of p-IAA/GCE in 0.1 M acetate buffer solution (pH 2.6) containing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 mM nitrite at 100 mVs⁻¹. The inset shows the linear relationship between the oxidation peak current and the concentration of nitrite. The Ag/AgCl electrode is used as a reference electrode.

trocatalytic activity may be attributed to the high specific surface area and excellent electron transfer ability of the p-IAA /GCE film, which led to the larger electroactive surface of the modified electrode for the detection of nitrite.

3.3. Effect of scanning rate

The effect of scan rate on the electrochemical response of nitrite cations at P-IAA/GCE was carried out to investigate the electrochemical process of nitrite at the modified electrode (Fig. 5). The anodic peak current (I_{pa}) at about 0.768 V was proportional to the square root of the scan rate (v^{1/2}) over the range of 25–400 mV/s. Linear relationship between the catalytic peak current and the square root of scan rate indicates that the oxidation of adrenaline is a diffusion controlled process (Fig. 5 inset).

3.4. Performance of the p-IAA/GCE for determination of nitrite

Determination of the concentration of nitrite at p-IAA/GCE was performed in pH 2.6 acetate buffer. Cyclic voltammograms of various concentrations of nitrite at p-IAA/GCE are given in Fig. 6. The anodic peak currents at 0.768 V were plotted against the bulk concentration of nitrite after the background subtraction. The response of anodic peak currents of nitrite at p-IAA/GCE was linear with the concentration of nitrite in the range of 0.5 mmol/L ~ 7.5 mmol/L (Fig. 6 inset). The correlation coefficient (r) and sensitivity coefficient of the calibration curve is 0.9962 and 1.3095 × 10⁻² A/M respectively.

Amperometric method was used to examine the sensitivity of p-IAA/GCE towards the detection of nitrite. Fig. 7 shows the amperometric i-t curve obtained for nitrite at p-IAA/GCE in a homogeneously stirred 0.1 M acetate buffer (pH 2.6) at an applied potential of 0.768 V. The p-IAA/GCE shows the initial current response

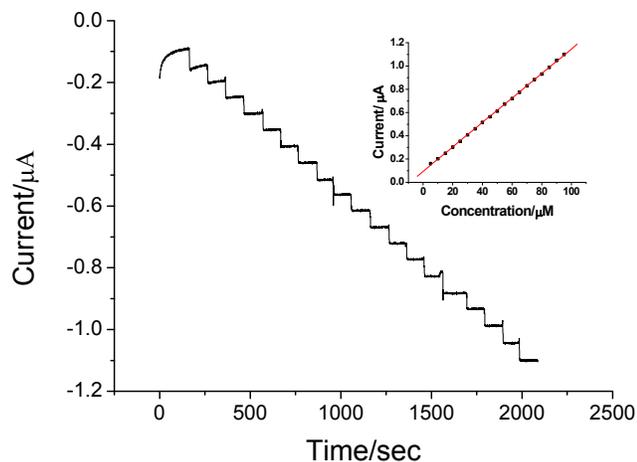


Figure 7. Amperometric responses of the p-IAA/GCE toward the successive additions of nitrite at 0.768 V. The inset summarizes the relation between response current and the nitrite concentration ($R = 0.9998$).

due to 5.0 μM nitrite and further addition of 5.0 μM nitrite in each step with a sample interval of 100 s, the current response increases and a steady state current response was attained within 3 s. Further, the amperometric current response was increased linearly with increasing nitrite concentration in the range of 5.0×10^{-7} – 5×10^{-4} M with a correlation coefficient of 0.9998 (Fig. 7 inset) and the detection limit was found to be 1×10^{-7} M ($S/N = 3$). The detailed comparison of nitrite detection performance using different nitrite sensors has been summarized in Table 1. The proposed method in this study presents the comparable performance with most of the reported nitrite sensors.

3.5. Reproducibility and stability of the p-IAA/GCE

The reproducibility of the p-IAA/GCE was investigated in the presence of 5.0×10^{-6} M nitrite in acetate buffer (pH 2.6). Amperometric experiments were repeatedly performed for 8 times with

the same p-IAA/GCE in the solution of 5.0×10^{-6} M nitrite. The relative standard deviation was 4.3%. Similarly, the fabrication reproducibility was estimated by using eight different electrodes. A solution containing 5.0×10^{-6} M nitrite was determined by eight electrodes in the same electrochemical cell, with a relative standard deviation of 5.4%, which indicated that the reproducibility of the electrode was excellent. When the electrode was used for approximately 40 times during 15 days only a small decrease of current sensitivity (about 8%) for 5.0×10^{-6} M nitrite was observed, which can be attributed to the excellent stability of the film. Interference studies were carried out with inorganic species.

3.6. Interference

No interference could be observed for the following materials: a 200-fold quantity of Na^+ , F^- , Ca^{2+} , Cl^- , Mg^{2+} , SO_4^{2-} , NH_4^+ , K^+ , CO_3^{2-} and NO_3^- , where the nitrite concentration was 5.0×10^{-5} M.

3.7. Real samples analysis

The practical application of p-IAA modified electrode was tested by measuring the concentration of nitrite in sausage samples. The sample preparation was following the literature report [8]. 5.0 g sausage crushed in advance was first treated with 12.5 mL borax saturated solution and then 2.5 mL of 30% $\text{Zn}(\text{Ac})_2$ solution to remove the protein. The resulting mixture was diluted to 50 mL and the filtrate was finally mixed with 0.1M acetate buffer (pH = 2.6) for nitrite determination. The typical results were summarized in Table 2. As compared to those measured by recommendatory spectroscopic method, relative error of 2.0% was obtained for the sample measurement using the proposed procedure, which indicating the developed electrode possesses the required accuracy for nitrite measurement and could be efficiently used for real sample determination.

4. CONCLUSIONS

This paper reports a highly sensitive and selective determination of nitrite using electropolymerized film of indole-3-acetic acid (p-IAA) on glassy carbon electrode (GCE) by amperometry. The resulting electrode exhibited substantial electrocatalytic effect on the oxidation of nitrite anions. Further, the amperometric current increases linearly while increasing the nitrite concentration from

Table 1. The comparison of the analytical characteristics of P-IAA/GCE with other electroanalytical techniques for nitrite detection.

Sensor type	Method	Linear range (μM)	Detection limit (μM)	Ref.
nano-Au/Ch/GCE	Differential-pulse voltammetry	0.4~750	0.1	7
Au/ZnO/MWCNTs/GCE	Amperometry	0.78~400	0.4	8
Nanodiamond powder electrode	Differential-pulse voltammetry	1000~20000	120	9
p-ATT/GCE	Amperometry	0.05~16	3.0×10^{-4}	10
Au-Fe(III)/GCE	Differential pulse amperometry	0.2~150	0.1	11
p-IAA/GCE	Amperometry	0.5~500	0.1	This work

Table 2. Real samples analysis by amperometry and UV-vis spectroscopy.

SausageSample	Chronoamperometry (mg/kg)	RSD of 5 repetitive experiments (%)	Spectroscopy (mg/kg)	Relative error (%)
	16.79	3.31%	17.14	2.0%

5.0×10^{-7} – 5×10^{-4} M and a detection limit was found to be 1.0×10^{-7} M (S/N = 3). The modified electrode was used to determine nitrite in the presence of 200-fold excess interferents such as Na^+ , F^- , Ca^{2+} , Cl^- , Mg^{2+} , SO_4^{2-} , NH_4^+ , K^+ , CO_3^{2-} and NO_3^- . The practical application of the present method was successfully applied to the determination of nitrite in sausage samples.

5. ACKNOWLEDGEMENTS

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