

Determination of nitrite with the electrocatalytic property to the oxidation of nitrite on thionine modified aligned carbon nanotubes

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Abstract

A thionine modified aligned carbon nanotubes (ACNTs) electrode was fabricated and was used to electrochemically determine nitrite. The thionine modified ACNTs electrode exhibited enhanced electrocatalytic behavior to the oxidation of nitrite. The electrochemical mechanism of the thionine/ACNTs electrode towards the oxidation of nitrite was discussed. The thionine modified ACNTs electrode exhibited fast response towards nitrite with a detection limit of 1.12×10^{-6} mol L⁻¹ and a linear range of 3×10^{-6} – 5×10^{-4} mol L⁻¹. The proposed method was successfully applied in the detection of nitrite in real samples.

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1. Introduction

Nitrite is ubiquitous within environment, food and physiological systems because it is commonly used as an additive in some foods [1] and as a corrosion inhibitor [2]. As nitrite is a potential toxicity through forming carcinogenic nitrosamine [3], determination of nitrite is important in environmental protection and public health. Therefore, many methods for nitrite determination have been developed in recent years, such as spectroscopic analysis [4–6], chromatography [7–9], Davis has reviewed the different methods of nitrite detection [10–12]. Of above-mentioned method, electrochemical detection techniques are favorable for nitrite determination [13–15]. However, most electrochemical methods are based on the reduction of nitrite and all of these methods suffer from poor sensitivity and are subject to several interferences [16,17]. Only few papers on the electrochemical oxidation of nitrite have

been reported [18–21]. These approaches that determine the nitrite offers several advantages, namely no interference from nitrate ion and from molecular oxygen, which are usually the major limitations in cathodic determination of nitrite. Also, the electrochemical reduction of nitrite give several products depending on electrode property of the catalyst employed, while its oxidation is a straight forward reaction, with nitrate being as the final product [22]. Since the nitrite oxidation involves a relatively higher over-potential at the surface of a bare glassy carbon electrode, the usefulness of detection of the oxidation of nitrite is limited. The chemically modified electrodes have been developed to decrease the over-potential for nitrite oxidation. Furthermore, modification of electrode surfaces provides a means of extending the dynamic range in analytical determinations.

Carbon nanotubes have attracted much attention during the past decade due to their unique mechanical, chemical and electrical properties [23,24]. The subtle electronic properties suggest that carbon nanotubes will have the ability to promote electron transfer reaction when used as an electrode in electrochemical reaction [25]. There have been

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several papers which demonstrated that carbon nanotubes have electrochemical properties which are equal or superior to many other electrode materials [26–29]. The electrocatalytic behavior of carbon nanotubes has been investigated by Gooding, Banks and Wang et al. [30–33]. Aligned carbon nanotubes (ACNTs) provide additional advantages for a maximized access of the nanotubes electrode surface and an efficient device construction, so ACNTs electrode has attracted much attention [34,35]. Moreover, to functionalize carbon nanotubes electrode with biomolecules or catalysts, a number of intriguing physicochemical approaches have recently been devised for modification of carbon nanotubes [36]. Thionine is a kind of purple dye, which can be easily dissolved in water and ethanol. Thionine has been used in many sensors, such as H_2O_2 biosensor [37], electrochemical sensor for immunoassay of carcinoembryonic antigen [38], and NADH biosensor [39]. Thionine molecules has been demonstrated to be easily adsorbed onto the surfaces of carbon nanotubes through strong π – π stacking force to introduce other molecules onto tube surfaces and enriching the chemistry of carbon nanotubes [40,41]. However, the thionine modified ACNTs electrode has never been reported and it is still unclear whether the electrode possesses significantly enhanced electrocatalytic property to nitrite.

In this paper, we introduce to fabricate a thionine modified ACNTs electrode by dipping ACNTs electrode in thionine solution simply. Electrocatalytic property to the oxidation of nitrite on the thionine modified ACNTs electrode has been investigated utilizing cyclic voltammetry and differential pulse voltammetry. The thionine modified ACNTs electrode, possessing maximized surface area and adsorbed abundant thionine molecules, exhibits a wonderful electrochemical response to trace nitrite.

2. Experimental

2.1. Chemicals and instrumentation

The nitrite was purchased from Shanghai Chemical Reagent Company (China). Stock solution of nitrite $1 \times 10^{-2} \text{ mol L}^{-1}$ was prepared by directly dissolving sodium nitrite in water and stored in the dark. Thionine was purchased from Sigma (US). The thionine solution (2 mg mL^{-1}) was prepared by dissolving 20 mg thionine in 10 mL water [40]. Other reagents were commercially available and were all of analytical reagent grade. Solutions were prepared with ultrapure water from the Aquapro system (Aquapro Co. China).

All electrochemical experiments including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with CHI 630 Electrochemical Analyzer (CH Instruments Inc. USA). The three-electrode electrochemical system is consisted of aligned carbon nanotubes working electrode, an Ag/AgCl reference electrode (saturated KCl) and a counter electrode made of platinum wire. Scanning electron microscopy (SEM) was used by the JEOL

JSM-5610LV (JEOL Co. Japan). Transmission electron microscopy (TEM) was used by the JEOL-JEM-2100F (JEOL Co. Japan).

2.2. Preparation of thionine modified ACNTs electrode

ACNTs grown on quartz substrate with inner diameter 30–40 nm, outer diameter 50–60 nm and length 7–8 μm were synthesized by chemical vapour deposition (CVD) according to the literature [42] and was sputtered with an around 200 nm of gold layer [43]. The slice of ACNTs that coated with Au was peeled off from the quartz substrate in 10% HF solution, then the well-aligned CNTs arrays with Au floor were cut into 1 mm^2 and purified by immersing them in 30 wt% nitric acid at 25°C for 2 h [44]. As a ACNTs electrode, a Cu wire was plastered onto the Au side ACNT slice using the electric glue. The exposed part of the Cu wire was covered with epoxy resin. The thionine modified ACNTs electrode was prepared by immersing an ACNTs electrode in thionine solution for 20 min, then washing it thoroughly with 0.1 mol L^{-1} PBS (pH 3.5) for 30 min at room temperature with softly stirring to remove the excess thionine molecules and airing for further use.

2.3. Measurement

PBS buffer (PH 3.5) was used as the supporting electrolyte for the determination of nitrite. Thionine modified ACNTs electrode was activated by the successive cyclic voltammetric sweeps between 0.45 and 1.1 V at 100 mV s^{-1} in the blank PBS buffer solution (pH 3.5). Determination of nitrite was carried out by differential pulse voltamograms from 0.5 to 1.0 V versus Ag/AgCl reference electrode.

3. Results and discussion

3.1. Characteristics of thionine modified ACNTs

Due to the large specific surface area of ACNTs, high quantity of thionine can be adsorbed onto ACNTs through strong π – π stacking force between these two kinds of conjugated frames. Fig. 1 shows the images of SEM and TEM (inset) of ACNTs and thionine modified ACNTs. On the ACNTs electrode, carbon nanotubes are vertically and orderly aligned together and have slick outer wall (shown in Fig. 1a and the inset). Comparison with the SEM image of Fig. 1a, the SEM image of Fig. 1b become blurred and individual carbon nanotube is looked like bigger feather, and the TEM image of Fig. 1b (inset) clearly shows that there is much fleecy stuff on the surface of carbon nanotubes. They indicate that high quantity of thionine has adsorbed onto the surface of ACNTs after immersing a ACNTs electrode in thionine solution. The dispersion experiment of carbon nanotubes in aqueous solution reveals that carbon nanotubes are always reunited with high-density large aggregates and difficult to be dispersed

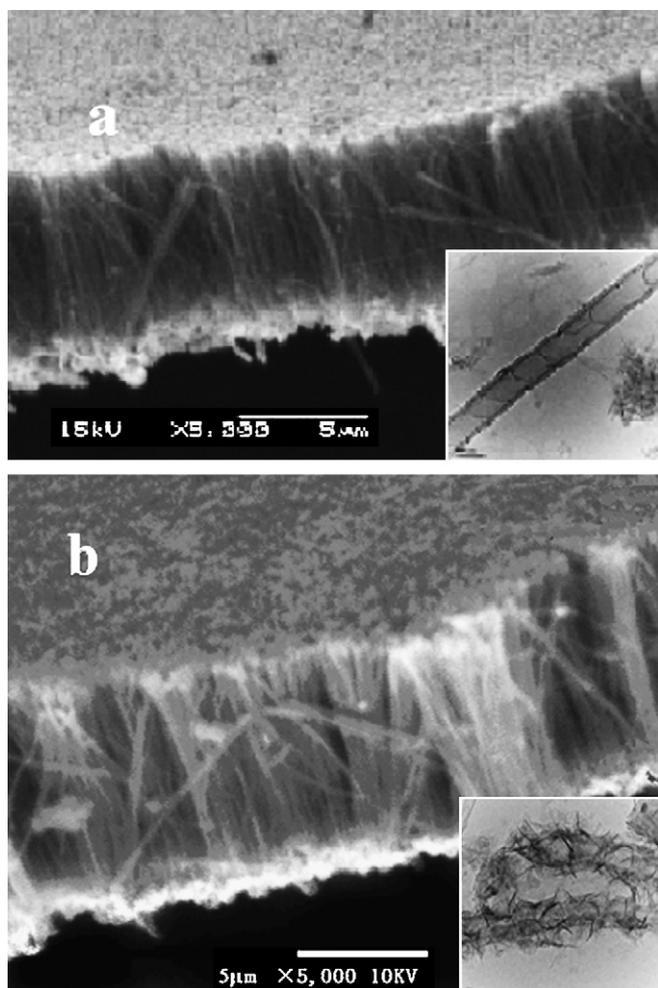


Fig. 1. SEM images of: (a) ACNTs, (b) thionine modified ACNTs. Inset: the TEM images.

in aqueous solution, but they are better wetted and dissolved well in thionine aqueous solution. Because thionine molecules are uniformly attached onto the individual ACNTs resulting to make the ACNTs surface more hydrophilic.

3.2. Electrocatalytic oxidation of nitrite on the thionine/ACNTs electrode

Fig. 2 shows the cyclic voltammograms of 1×10^{-4} mol L^{-1} nitrite in PBS buffer solution (pH 3.5) on glass carbon electrode (GCE), ACNTs electrode and thionine modified ACNTs electrode. As shown in Fig. 2A, on GCE there is an irreversible faintness oxidation peak at 0.90 V which is corresponding to the conversion of NO_2^- to NO_3^- through a two-electron oxidation process [45]. While on the bare ACNTs electrode, the oxidation peak potential of nitrite shifts negatively to 0.80 V and the peak current increased notably (Fig. 2B). The reduction of over-potential and the enhancement of peak current indicate that the ACNTs modified electrode can efficiently promote the electro-oxidation of NO_2^- . The better performance can be explained

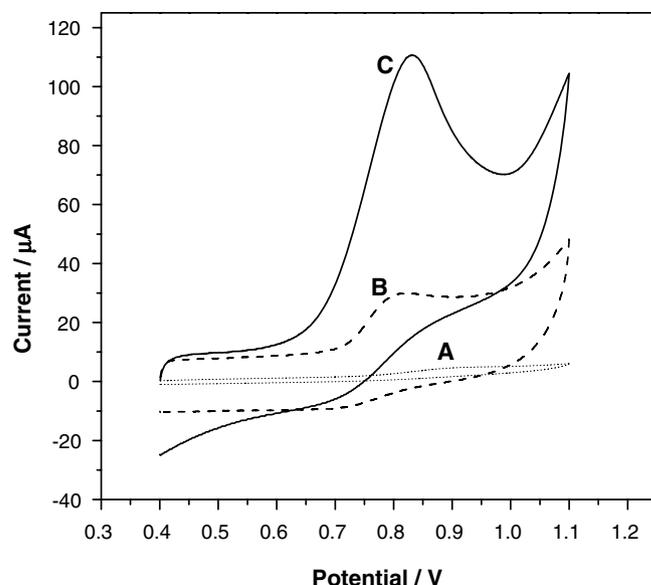
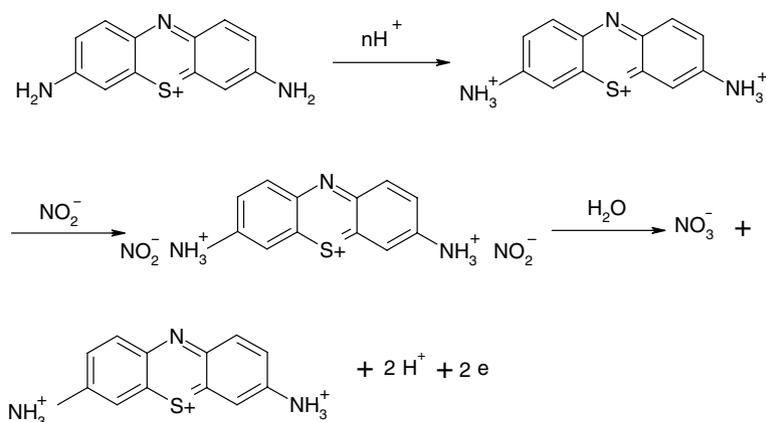


Fig. 2. Cyclic voltammograms of 1×10^{-4} mol L^{-1} NO_2^- on: (A) glassy carbon electrode, (B) ACNTs electrode, (C) thionine modified ACNTs electrode in PBS buffer solution (pH 3.5). Scan rate: 100 mV s^{-1} .

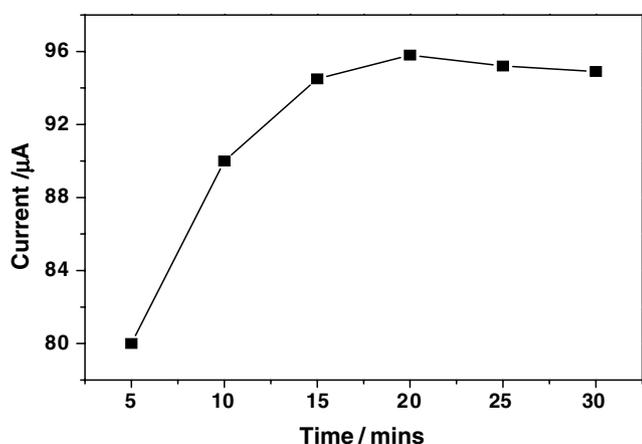
that CNTs have high specific surface area and novel electronic transfer ability, which leads to the larger electroactive surface of the modified electrode for detecting the nitrite. Noticeably, Fig. 2C shows that on thionine modified ACNTs electrode the oxidation current of nitrite is increased several times as much as that on bare ACNTs electrode, which was attribute to the thionine that has obviously pre-concentration effect on nitrite. The electrochemical reaction process of nitrite on thionine modified ACNTs electrode could be expressed as Scheme 1. One thionine molecule has two amino groups which have high reactivity to several function groups. In acidic solution, thionine is positively charged due to the protonation of NH_2 . The NH_3^+ in the structure of thionine attracts negatively charged ions such as NO_2^- and it is possible to accumulate nitrite ions on the electrode surface. The modified electrode promoted the oxidation of NO_2^- , and thus the oxidation peak current of NO_2^- increased sharply.

3.3. Effect of the amount of the thionine as modifier

The amount of thionine coated the ACNTs is expected to affect the oxidation current of nitrite. To investigate the effect of the amount of thionine on the oxidation current, a ACNTs electrode was immersed in 2 mg mL^{-1} of thionine solution for different time to form thionine/ACNTs electrode. 5, 10, 15, 20, 25, 30 min was choose for thionine/ACNTs preparation. As shown in Fig. 3, the oxidation current of 1×10^{-4} mol L^{-1} nitrite increases gradually with the time increased and at 25 min the oxidation current achieved the peak, which indicates that until 25 min, the amount of thionine attached on the ACNTs comes to a head and the catalytic effect to nitrite is the best one. When adsorb time increased continuously, the catalytic



Scheme 1. Electrocatalytic mechanism of thionine on ACNTs electrode to the oxidation of nitrite.

Fig. 3. Effect of the time that the ACNTs immersed in thionine solution on the oxidation current of nitrite. The concentration of nitrite is $1 \times 10^{-4} \text{ mol L}^{-1}$ and other conditions are the same as in Fig. 2.

effect attend to a platform. Therefore, 2 mg mL^{-1} of thionine was applied to coat 1 mm^2 ACNTs electrode for 25 min in the modification process.

3.4. Effect of pH of the supporting electrolyte

It was discovered that the oxidation peak current of nitrite in PBS buffer solution was more sensitive and the peak shape was more preferable than in the other supporting electrolytes such as B–R buffer solution and acetum buffer solution. In this experiment, acidity of PBS buffer solution is essential. In order to find the effect of the pH value of the PBS buffer to the detection of nitrite, different pH value of PBS was investigated. As shown in Fig. 4, the oxidation current response of nitrite is greatly influenced by pH value, apparently. The peak current increases with the increasing of pH and the highest current is observed in pH 3.5. Then there is a platform between the pH value 3.5 and 4.6 which indicates that the surface character of the electrode attains balance at the pH value. When the pH value kept up increasing, the oxidation current fell on the contrary. The phenomena show that with the increase

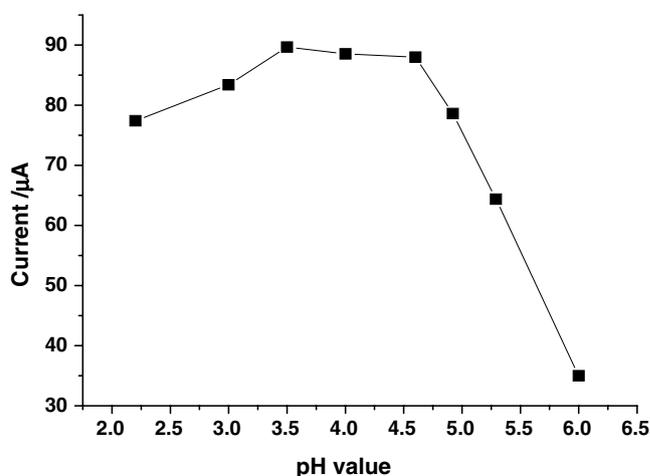
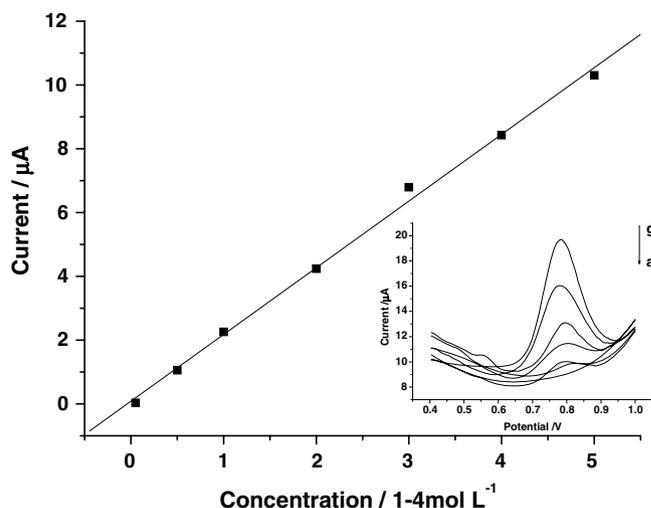
Fig. 4. Effect of the pH value of PBS buffer solution to the detection of $1 \times 10^{-4} \text{ mol L}^{-1}$ nitrite.Fig. 5. The linear range of nitrite detection. The inset is the differential pulse voltammetry responses of nitrite: (a) 0 mol L^{-1} ; (b) $3 \times 10^{-6} \text{ mol L}^{-1}$; (c) $1 \times 10^{-4} \text{ mol L}^{-1}$; (d) $2 \times 10^{-4} \text{ mol L}^{-1}$; (e) $3 \times 10^{-4} \text{ mol L}^{-1}$; (f) $4 \times 10^{-4} \text{ mol L}^{-1}$; (g) $5 \times 10^{-4} \text{ mol L}^{-1}$.

Table 1
Results for determination of nitrite in the sausage samples

Samples	Actual found (10^{-5} mol L $^{-1}$)	Added (10^{-5} mol L $^{-1}$)	Found after adding (10^{-5} mol L $^{-1}$)	RSD (%, $n = 5$)	Recovery (%, $n = 5$)	Concentration of nitrite in sausage (mg kg $^{-1}$)
1	0.225	2.00	2.15	2.12	96.3	15.5
2	0.287	2.00	2.33	2.26	102.2	19.8
3	0.277	2.00	2.18	1.99	95.2	19.1

of pH value, the NH $_2$ group of thionine can not convert to NH $_3^+$ effectively. So, the following experiments were carried out in pH 3.5 PBS buffer solution.

3.5. Linear range of nitrite detection, stability and reproducibility

The oxidation current performed with DPV has been employed to determine the nitrite concentration. Fig. 5 shows the linear relation between the oxidation peak current at 0.8 V and the nitrite concentration. The inset of Fig. 5 is the DPV of different concentration of nitrite. The results shows that peak current is proportional to the concentration of nitrite ranging from 3.0×10^{-6} to 5.0×10^{-4} mol L $^{-1}$. The linear regression equation of the former is $y = 0.023x - 8.75 \times 10^{-8}$ (x , the concentration of the nitrite, 10^{-5} mol L $^{-1}$, y , the oxidation current of the nitrite, μ A), with a correlation coefficient of $r = 0.997$. The detection limit ($S/N = 3$) is 1.12×10^{-6} mol L $^{-1}$.

To prove the precision of the method, electrochemical experiments were repeatedly performed 11 times with the same thionine modified ACNTs electrode. It has been found that the relative standard deviation of the response current of nitrite for five repetitive measurements was about 2.75% which indicates that the optimized thionine modified ACNTs electrode has a good operational stability. The operational stability of the thionine modified ACNTs electrode was measured by DPV in 0.1 mol L $^{-1}$ phosphate pH 3.5 buffer containing 1×10^{-5} mol L $^{-1}$ nitrite. The stability of thionine modified ACNTs electrode was found stable for over two week and retaining 94.5% reproducibility when stored at 4 °C.

3.6. Applications

Possible interference for the detection of nitrite on thionine modified ACNTs electrode was investigated by addition of various ions in to the PBS solution (pH 3.5) containing 1×10^{-5} mol L $^{-1}$ nitrite. The results showed that most of the ions, such as K $^+$, Na $^+$, Ag $^+$, Mg $^{2+}$, Ca $^{2+}$, Zn $^{2+}$, Ni $^{2+}$, F $^-$, Cl $^-$, NO $_3^-$, SO $_4^{2-}$, H $_2$ PO $_4^-$, HPO $_4^{2-}$, and PO $_4^{3-}$ did not interfere with the determination in a 100-fold concentration. We found 50-fold amount of Cd $^{2+}$ and Br $^-$, 20-fold amount of S $_2$ O $_3^{2-}$, and 10-fold amount of I $^-$ exhibit serious interference.

Then, the method was applied to determine nitrite in the sausage samples. The sausage was crush adequately in the

mortar. Crushed sausage, 5.000 g of 12.5 mL of borax saturated solution and some hot ultrapure water was mixed and then shook gently. Afterward, 2.5 mL of 30% ZnSO $_4$ solution dropped into it and dilute to 500 mL with water. 5 min later, the upper fattiness was thrown away and the clear solution was filtrated. At last the filtrate was added into 0.1 mol L $^{-1}$ PBS (pH 3.5) for the detection. Recovery studies were carried out on samples added with the determinate amounts of nitrite standard solution (5.00×10^{-5} mol L $^{-1}$). The results are summarized in Table 1: The “Actual found” is the concentration of the sample solution which measurement units is mol L $^{-1}$. The “Added” refers to the value of concentration of certain nitrite standard solution on the total concentration of the sample. The “Found after adding” is the detection result after the adding of the standard solution. The “recoveries” are derived by making the difference between “Found after adding” and “Actual found” to divide the “Added”.

4. Conclusions

Significantly electrochemical response to trace nitrite on thionine modified ACNTs electrode, which possesses maximized surface area and adsorbed abundant thionine molecules, has been observed in this work. This modified electrode provides a sensitive method for the detection of nitrite and can avoid interference from nitrate ion and from molecular oxygen. Compared with the nitrite oxidation-based nitrite sensor reported previously, this method exhibits wider linear range, lower detecting limit.

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