Amperometric Determination of Nitrite at Poly(Methylene Blue)-Modified Glassy Carbon Electrode

Guang-Ri Xu,[†,*] Guifang Xu,[†] Ming-Lu Xu,[†] Zhengqing Zhang,[†] Yuan Tian,[‡] Han Nim Choi,[§] and Won-Yong Lee[§,*]

[†]*Henan Institute of Science and Technology, Xinxiang 453003, P.R. China.* [*]*E-mail: xugr70@hotmail.com*
[‡]*School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, China*
[§]*Department of Chemistry and Center for Bioactive Molecular Hybrids, Yonsei University, Seoul 120-749, Korea*
[*]*E-mail: wylee@yonsei.ac.kr*

Electrochemical characteristics of nitrite ion were investigated at a poly(methylene blue)-modified glassy carbon electrode by cyclic voltammetry and differential pulse voltammetry. The poly(methylene blue)-modified glassy carbon electrode exhibited enhanced anodic signals for nitrite. The effects of key parameters on the detection of nitrite were evaluated at the modified electrode, such as pH, accumulation time, and scan rate. Under optimum condition, the chemically modified electrode can detect nitrite in the concentration range 2.0×10^{-6} to 5.0×10^{-4} M with the detection limit of 2.0×10^{-6} M and a correlation coefficient of 0.999. The detection of nitrite using the chemically modified electrode was not affected by common ions such as Na^+, K^+, Ca^{2+}, Cl^-, HPO_4^{2-} and $H_2PO_4^-$. The modified electrode showed good stability and reproducibility. The practical application of the present method was successfully applied to the determination of nitrite ion in cabbage samples.

Key Words : Methylene blue, Nitrite, Cyclic voltammetry, Differential pulse voltammetry, Chemically modified electrode

Introduction

Nitrite (NO_2^-) is widespread in natural environment and food because it is commonly used as food preservative.[1] Excess of nitrite in the human blood could be toxic because nitrite can interact with amine to form carcinogenic nitrosoamines.[2] Therefore, it is important to strictly control the nitrite content in food for keeping the human health. Many methods such as spectrophotometry,[3,4] chromatography,[5,6] and electrochemical sensors,[7-16] have been developed for nitrite determination. Among those methods, electrochemical methods are most favorable in terms of simplicity, speed, and cost effectiveness. The electrochemical detection methods of nitrite are based on either oxidation or reduction. The reductive detection methods of nitrite are poor in sensitivity and are susceptible to the interference such as reductions of nitrate (NO_3^-) and molecular oxygen.[11] The electrochemical oxidation of nitrite at bare electrodes commonly requires high over-potential and the electrode is also poisoned by the species formed during the oxidation process. Therefore, chemical modifications of electrode surface have been widely tried in order to decrease the overpotential and increase the sensitivity for the nitrite oxidation. Among the various modification methods, chemically modified electrodes based on metal complex,[7-9] functionalized electropolymerized films[10] or composite films containing carbon nanotubes[11-14] are most widely studied. Although aforementioned methods for the preparation of chemically modified electrodes are effective, new fabrication schemes providing improved sensitivity, selectivity and long-term stability are highly desired to broaden the application of electrochemical nitrite sensors in analytical sciences. Here, we report on a simple electrochemical nitrite sensor based on poly(methylene blue)-modified glassy carbon electrode. The poly(methylene blue)-modified electrodes are well known and commonly used as an electrocatalyst or electron transfer mediator.[17-19] The poly(methylene blue)-modified electrode can be easily prepared by simple electrochemical process. In the present work, the electrochemical behavior of the poly(methylene blue)-modified electrode towards nitrite oxidation have been characterized for the first time. Furthermore, the attractive analytical performance of the present electrochemical nitrite sensor has been evaluated in terms of response time, sensitivity, and long-term stability relative to the other electrochemical nitrite sensor. Finally, the present electrochemical sensor was applied to the determination of nitrite in a cabbage sample since cabbage is one of the main materials for fruit juice and it has known to contain a high concentration of nitrite.

Experimental

Reagents. All chemicals were analytical grade and were used as supplied without further purification. Methylene blue (MB), sodium nitrite, potassium dihydrogen phosphate, potassium hydrogen phosphate, citrate acid, sodium nitrate and ethanol were purchased from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China). A series of buffer having different pH values were prepared form disodium hydrogen phosphate-citrate acid buffer (DPCBS). Deionized water

was purchased from Xuefeng Water Treatment Company (Zhengzhou, China). All experiments were carried out at room temperature.

Instrumentation. All electrochemical experiments were performed with a CHI760C electrochemical workstation (Shanghai Chenhua Instruments, Shanghai, China) connected to a computer. The classical three-electrode system consisted of a KCl saturated Ag/AgCl electrode as the reference electrode, a platinum electrode as the auxiliary electrode, and a poly(methylene blue)-modified glassy carbon (GC) electrode (d = 2.0 mm or 3.0 mm) as the working electrode.

Preparation of Poly(Methylene Blue)-Modified Electrode. A GC disk electrode (Tokai Carbon, Japan) was consecutively polished with aqueous alumina slurries of 1.0, 0.3 and 0.05 μm until mirror finish. Electrode was thoroughly rinsed with water in each polishing step. The polished electrode was sonicated in ethanol and deionized water, respectively for 5 min and dried at room temperature. The methylene blue was electropolymerized from 1.0 mM methylene blue solution prepared in 2.5×10^{-2} M phosphate buffer solution (pH = 6.8) by cycling the electrode potential from −1.00 and +0.60 V at a scan rate of 100 mV/sec according to the literature.[17] The thickness of the polymer was controlled by the number of potential cycling. After electropolymerization, the electrodes were thoroughly rinsed with buffer to remove any remaining monomeric methylene blue. When not in use, the electrode was stored in deionized water.

Real Sample Test. GC/MB electrodes have been used to quantify nitrite in a real sample. Cabbage was purchased in a local supermarket, which was cut into little pieces. Cabbage (1000 g) was placed in a mortar, then grinded into pulp. The pulp was placed in disodium hydrogen phosphate - citric acid buffer (pH = 3.81) overnight. The solution was consecutively filtered with gauze, filter paper, and 0.22 μm micropore membrane. The filtrate was electrochemically detected.

Results and Discussion

Electrochemical Oxidation of Nitrite. The poly(methylene blue)-modified electrode was prepared from 1.0 mM monomeric methylene blue solution by cycling the electrode potential from −1.00 and +0.60 V at a scan rate of 100 mV/ sec in 2.5×10^{-2} M phosphate buffer according to the literature.[17] As shown in Figure 1, the growth of the polymer film was confirmed by a decrease in the redox waves at −0.05 V and −0.24 V for the monomeric methylene blue and by an increase in the redox waves at 0.05 V and −0.04 V for the poly(methylene blue) film. Figure 2 shows cyclic voltammograms of 2.0×10^{-3} M nitrite at the poly(methylene blue)-modified electrode and at bare GC in 2.5×10^{-2} M phosphate buffer (pH 3.8). The voltammetric behavior of nitrite at the bare GC electrode showed irreversible electrode reaction with sluggish and small response at +0.88 V corresponding to the two-electron oxidation process of NO_2^- to NO_3^-.[20] While at the poly(methylene blue)-modified GC
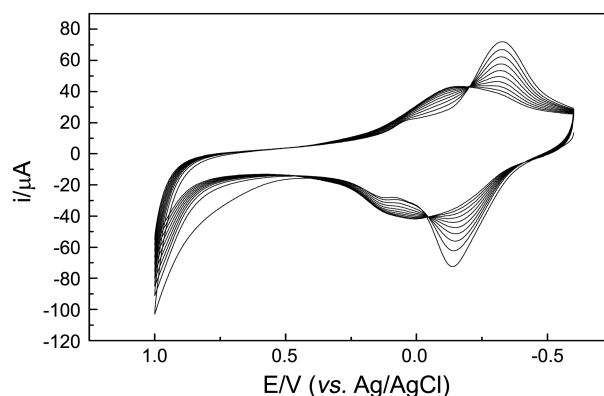

Figure 1. Cyclic voltammograms of the electropolymerization of methylene blue at a glassy carbon electrode with a scan rate of 100 mV/s in 2.5×10^{-2} M phosphate buffer at pH 6.8; electrode diameter = 2.0 mm.
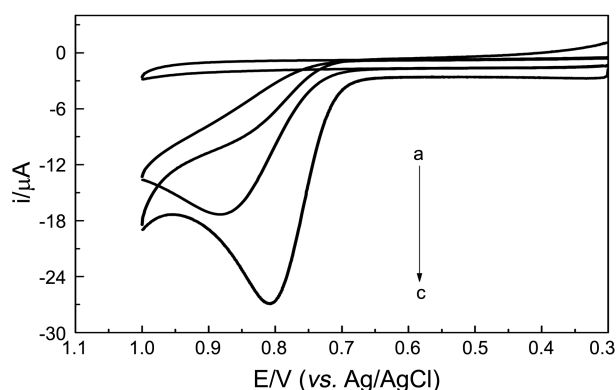

Figure 2. Cyclic voltammograms of a bare GC electrode in hydrogen phosphate-citrate acid buffer (pH 3.81) in the absence (a) and the presence (b) of 2.0×10^{-3} M nitrite and also the cyclic voltammogram of the poly(methylene blue)-modified GC electrode in the presence of 2.0 mM nitrite (c). Scan rate: 20 mV/s; immersion time: 45 s; room temperature; electrode diameter = 2.0 mm.

electrode, irreversible oxidation peak potential was shifted to lower potential at 0.81 V and the the anodic peak current obviously increased at least twice larger than that obtained at a bare GC electrode. The decrease in overpotential and the increase in the peak current indicate that the poly(methylene blue)-modified GC electrode can efficiently promote the electrooxidation of nitrite as reported previously for NADH in the literature.[18]

$$PMB_{ox} + NO_2^- + 2H_2O \rightarrow NO_3^- + PMB_{red} \qquad (1)$$

$$PMB_{red} \rightarrow PMB_{ox} + 2H^+ + 2e^- \qquad (2)$$
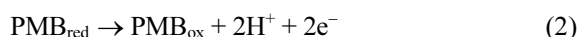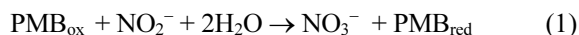
In addition, the enhancement of the oxidation current of nitrite could be partly attributed to the preconcentration capability of the poly(methylene blue)-modified GC electrode towards nitrite. Since two amino groups of the methylene blue possess pK_a values in the range 4.5-6.0,[17] the poly(methylene blue) is positively charged in acidic solution. Therefore, the positively charged poly(methylene blue) film on GC electrode attracts the negatively charged nitrite ion
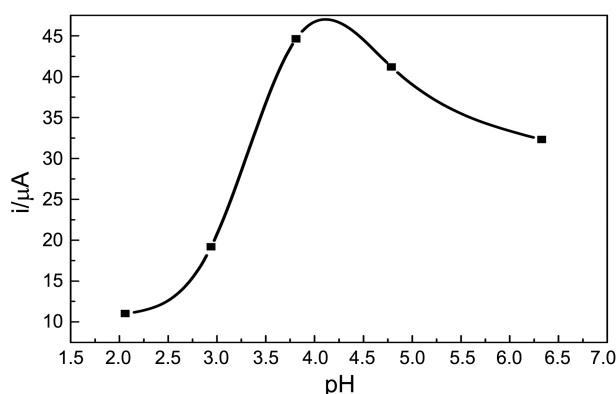
Figure 3. Effect of pH on the oxidation current of nitrite ion at poly(methylene blue)-modified glassy carbon electrodes. Experimental conditions: 2.0×10^{-3} M nitrite ion in disodium hydrogen phosphate-citrate acid buffer; Scan rate: 20 mV/s; immersion time: 45 s; room temperature; electrode diameter = 3.0 mm.
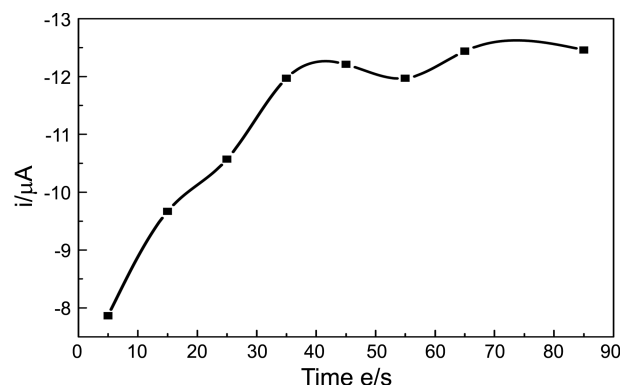

Figure 4. Effect of accumulation time on the oxidation signal of nitrite obtained at poly(methylene blue)-modified glassy carbon electrodes. Experimental conditions: 1.0×10^{-3} M nitrite ion in disodium hydrogen phosphate-citrate acid buffer (pH 3.81); Scan rate: 20 mV/s; room temperature; electrode diameter = 2.0 mm.

(NO$_2^-$) via electrostatic interaction and thus it is possible to accumlate nitrite ions on the electrode surface.

The number of potential cycling in the preparation poly(methylene blue)-modified GC electrode has affected the oxidation signal for nitrite. The optimum number of potential cycling was found to be 10, in that case the poly(methylene blue)-modified GC electrode exhibited the largest oxidation current for nitrite solution. Further potential cycling (more than 20 cycles) did not increase the electrochemical signal, but rather slightly decreased them.

Effect of pH. The effect of pH on the current response of nitrite has been examined. As shown in Figure 3, the oxidation peak current is greatly affected by buffer solution pH. The oxidation peak current increases with the increase of pH from 2.0 to 3.8 and the highest current is observed at around pH 3.8. Then, the oxidation peak current slowly decrease with the increase of pH in the range up to pH 6.5. The results indicate that the poly(methylene blue) film on GC electrode is positively charged at pH 3.8 and thus attracts the negatively charged nitrite ion but the poly(methylene blue) film on GC electrode is not so stable in strong acidic conditions at pH below 3.8. Therefore, all the subsequent experiments are carried out in phosphate buffer at pH 3.8.

Effect of Accumulation Time. Since the enhancement of the oxidation peak current is related to the the preconcentration capability of the poly(methylene blue)-modified GC electrode towards nitrite, the accumulation time can strongly affects the current signal. The poly(methylene blue)-modified GC electrode has been soaked into the nitrite solution for the accumulation. As shown in Figure 4, the oxidation peak current of nitrite ion remarkably increases with the increase of accumulation time up to 45 s and then the peak reaches a platau after 45 s, which indicates that the accumulation process is so rapid and effective. In all the subsequent experiments, the accumulation time was 45 s.

Scan Rate Study. The effect of pH on the oxidation peak current of nitrite has been examined. Figure 5 exhibits the cyclic voltammograms of nitrilte ion obtained on the poly(methylene blue)-modified GC electrodes in phosphate
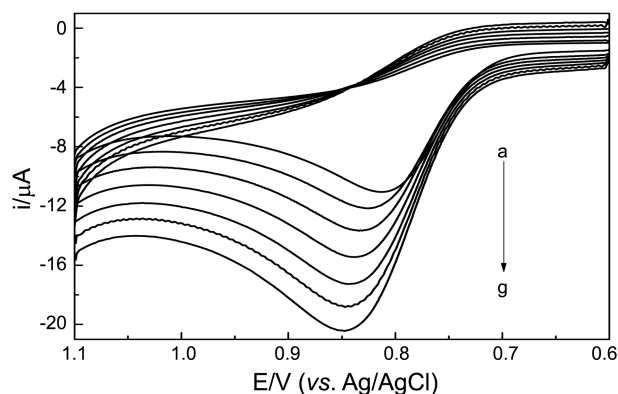
Figure 5. Effect of scanning rate on the determination of nitrite at poly(methylene blue)-modified glassy carbon electrodes. Experimental conditions: scanning rate (a-g): 10, 20, 30, 40, 50, 60, 70 mV/s, 1.0×10^{-3} M nitrite in disodium hydrogen phosphate-citrate acid buffer (pH 3.81); immersion time: 45 s; room temperature; electrode diameter = 2.0 mm.

buffer (pH 3.81) at different scan rates ranging from 10 mV to 70 mV. As the scan rate increases, the oxidation current increase. The increase in the oxidation peak current is linearly dependent upon the scan rate (v) with the equation: $I_p = -0.130 v - 7.38$, r = 0.992 (graph is not shown). It can be inferred that the current is adsorption-controlled current. It is also indicats that the negatively charged nitrite ion is effectivly accumulated within the positively charged poly(methylene blue) film on GC electrode surface. While, when the scan study is carried out in the relative high scan rate ranging 100 mV to 700 mV, the increase in the oxidation peak current is linearly dependent upon the square root of scan rate (v) with the equation: $I_p = -0.225 v - 0.406$, r = 0.983 (graph is not shown). It can be inferred that the current is typical diffusion-controlled current in high scan region.

Response Characteristics. The oxidation peak current obtained with differential pulse voltammetry (DPV) has been used to construct the calibration curve of nitrite standard solution. DPV conditions were 25 mV pulse height, 4.0 mV step height and 15 Hz frequency. As shown in

(a)

(b)
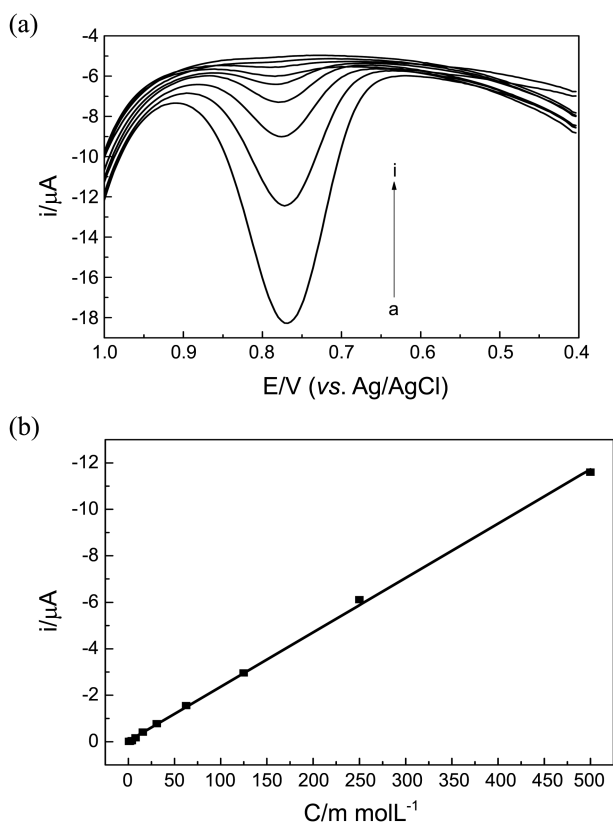
Figure 6. (A) Differential pulse voltammograms of various concentrations of nitrite at poly(methylene blue)-modified glassy carbon electrodes. Experimental conditions: (a) 5.0×10^{-4} mol/L, (b) 2.5×10^{-4} mol/L, (c) 1.3×10^{-4} mol/L, (d) 6.3×10^{-5} mol/L, (e) 3.1×10^{-5} mol/L, (f) 1.6×10^{-5} mol/L, (g) 7.8×10^{-6} mol/L, (h) 3.9×10^{-6} mol/L, (i) 2.0×10^{-6} mol/L in disodium hydrogen phosphate-citrate acid buffer (pH 3.81); Scan rate: 20 mV/s; immerging time: 45 s; room temperature; electrode diameter = 2.0 mm. (B) Calibration curve of nitrite.

Figure 6, the oxidation peak current of nitrite is linearly proportional to the concentration of nitrite ion ranging from 2.00×10^{-6} M to 5.00×10^{-4} M (The linear regression equation: $i = -2.34 \times 10^{-2} C + 2.44 \times 10^{-8}$, r = 0.999). The detection limit (S/N = 3) for nitrite is 2.00×10^{-6} M. The linear dynamic range and detection limit obtained at the present poly(methylene blue)-modified GC electrode without the incorporation of nanostructured materials are clearly comparable to those reported in the literature,[7-14] which is a distinctive advantage of the present method.

The possible interference from several common ions in the detection of nitrite ion was tested by adding various ions into the 0.5 mM nitrite solution in phosphate buffer solution at pH 3.8. The concentrations of tested interference ions are 100-fold higher than that of nitrite. The tested results show that most common ions such as Na^+, K^+, Ca^{2+}, Cl^-, HPO_4^{2-} and $H_2PO_4^-$ does not affect the detetion of nitrite as summarized in Table 1. While, in the presence of 5-fold higer concentration of nitrate ion, the electrochemical response of the nitrite is 25% higher than that in the absence of nitrate ion, which could be attributed the conversion of unstable nitrate into nitrite leading to the increased local

Table 1. Effect of interfering ions in the determination of nitrite

Interferent	[interferent]/[NO_2^-]	Nitrite recovery (%)
Na^+	100	99.6 ($\pm$ 0.2)
K^+	100	100.2 ($\pm$ 0.1)
Ca^{2+}	100	99.9 ($\pm$ 0.1)
Cl^-	100	99.8 ($\pm$ 0.1)
HPO_4^{2-}	100	100.7 ($\pm$ 0.2)
$H_2PO_4^-$	100	99.5 ($\pm$ 0.3)
Nitrate	5.0	125 ($\pm$ 3.8)

concentration of nitrite in solution.

The present poly(methylene blue)-modified glassy carbon electrode was quite stable for 2 month period without significant signal drop when it stored in phosphate buffer solution (pH 6.8) at 4 °C.

Determination of Nitrite in Real Samples. The present method was appplied to the determination of nitrite in the cabbage samples. The cabbage was cut into little pieces and 1000 g of the cabbage was crushed in a mortar. The crushed cabbage was mixed with 250 mL of disodium hydrogen phosphate-citric acid buffer (pH = 3.8) and then shaken gently overnight. The sample was filtered with gauze, filter paper, and finally with 0.22 μm micropore membrane filter. The filtrate was then analyzed. Standard addition method was used in the determination of nitrite in the cabbage samples. The concentration of cabbage samples are found to be 1.09 ± 0.03 mg/kg (n = 5). This result is quite similar to the previously reported value.[21] In addiiton, the present method was quite reproducible with the relative standard deviation of 2.97%.

Conclusions

The poly(methylene blue)-modified glassy carbon electrode has been used for a highly sensitive and selective electrochemical determination of nitrite in cabbage samples. Due to the preconcentration effect and electrocatalytic activity of the poly(methylene blue) film towards nitrite ion, the present nitrite sensor exhibited the increased electrochemical response. Thus, the present nitrite sensor based on the poly(methylene blue)-modified glassy carbon electrode displayed a reasonably good sensitivity along with a detection limit (S/N = 3) of 2.0×10^{-6} M without a complicated incorporation of CNT or nanoparticles. Moreover, the present nitrite sensor is almost free from the interference by common ions such as sodium ion, potassium ion, calcium ion, chloride ion, and phosphate ion. The sensitivity of the present nitrite sensor along with its good selectivity and reproducibility might allow us for its potential use in the determination of nitrite in food and environmental samples.

References

1. Alonse, B.; Etxaniz, B.; Martinez, M. D. *Food. Addit. Contam.* **1992**, *9*, 111.
2. Bruning-Fann, C. S.; Kaneene, J. B. *Vet. Hum. Toxicol.* **1993**, *35*, 5211.
3. Tarafder, P. K.; Rathore, D. P. S. *Analyst.* **1988**, *113*, 1073.
4. Wang, G. F.; Satake, M.; Horita, K. *Talanta* **1998**, *46*, 671.
5. Matteo, V. D.; Esposito, E. *J. Chromatogr. A* **1997**, *789*, 213.
6. Ito, K.; Takayama, Y.; Makabe, N.; Mitsui, R.; Hirokawa, T. *J. Chromatogr. A* **2005**, *1083*, 63.
7. Caro, C. A.; Bedioui, F.; Zagal, J. H. *Electrochim. Acta* **2002**, *47*, 1489.
8. Pournaghi-Azar, M. H.; Dastangoo, H. *J. Electroanal. Chem.* **2004**, *567*, 211.
9. Santos, W.; Sousa, A.; Luz, R; Damos, F.; Kubota, L.; Tanaka, A.; Tanaka, S. *Talanta* **2006**, *70*, 588.
10. Kalimuthu, S.; John, S. A. *Electrochem. Commun.* **2009**, *11*, 1065.
11. Jiang, L.; Wang, R.; Li, X.; Jiang, L.; Lu, G. *Electrochem. Commun.* **2005**, *7*, 597.
12. Zhao, K.; Song, H.; Zhung, S.; Dai, L.; He, P.; Fang, Y. *Electrochem. Commun.* **2007**, *9*, 65.
13. Xiao, F.; Mo, Z.; Zhao, F.; Zeng, B. *Electrochem. Commun.* **2008**, *10*, 1740.
14. Zhu, N.; Xu, Q.; Li, S.; Gao, H. *Electrochem. Commun.* **2009**, *11*, 2308.
15. Wang, S.; Yin, Y.; Lin, X. *Electrochem. Commun.* **2004**, *6*, 259.
16. Liu, L.; Tian, L.; Xu, H.; Lu, N. *J. Electroanal. Chem.* **2006**, *587*, 213.
17. Karyakin, A. A.; Strakhova, A. K.; Karyakina, E. E.; Varfolomeyev, S. D.; Yatsimirsky, A. K. *Bioelectrochem. Bioenerg.* **1993**, *32*, 35.
18. Silber, A.; Hampp, N.; Schuhmann, W. *Biosens. Bioelectron.* **1996**, *11*, 215.
19. Brett, C. M. A.; Inzelt, G.; Kertesz, V. *Anal. Chim. Acta* **1999**, *385*, 119.
20. Chen, S. M. *J. Electroanal. Chem.* **1998**, *457*, 23.
21. Wang, F.; Wang, S. Z.; Zhu, L. *Chinese Vegetables* **2009**, *14*, 17.