ISSN : 0974-7419 Volume 11 Issue 1

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 11(1) 2012 [06-11]

Electrochemical detection of hypoxanthine using chitosan-multiwall carbon nanotubes modified electrode

Lizhu Yang[1,2*], Xia Wang[1], Tiantian Zhang[2], Jing Zhu[1], Xiaofeng Yang[2]

[1]Department of Chemistry, East China Normal University, Shanghai, 200062, (P.R.CHINA)

[2]School of Pharmacy, Wenzhou Medical College, Wenzhou, 325035, (P.R.CHINA)

E-mail : yanglz3000@yahoo.com.cn

Received: 24th July, 2011 ; Accepted: 24th August, 2011

ABSTRACT

A novel chitosan-carboxylated multiwall carbon nanotube modified glassy carbon electrode (CTS/MWNT/GCE) was developed to investigate the oxidation behavior of hypoxanthine using cyclic voltammetry and linear sweep voltammetry modes. The electrochemical behavior of hypoxanthine was investigated with great detail. Under the optimized conditions, the oxidation peak current is proportional to the concentration of hypoxanthine over the range from 3.0×10^{-7} mol/l to 2.0×10^{-4} mol/l with a determination limit (S/N=3) 8.0×10^{-8} mol/l. The proposed method was successfully applied in the detection of hypoxanthine in fish samples.

KEYWORDS

Hypoxanthine;
Chitosan;
Multiwall carbon nanotubes;
Cyclic voltamograms;
Linear sweep voltamograms.

INTRODUCTION

Chitosan, which is derived from chitin (a naturally occurring polysaccharide found in insects, arthropods and crustaceans) by de-acetylation, has excellent biological compatibility, and has been extensively used as modifier due to its amino groups and hydroxy groups[1, 2]. Besides, chitosan is a natural polymer, using it as a dispersant is the requirement of environment of protection. Carbon nanotubes (CNT) are new and interesting members of the carbon family offering unique mechanical and electronic properties combined with chemical stability. The active site being the end of the tubes make CNT hold excellent properties[3], so CNT modified solid electrode has attracted much attention. But the insolubility of it in most solvents restrained its application in electroanalysis[4].

The development of detection for hypoxanthine (HX) is of medical and biological importance[5, 6]. On the other hand, the levels of these compounds are generally used in the food industry as an index for evaluating meat or fish freshness[7]. A great number of methods have been developed for the determination of hypoxanthine including high performance liquid chromatography (HPLC)[8], chromatography[9], spectrophotometry[10]. Additionally, various electrochemical methods using different electrodes have also been reported because of high sensitivity and extreme simplicity. For example, a electrochemiluminescent (ECL) biosensor based on an electrically heated carbon paste electrode (HCPE) that was surface modified by xanthine oxidase (XOD) was designed for hypoxanthine detection[11]. The linear range is from 8.0×10^{-7} mol/l to 3.0×10^{-4} mol/l at a temperature of 25°C and from 6.0×10^{-7} mol/l to

2.0×10^{-4} mol/l at a temperature of 35°C. A Pd-IrO_2 modified electrode and a mesoporous TiO_2-modified carbon paste electrode were employed for the determination of hypoxanthine[12, 13].

In this paper, a novel chitosan-carboxylated multiwall carbon nanotube (MWNT) modifier was obtained by putting carboxylated MWNT into chitosan solution (0.5%), achieving a chitosan-carboxylated MWNT modified glassy carbon electrode (CTS/MWNT/GCE). The CTS/MWNT/GCE was used for the detection of hypoxanthine. Compared with a bare electrode, the CTS/MWNT/GCE showed a significant enhancement effect on the oxidation peak current of hypoxanthine, and an electrochemical method was proposed to detect hypoxanthine in fish samples. The new procedure possesses several advantages, such as easy modification of the electrode, a lower detection limit, excellent reproducibility, highly stability and low cost.

EXPERIMENTAL

Apparatus and reagents

All the electrochemical measurements were performed with a CHI 650B electrochemical work station (Shanghai Chenhua Co., China). A conventional three-electrode system was used with a modified or bare GC electrode (3mm in diameter) as working electrode, a platinum wire electrode as counter electrode and a saturated calomel electrode as a reference electrode. A PHS-3C PH Meter (Shanghai precision & scientific instrument Co., LTD, China) combined PH electrode was used for pH measurement. All potentials were referred to the SCE.

The chitosan solution (0.5%) was prepared by dissolving 0.01g chitosan (purchased from Fluka, deacetylation degree≥85%) in 2 ml 2 mol/l acetic acid[14]. The MWNT used in this work (obtained from the Institute of Nanometer, China Central Normal University) were synthesized by the catalytic pyrolysis. And purification begins with a 7 h reflux under the condition of magnetic heat stirring in 2 mol/l nitric acid, then ultrasonic dispersing for 1 h in thick muriatic acid, washing with redistilled water until a neutral solution is gained[15]. Hypoxanthine (purchased from Sinopharm Chemical Reagent Co., LTD, China) was dissolved in 0.01 mol/l NaOH to form 1.0×10^{-2} mol/l stock solution and then stored at 4°C. In the experiment, hypoxanthine stock solution was diluted to working solutions at desired concentration using redistilled water. All the chemicals were used without further purification and all the solutions were prepared with redistilled water. All experiment were carried out at room temperature (approx.25 °C).

Preparation for chitosan-MWNT modified GC electrode

0.2 mg carboxylated MWNT was putted into 2 ml chitosan solution (0.5%), ultrasonic agitation for a few minutes, then a black chitosan-MWNT suspension was obtained. Before being modified, the GCE was polished with 0.3 and 0.05μm aluminum slurry, rinsed thoroughly with redistilled water, then ultrasonically rinsed with acetone, alcohol and redistilled water for 1 min each, and dried under an infrared lamp. After the GCE was cooled, it was smeared evenly with 10 μl of a chitosan-MWNT solution by a micro-syringe, and then dried under an infrared lamp for 10 min. After cooling, the chitosan-MWNT modified GC electrode (CTS/MWNT/GCE) could be used. The chitosan modified GCE (CTS/GCE) was prepared by the same method.

Experimental procedure

A 0.1 mol/l phosphate buffer solution (pH=7.0) was used as the supporting electrolyte in a conventional electrochemical cell. At the begin beginning of experiment, a certain volume of hypoxanthine standard solution was placed into the cell to make up 10 ml mixture solution. The accumulation was carried out at open circuit via stirring the solution for 2 min, and then kept quiet for 10 s. The voltammograms were recorded between 0.0 V and 1.0 V at a scan rate of 100 mV/s After each measurement, the modified electrode was activated by successive cyclic voltammetric sweeps between 0.0 V to 1.0 V at 100 mV/s in a blank phosphate buffer solution (pH=7.0).

RESULTS AND DISCUSSION

Voltammetric responses of hypoxanthine at CTS/MWNT/GCE

Figure 1 Shows the successive cyclic

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voltammograms of hypoxanthine at chitosan-MWNT modified GCE in 0.1 mol/l phosphate buffer solution (pH=7.0), During the first anodic sweep from 0.0 to 1.0 V at scan rate of 100 mV/s, an oxidation peak at 0.78V is observed for 2.0×10^{-6} mol/l hypoxanthine. On the reverse scan, no corresponding reduction peak is observed, indicating that the electrochemical oxidation of hypoxanthine is totally irreversible. Otherwise, the oxidation peak current obviously decreases during the second cyclic potential sweep, maybe attributed to the adsorption of oxidative product at the electrode surface.

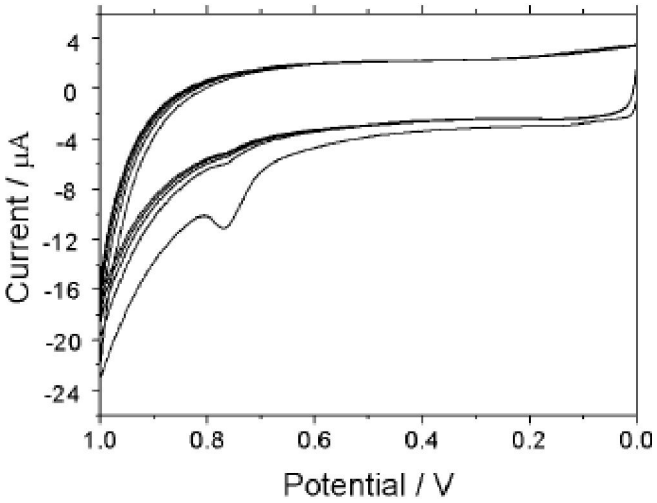

Figure 1 : Successive cyclic voltammograms of 2.0×10^{-6} mol/l HX at CTS/MWNT/GCE in 0.1 mol/l PBS (pH=7.0) Scan rate: 100 mV/s

Cyclic voltammograms of hypoxanthine at the chitosan-MWNT modified GCE, chitosan modified GCE and bare GCE were shown in Figure 2. At the bare GCE, a weak irreversible wave with oxidation peak potential at 0.78V (curve 2a), and at the CTS/GCE, there is no electrochemistry response (curve 2b), while at the CTS/MWNT/GCE, the oxidation peak current increased significantly (curve 2c), which implied that chitosan-MWNT modified GCE held the properties that was favorable to the oxidation of hypoxanthine. Better performance of hypoxanthine on CTS/MWNT/GCE was attributed to the MWNT potential porous leading to the larger electroactive surface area and thus greater response of hypoxanthine[3].

Effect of the supporting electrolyte and solution pH

As a key factor affecting the electrochemical responses of 2.0×10^{-6} mol/l hypoxanthine, different supporting electrolytes were tested by CV, including 0.1 mol/l phosphate buffer solution (PBS, pH 4.5-8.5), 0.1 mol/l sodium acetate-acetic acid buffer solution (NaAc-HAc, pH 4.0-6.0) and other different acids and alkalies, such as H_2SO_4 (0.1 mol/l), HCl (0.1 mol/l), $HClO_4$ (0.1 mol/l) and NaOH (0.1 mol/l). It was found that the oxidation peak current and the voltammogram shape were best defined in phosphate buffer solution. Therefore, a phosphate buffer solution was used for the detecting hypoxanthine.

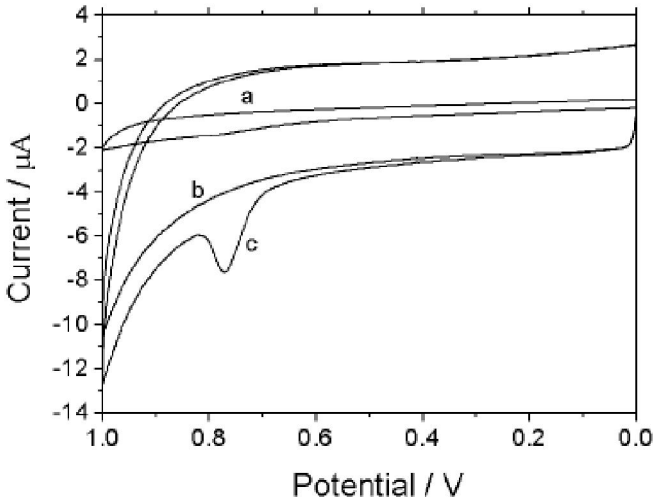

Figure 2 : Cyclic voltammograms of 2.0×10^{-6} mol/l HX in 0.1 mol/l PBS (pH=7.0) at (a) bare GCE, (b) CTS/GCE, (c) CTS/MWNT/GCE. Scan rate: 100 mV/s

The pH effect of the supporting electrolyte on the peak potential and the peak current for the oxidation of hypoxanthine was studied, as was shown in Figure 3. As pH value increasing from 4.5 to 6.5, the oxidation peak current of hypoxanthine obviously increased. When further improving the pH value from 6.5 to 8.5, the oxidation peak current changed very slightly. In addition, as pH value increasing from 4.5 to 8.5, the E_{pa} shifted linearly to more negative potential, obeying the following equation: E_{pa} = 1.2106-0.06158pH. The slope of E_{pa}/pH is -61.58 mV, indicating that the number of electrons and protons involved in the oxidation of hypoxanthine is the same.

Effect of the amount of chitosan-MWNT modifier at the CTS/MWNT/GCE

The amount of chitosan-MWNT suspension on the surface of electrode affected the oxidation peak current of hypoxanthine. Figure 4 showed that the peak current of hypoxanthine enhanced when the amount of

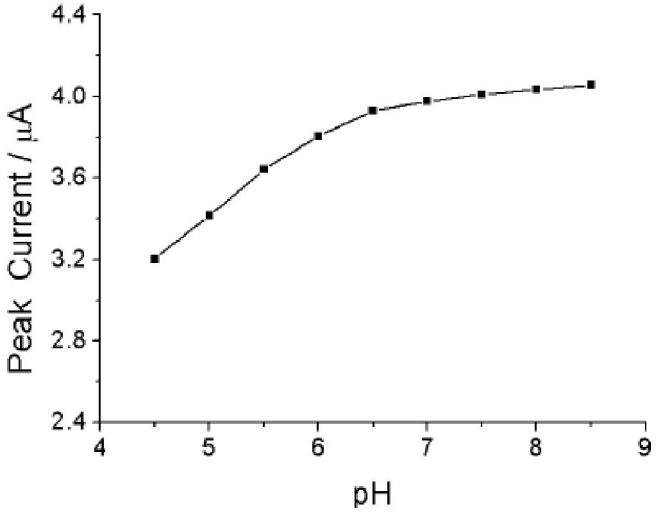

Figure 3 : Effect of the solution pH on oxidation peak current of 2.0×10^{-6} mol/l HX at CTS/MWNT/GCE in 0.1 mol/l PBS (pH=7.0). Scan rate: 100 mV/s

chitosan-MWNT suspension increased, which probably because of the presence of electro-reactive sites which increased with the amount of MWNT[3]. The peak current is very high when used 8μl of chitosan-MWNT suspension. Further increasing the amount of the chitosan-MWNT suspension, the peak current almost keep stable. However, when it exceeds 12 μl, the background current enhanced while the peak current conversely decrease. These because the film on the electrode surface was too thick, which prevented the electron transfer between hypoxanthine and electrode. In this paper, 10 μl of chitosan-MWNT suspension was used for voltammetric determination of hypoxanthine.

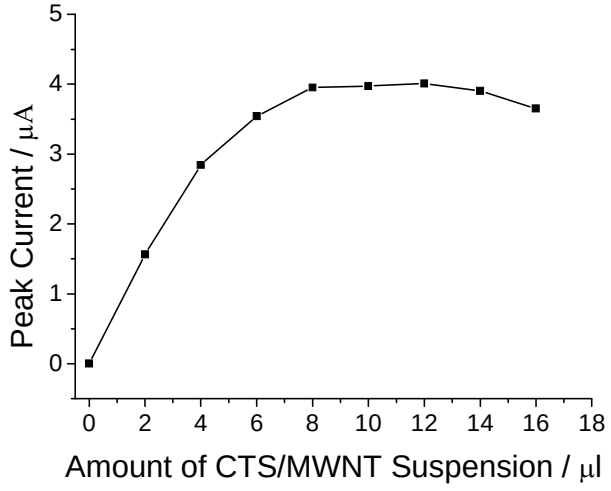

Figure 4 : Effect of amount of chitosan-MWNT suspension on oxidation peak current of 2.0×10^{-6} mol/l HX in 0.1mol/l PBS (pH=7.0). Scan rate: 100 mV/s

Effect of accumulation conditions

Accumulation is a simple and effective way to enhance the determination sensitivity. Accumulation potential and accumulation time have to be considered in a common accumulation step. The oxidation peak current of 2.0×10^{-6} mol/l hypoxanthine was measured after 2 min accumulation under different potential as well as open circuit. The peak currents almost keep unchanged, revealing that the accumulation potential has no influence on the oxidation peak current of hypoxanthine at the CTS/MWNT/GCE. Thus, the accumulation step was performed under open circuit.

Figure 5 shows the dependence of oxidation peak current on the accumulation time. when the accumulation time increased from 0 to 2 min, the oxidation peak current increased obviously. However, with further increasing accumulation time the oxidation peak current changes slightly. Considering both the sensitively and working efficiency, an accumulation time of 2 min was chosen as the optimal accumulation time.

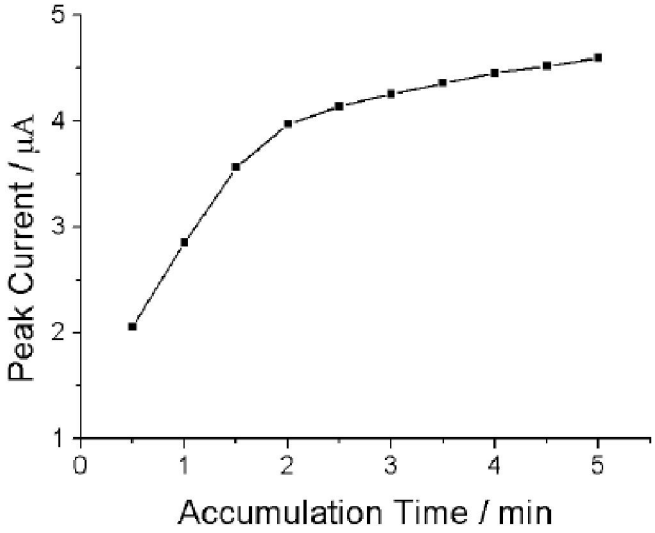

Figure 5 : Effect of accumulation time on oxidation peak current of 2.0×10^{-6} mol/l HX at CTS/MWNT/GCE in 0.1 mol/l PBS (pH=7.0). Scan rate: 100 mV/s

Effect of scan rate

The relation between peak current and scan rate had become the diagnostic criteria in distinguishing controlled step of electrode process for oxidation reactions occurred at electrode surface. On the other hand, although the peak current increased with the increase of scan rate the background current was enhanced too. High scan rate was not suitable for the measurement of

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peak current. In this work, effects of scan rate (υ) on the oxidation peak current (i_{pa}) of 2.0×10^{-6} mol/l hypoxanthine were examined by CV in 0.1 mol/l PBS (pH=7.0). The oxidation peak current of hypoxanthine was proportional to the scan rate in the range from 25 to 200 mV/s and could be expressed as follows: i_{pa} (μA) = 3.176 υ (mV/s) + 0.917 (R = 0.999). Thus, the electrode process was controlled by the adsorption step and the scan rate 100 mV/s was chosen for quantitative analysis of hypoxanthine.

Calibration gragh, detection limit, and reproducibility

The relationship between the oxidation peak current (i_{pa}) of hypoxanthine and its concentration (C) was investigated in 0.1 mol/l phosphate buffer solution (pH=7.0) by SLV. Figure 6 showed the current response of different concentration of HX. The linear graph increases from 3.0×10^{-7} mol/l to 2.0×10^{-4} mol/l with a regression equation of i_{pa} (μA) = $1.385+1.307\times10^{6}C_{HX}$ (mol/l) (r=0.998). The detection limit (S/N=3) is 8.0×10^{-8} mol/l.

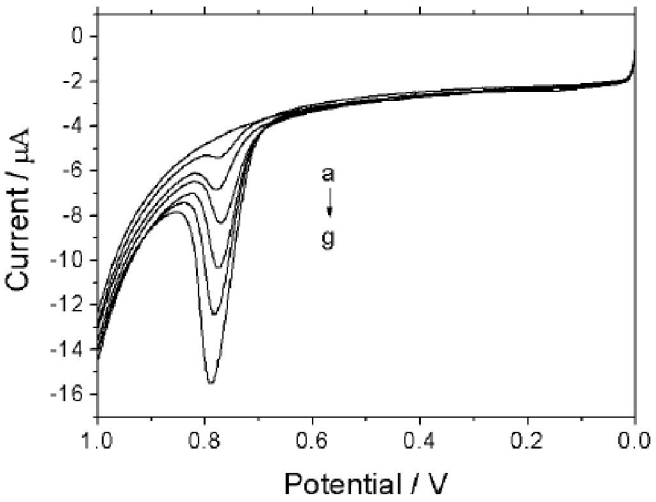

Figure 6 : Linear sweep voltamograms of different concentration of HX in 0.1 mol/l PBS (pH=7.0): (a) 0, (b)5.0×10^{-7} mol/l, (c) 1.0×10^{-6} mol/l, (d) 2.0×10^{-6} mol/l, (e) 4.0×10^{-6} mol/l, (f) 5.0×10^{-6} mol/l and (g) 1.0×10^{-5} mol/l at CTS/MWNT/GCE. Scan rate: 100 mV/s

The relative standard deviation (RSD) of 3.5% for 10-times parallel detections of 2.0×10^{-6} mol/l hypoxanthine suggests excellent reproducibility of CTS/MWNT/GCE. Additionally, the reproducibility between multiple electrode preparations was estimated by comparing the oxidation peak current of 2.0×10^{-6} mol/l hypoxanthine. The RSD is 4.4% for the same CTS/MWNT/GCE modified 10 times, revealing that the method for preparation CTS/MWNT/GCE has good potential applications. The long-term stability of the CTS/MWNT/GCE was tested by measuring the current response at a fixed hypoxanthine concentration of 2.0×10^{-6} mol/l over 10 days. The CTS/MWNT/GCE was used daily and stored in air. After 10 days, the current response only decreases 4.5%, suggesting that CTS/MWNT/GCE reported in this work has long-term stability.

Interference

The possible interferences of other biomolecules on the determination of hypoxanthine were studied under the above-optimized conditions. It was found that 100-fold concentration of ascorbic acid and uric acid, 50-fold of concentration xanthine, urea, glucose, lactic acid, serine, oxalic acid, guanine and adenine, and 20-fold concentration dopamine and cysteine almost have no influence on the determination of 2.0×10^{-6} mol/l hypoxanthine (signal change below 5%), which demonstrated the good selectivity of the CTS/MWNT/GCE developed for the detection of hypoxanthine.

Determination of samples

The performance of the CTS/MWNT/GCE for the analysis of real fish samples was tested. Fish tissue samples were macerated with 0.5 mol/l perchloric acid for about 1 h. After centrifugation at 8000 rpm for 10 min, the supernatant extract was transferred into 0.1 mol/l phosphate buffer solution of pH 7.0. Then before determination, the extracts were diluted to appropriate concentration. In order to testify the accuracy of this method, hypoxanthine standard solution was added into the sample, and the recovery was tested. TABLE 1 summarized the analytical results and the recovery of the sensor performance. The recovery is in the range

TABLE 1 : Determination of hypoxanthine in fish samples.

Samples NO.	Found (μmol/l)	Added (μmol/l)	Total (μmol/l)	Recovery (%)
1	20.0	20.0	40.5	102.5
2	24.5	20.0	45.3	103.2
3	18.7	20.0	39.1	102.1
4	16.5	20.0	36.2	98.2
5	25.3	20.0	44.9	98.4

from 98.2% to 103.2%, suggesting that this newly developed method has good accuracy.

CONCLUSIONS

In this work, a natural polymer, chitosan, has been used as a dispersant of MWNT. Chitosan-carboxylated MWNT film was very homogeneous and stable on the surface of glassy carbon electrode. The chitosan-carboxylated MWNT modified GCE is specific to hypoxanthine with other homogeneous species hardly interfering. Furthermore, its sensitivity, repeatability and stability are satisfactory. We firmly believe that further application of the chitosan-carboxylated MWNT modified GCE will be explored in the future.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Foundation of Wenzhou Science & Technology Bureau (Grant No. Y20100051) for financial support of this work.

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