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Sensitive detection of hydroxylamine at a simple baicalin carbon nanotubes modified electrode

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ABSTRACT

A baicalin multi-wall carbon nanotubes (BaMWCNT) modified glassy carbon electrode (GCE) for the sensitive determination of hydroxylamine was described. The BaMWCNT/GCE with dramatic stability was firstly fabricated with a simple adsorption method. And it showed excellent catalytic activity toward the electrooxidation of hydroxylamine. The amperometric response at the BaMWCNT/GCE modified electrode increased linearly to hydroxylamine concentrations in the range of 0.5 μM to 0.4 mM with a detection limit of 0.1 μM. The modified electrode was applied to detection hydroxylamine in the tap water, and the average recovery for the standards added was 96.0%.

1. Introduction

Hydroxylamine belongs to an important class of reducing agents, which are commonly used for synthesis of pharmaceutical intermediates and final drug substances. However, hydroxylamine is a well-known mutagen, moderately toxic and harmful to microorganisms that could interfere with biological sewage plant performance [1–3]. Because of the industrial and pharmacological significance of hydroxylamine, a sensitive, simple and inexpensive method is required for its reliable measurement. Some methods have been reported for the determination of low levels of hydroxylamine. These include various gas or liquid chromatography with different detectors [4–9], spectrophotometric determination after derivatization [10,11], flow injection analysis with biamperometry at platinum electrodes [12]. In the electrochemical methods, different modified electrode was applied to realize the sensitive determination of hydroxylamine at low overpotential [13–20].

It is confirmed that *o*-quinones can be quite active in the electrocatalytic oxidation of some molecules such as oxygen [21,22], dihydronicotineamide adenine dinucleotide [23,24], and also hydroxylamine [16]. Zare's group had done a lot on the modified electrodes with compounds having an *o*-quinone moiety for the electrocatalytic oxidation of hydroxylamine [25–27]. Baicalin, a flavonoid compound derived from the root of *Scutellaria baicalensis Georgi*, has been proved to have many biological activities such as the anti-inflammatory and antipyretic effects [28,29]. From previous studies, *o*-quinones would be produced when baicalin was oxidized by electrochemical method [30,31], which suggested the catalytic activity of baicalin toward the electrooxidation of hydroxylamine. As the most popular type nano-material, multi-wall carbon nanotubes (MWCNT) have led to wide research activities in the area of sensor applications for the tremendous catalytic activity for many molecules, the high surface area and the other advantages [32,33]. It is accepted that excellent electrode performance would be provided when quinone was integrated with MWCNT on one electrode surface [17,26,31,34].

In this work, we reported the preparation, electrochemical behavior and electrocatalytic activity toward the oxidation of hydroxylamine of the baicalin MWCNT (BaMWCNT) modified glassy carbon electrode (GCE). The amperometric performance of BaMWCNT/GCE gives several distinct advantages when compared with the other modified electrodes from available references, including short response time and good limit of detection for hydroxylamine. Thus, the modified electrode was used to detect hydroxylamine in spiked tap-water samples.

2. Experimental

Baicalin stock solution, (1.0 mM), was prepared by dissolving 0.0223 g of the flavonoid compound (Sigma, USA) in ethanol in a 50 ml volumetric flask. Hydroxylamine stock solution was prepared by dissolving 0.3475 g hydroxylamine chloride (Tianjin Fucheng Chemical reagent company, China) in water, and diluting to 50 ml with water. MWCNT (10–30 nm diameter, 0.5–40 μm length, 95%

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nominal purity) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China), and purified by stirring in a 2 M nitric acid solution for 20 h prior to use in all cases [34]. 5.0 mg pretreated MWCNT was well dispersed into 5 ml N,N-dimethylformamide with the help of ultrasonic agitation. The 0.1 M phosphate buffer solutions (PBS, pH 7.0), which were made up from Na_2HPO_4 and NaH_2PO_4, were always employed as supporting electrolyte except that the pH-dependent experiments were carried out in PBS with various pH values. All other reagents were of analytical grade and were used without further purification. All solutions were prepared with the ultrapure water obtained from a Milli-QG (Millipore Corp. U.S.A.) water-purification system.

Electrochemical experiments were performed with a CHI660A electrochemical system (Chenhua Instruments, Shanghai, China) in a conventional three-electrode cell. The working electrode was a modified or unmodified GCE (Model CHI104, 3 mm diameter). A platinum wire was used as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All potentials were measured and were reported versus SCE. A DL-180 ultrasonic apparatus (ShiPuTian Electronic Apparatus Company, Xiangshan, Zhejiang, China) was employed to prepare the MWCNT suspension. All the pH measurements were made with an Orion model SA 720 digital ionmeter.

The MWCNT/GCE was prepared similar to the previous work. In brief, the GCE was polished with 0.05 μm aluminum slurry on chamois leather, rinsed thoroughly with water, then sonicated in water for 3 min. The MWCNT film was prepared by casting 8 μL of the above MWCNT suspension on the GCE surface, and then dried under an infrared heat lamp.

3. Results and discussion

3.1. Preparation of BaMWCNT/GCE

As is well known, MWCNT are a type of nano-carbon with nano-sized tubular microstructure, large specific surface area, and highly activated surface, and these properties pledge the strong adsorption ability of MWCNT. Thus, adsorption method was applied to prepare the BaMWCNT/GCE. The BaMWCNT/GCE was prepared by immersing the MWCNT modified GCE into the above baicalin stock solution for a certain time, rinsed with water, and then placed in a pH 7.0 (0.1 M) PBS to be used.

With the aim of developing a stable sensing interface, the quantity of baicalin modified on the GCE was investigated. The time of adsorption of the MWCNT modified electrode in 1.0 mM baicalin stock solution affects the surface coverage of the catalyst adsorbed on the electrode surface. The effect of the time of adsorption was investigated from 3 to 100 s. In all cases, the surface coverage (Γ) was evaluated from the cyclic voltammograms recorded at 5 mV s^{-1} and using the equation $\Gamma = Q/nFA$, where Q is the charge obtained by integrating the anodic peak under the background correction, A the geometric area of the electrode, i.e. 0.070 cm^2, and the other symbols have their usual meaning. As shown in Table 1, the anodic current increased gradually and then slowly with the increase of the adsorption time, while Γ increases slowly with the time of adsorption up to 100 s, and the maximum Γ (Γ_m) is 2.65×10^{-9} mol cm^{-2}, this value is about 16-fold of the Γ_m (1.65×10^{-10} mol cm^{-2}) of baicalin at a bare GCE [30], and the surface coverage reaches 63% within 5 s, these findings suggested that baicalin adsorbed strongly at the MWCNT surface. As concerned the electrocatalytic oxidation current of BaMWCNT/GCE to hydroxylamine showed in Table 1, the catalytic current increased only about 10% from 5 s to 100 s of the adsorption time, though the anodic peak current of baicalin on BaMWCNT/GCE increased dramatically. This may be because not all baicalin adsorbed on the MWCNT were effective for the electrooxidation of hydroxylamine. Thus, the BaMWCNT/GCE was prepared by immersing the MWCNT/GCE in a 1.0 mM baicalin stock solution for 5 s.

Table 1
Effect of adsorption time of MWCNT/GCE in the baicalin stock solution.

Adsorption time (s)	Anodic peak current (μA)	Surface coverage (10^{-9} mol cm^{-2})	Catalytic current (μA)
3	2.837	1.378	13.15
5	3.564	1.669	13.67
10	3.945	1.768	13.92
20	4.604	1.886	13.98
35	5.170	2.151	14.12
50	5.477	2.412	14.42
80	6.073	2.635	15.14
100	6.125	2.652	15.20

The working stability of the BaMWCNT/GCE was verified by monitoring the remaining peak potential and current after successive sweeps of cyclic voltamograms. The peak height and peak potential of the surface immobilized film, by cycling the electrode potential over the range – 0.1–0.3 V, remained nearly unchanged. The peak current is almost 99.6% after 20 cycles in electrolyte solution with scan rate 5 mV s^{-1} (Fig. 1). On the other hand the storage stability of the chemically modified electrodes was very good as the electrodes were found to have reserved 95% of their initial activity for more than one month when kept in pH 7.0 PBS at room temperature. The high stability of the modified electrode is related to the chemical stability of the MWCNT films, the strong adsorption of baicalin on MWCNT film and the π–π interaction between the MWCNT and the hydroxyl groups in baicalin molecule [35].

3.2. Electrochemical behavior of the BaMWCNT/GCE

A single and well-defined redox couple was observed on the typical cyclic voltammograms of the BaMWCNT/GCE (Fig. 2). The peak potential of anodic and cathodic peak was 0.119 and 0.108 mV at the scan rate of 5 mV s^{-1}, respectively, with an 11 mV of the peak-to-peak separation. The peak current of the cathodic and anodic peak was almost equal. The formal potential was 114 mV versus SCE, and this electrochemical behavior is similar with our previous results of baicalin in solution at a bare GCE [30]. It is believed that the electrochemical oxidation mechanism of polyphenol such as baicalin was closely related with the phenolic hydroxyl group in the structure of the compound. The ortho-hydroxyl groups are often electrooxidized simultaneously at a low potential [36]. Consequently, this pair of peaks of BaMWCNT/GCE was ascribed to

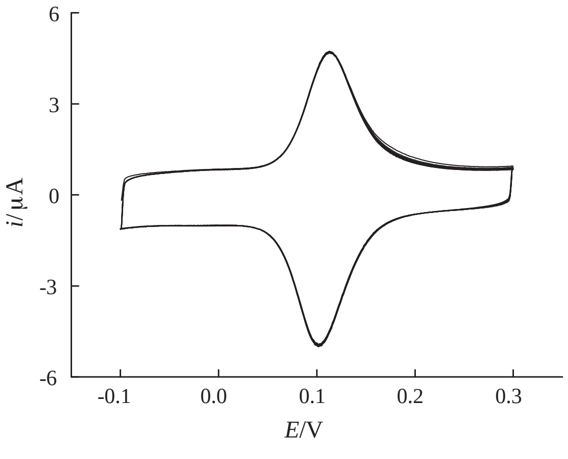

Fig. 1. 20 successive sweeps of cyclic voltammograms of a BaMWCNT/GCE in 0.1 M phosphate buffer (pH 7.0) at scan rate of 5 mV s^{-1}.

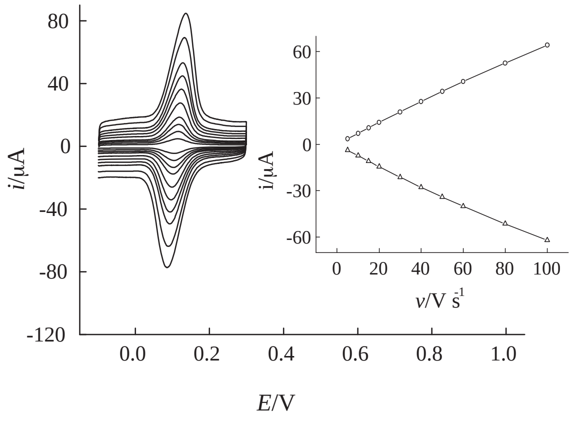

Fig. 2. Cyclic voltammograms of a BaMWCNT/GCE in 0.1 M phosphate buffer (pH 7.0) at scan rate of 5–100 mV s^{-1}. Insert is the variation of anodic and cathodic peak currents versus scan rates.

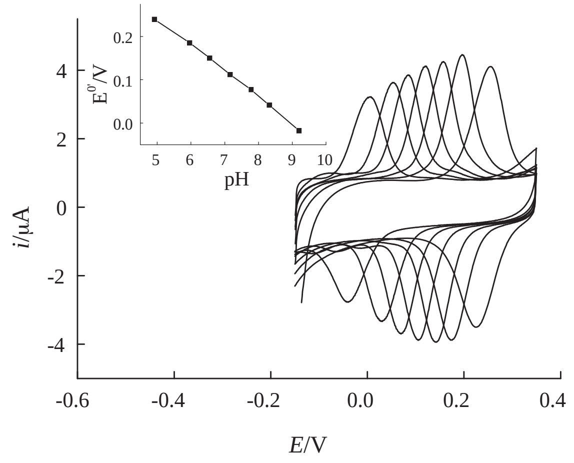

Fig. 3. Cyclic voltammograms of a BaMWCNT/GCE in 0.1 M phosphate buffer with different pH values, scan rate 5 mV s^{-1}. Insert is the relationship between the formal potential of baicalin and pH.

the redox of the 5,6-dihydroxyl substitute (catechol substitute) on the A-ring, which corresponds to an electrochemically reversible process involving two electrons and two protons [30,37].

The cyclic voltammograms of the modified electrode were recorded in pH 7.0 PBS at various potential scan rates. As it can be seen in Fig. 2, the small peak-to-peak separation was observed at the BaMWCNT/GCE. The ratio of cathodic to anodic peak currents at various sweep rates is almost unity. The insert of Fig. 2 shows that the anodic and cathodic peak currents are directly proportional to the scan rate up to 100 mV s^{-1}. All these show that the voltammetric peaks are typical of a surface confined redox couple. The peak-to-peak potential separation was almost invariant for scan rate below 100 mV s^{-1}, suggesting facile charge transfer kinetics over this range of sweep rate [38,39]. At higher sweep rates, the plot of peak current versus scan rate deviates from linearity and the peak currents become proportional to the square root of the scan rate (not shown), indicating a diffusion-controlled electrode process. Based on the Laviron's theory, it is possible to determine the transfer coefficient (α) by measuring the shift of the peak potentials with scan rate v for $n\Delta E_p > 200$ mV. The evaluated values for the kinetic parameters α_a and α_c (anodic and cathodic transfer coefficients) from the slope of E_p–log v curves (not shown) is 0.65 and 0.42, respectively.

To investigate the effect of pH on the electrochemical behavior of the modified electrode, cyclic voltammograms using the BaMWCNT/GCE in solutions of varying pH from 4.9 to 9.2 were recorded at 5 mV s^{-1} (Fig. 3). As expected, the formal potential of the surface redox couple, taken as the average of anodic and cathodic peak potentials, was pH-dependent, with a slope of 60.8 mV/pH unit in a wide range, which is very close to the anticipated Nernstian value of 59 mV for an equal electron and proton process [38]. In addition, the variation in peak current was modest, and the peak separation did not increase rapidly with the decrease or increase of the solution pH, i.e. only 49 mV in pH 9.2 solution, indicating that the modified electrode showed acceptable electrochemical redox reversibility in different medium besides the solution with neutral pH.

3.3. Electrocatalytic oxidation of the BaMWCNT/GCE to hydroxylamine

The representative cyclic voltammograms of the BaMWCNT/GCE in the absence and presence of 1.0 mM hydroxylamine were showed in Fig. 4. In the presence of hydroxylamine, there is a great increase in the anodic current at around the same potential where the quinone–hydroquinone redox electrochemical reaction occurred, whereas the cathodic current almost disappeared. As explained in the literature [24], this is definitely caused by the fact that the hydroxylamine in the bulk diffuses toward the electrode and reduces the oxidized form of baicalin (quinone) in the MWCNT. While the regeneration of the electroactive species, baicalin, occurred in a given reaction rate leads the peak current enhancement. The anodic peak potential for the electrocatalytic oxidation for hydroxylamine at this modified carbon electrode is about 100 mV, while at the bare GCE hydroxylamine showed no distinct peak until 1000 mV [17]. This dramatic decrease in overpotential and enhancement of peak current for hydroxylamine oxidation indicate a strong catalytic effect of modified baicalin and MWCNT. The anodic current on the cyclic voltammograms of the modified electrode in solutions containing different concentrations of hydroxylamine was measured and the catalytic current versus hydroxylamine concentration was linear in the concentration range of 0.05–2.0 mM. Thus, the BaMWCNT/GCE could be applied to the determination of high concentration hydroxylamine by cyclic voltammetry.

3.4. Amperometric response of hydroxylamine at BaMWCNT/GCE

Fig. 5 presents the amperometric response at BaMWCNT/GCE held at 0.20 V in stirred pH 7.0 PBS for successive addition of different amount of hydroxylamine. The modified electrode responded

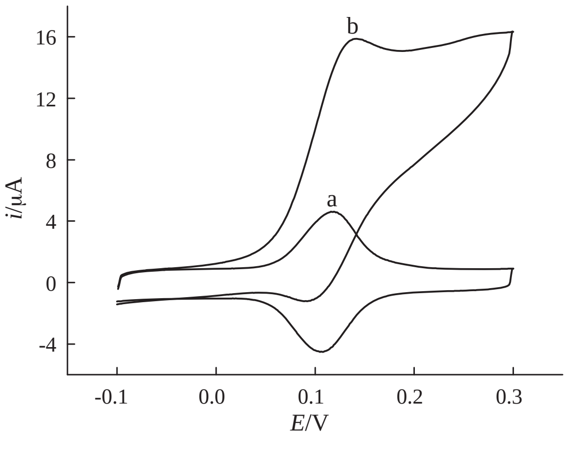

Fig. 4. Cyclic voltammograms of a BaMWCNT/GCE in 0.1 M phosphate buffer (pH 7.0) in the absence (a) and presence (b) of 1.0 mM hydroxylamine, scan rate 5 mV s^{-1}.

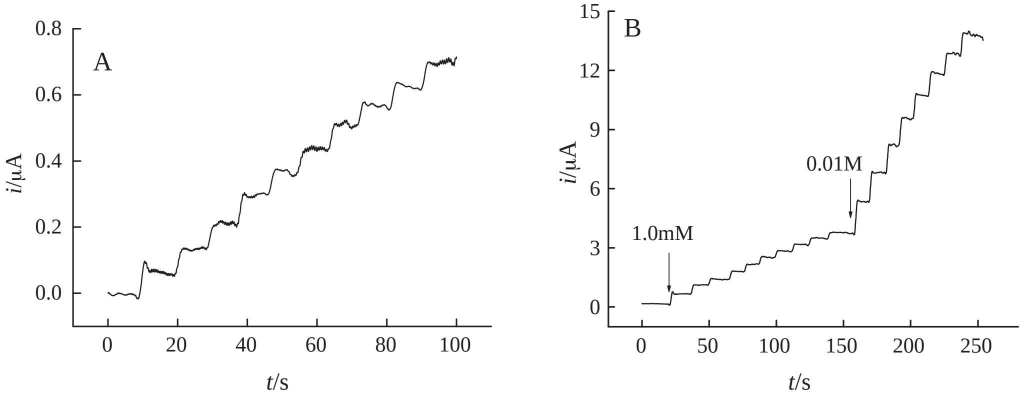

Fig. 5. Amperometric response at BaMWCNT/GCE held at 0.20 V for successive addition of (A) 50 μL of 0.1 mM hydroxylamine in stirred phosphate buffer solution and (B) 1.0 mM and 0.01 M hydroxylamine solution in to the final solution of A step.

Table 2
Comparison of the analytical parameters obtained at different modified electrode from available references with the proposed electrode in this work.

Electrode	Sensitivity ($\mu A\,mM^{-1}$)	Linear range[a] (μM)	Detection limit (μM)	Method	Reference
RuON-GCE	–	4.0–33.8	0.45	Amperometry	[13]
ZnO/MWCNT	–	0.4–19,000	0.12	Amperometry	[15]
CGA–MWCNT–GCE	–	11.8–74.1	1.4	Amperometry	[17]
RMWCNT/GCE	28.8	1.0–33.8	1.0	Amperometry	[26]
NiCoHCF/GCE	4.94	20–200	0.23	DPV	[40]
CMCPE	0.30	60–1000	10.75	Amperometry	[25]
Au/PPy/GCE	63.9	1–500	0.21	DPV	[20]
BaMWCNT/GCE	27.4	0.5–400	0.1	Amperometry	This work

[a] For the multi-linear ranges electrodes, only the first linear range was given.

linearly to hydroxylamine concentrations between 0.5 μM and 0.4 mM with a sensitivity of 27.4 $\mu A\,mM^{-1}$. The relative standard deviation for six successive measurements of 0.05 mM hydroxylamine was 4.8%. The limit of detection, estimated from the value of three times the standard deviation of the background noise, was 0.1 μM. The current reached its steady-state current within 3 s, which indicated a fast electron transfer process between GCE and hydroxylamine. Table 2 compared the analytical parameters obtained at different modified electrode from available references with the proposed electrode in this work. It can be seen that the BaMWCNT/GCE indicated superior sensitivity and detection limit, compared not only with the metal hexacyanoferrate, but also the other quinone-monity compound such as coumestan and chlorogenic acid modified electrodes, and showed comparable detection limit with the ZnO/MWCNT modified electrodes.

3.5. Selectivity of BaMWCNT/GCE

An important problem in determining hydroxylamine is when it presents with other electroactive compounds in a variety of industrial processes. Therefore, the effect of various species on the determination of 0.05 mM hydroxylamine was tested at the BaMWCNT/GCE by amperometry. The tolerance limit is defined as the molar ratio of the additive/hydroxylamine causing less than 5% relative error. The following ions did not interfere even when their concentration was a 1000-fold excess over hydroxylamine: Mg^{2+}, Ca^{2+}, NO_3^-, SO_4^{2-}, CO_3^{2-}, CH_3COO^-, SCN^-, F^-, Cl^- and Br^-, and the other ions such as NO_2^- and $S_2O_3^{2-}$ interfered the amperometric response when their concentration was a 100-fold excess over hydroxylamine. In addition, similar amount of I^- and hydrazine show distinct interference to the determination of hydroxylamine.

3.6. Preliminary application of the BaMWCNT/GCE

The method proposed was applied to the determination of hydroxylamine in tap water. The tap water samples were taken from the local laboratory and spiked with contain amount of hydroxylamine, then the samples were analyzed by amperometry (Fig. 6a and c). To be compared, amperometric responses at BaMWCNT/GCE in stirred PBS (pH 7.0) for addition of equivalent of standard hydroxylamine solution were also recorded at intervals (Fig. 6b and d). The average recovery for the hydroxylamine in the tap water samples and in the ultrapure water is 96.0% and 98.9%, respectively. The tap water spiked with equivalent amount of hydroxylamine was also determined by the spectrophotometry [10]. There is no significant difference between the results obtained by amperometry and spectrophotometry, proving the applicability of established method based on the BaMWCNT/GCE (Fig. 6).

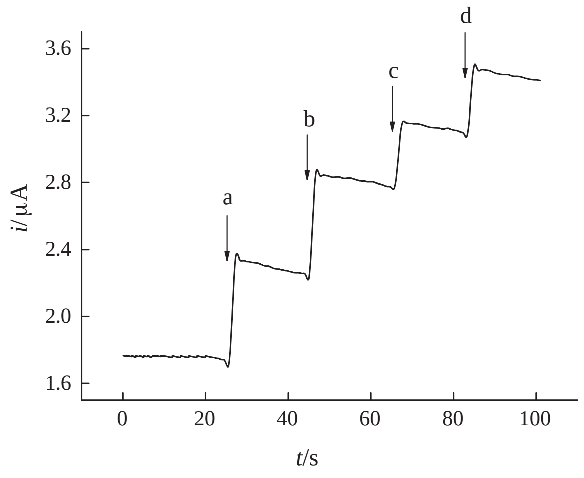

Fig. 6. Amperometric response at BaMWCNT/GCE in stirred phosphate buffer solution (pH 7.0) for addition of 50 μL (a and b) and 30 μL (c and d) 1.0 mM hydroxylamine standard (a and c) and tap water (b and d) solution.

4. Conclusions

A BaMWCNT/GCE was successfully fabricated by adsorption of baicalin on the MWCNT modified GCE. The modified electrode exhibits excellent electrocatalytic behavior toward hydroxylamine oxidation in aqueous PBS. It has been shown that cyclic voltammetry and amperometry can be used as analytical methods for hydroxylamine determination in quiet and moving solution, respectively, with broad linear range, high sensitivity, low detection limit and good stability.

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