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Selective Determination of Epinephrine in the Presence of Ascorbic Acid on a Carbon Nanotubes Modified Electrode Based on the Enhancement Effects of the Hexadecyl Trimethyl Ammonium Bromide

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A modified glassy carbon electrode (GCE) was fabricated by covered with a layer of multi-wall carbon nanotubes (MWCNTs) coated with hexadecyl trimethyl ammonium bromide (CTAB). The modified electrode showed excellent electrochemical catalytic properties for the redox of epinephrine (EP) and ascorbic acid (AA). In the presence of CTAB, the peak separation between EP and AA can be broadened to 256 mV by the CTAB. Based on this, a sensitive method for determination of EP and AA was set up. The detection limits were 3.0×10^{-8} mol/L and 1.0×10^{-4} mol/L (S/N=3) for EP and AA, respectively. The proposed method was used to detection of EP and AA in injections with simplicity, high sensitivity and selectivity.

Keywords: Selective determination; Epinephrine; Ascorbic acid; Hexadecyl trimethyl ammonium bromide; Multiwall carbon nanotube

1. INTRODUCTION

Surfactants are a kind of amphiphilic molecule with a hydrophilic polar head on one side and a long hydrophobic tail on the other. It can be adsorbed on the hydrophobic surface of electrode altering the properties of electrode/solution interface and in turn heavily influencing the electrochemical processes of electroactive species [1-4], and the amphiphilic surfactant molecules self-assembled on solid surfaces can be used as models for biological membranes [5] to simulate various

biological/industrial processes [6-8]. Hexadecyl trimethyl ammonium bromide (CTAB) is an important cationic surfactant and was mainly used in DNA extraction.

Epinephrine (EP), as an important catecholamine neurotransmitter, exists in nervous tissues and body liquid. Its concentration level change induced by metabolism handicap would arouse many diseases, such as Parkinson's disease, schizophrenia and HIV infection [9-11]. Therefore, to develop simple and fast method for determination of EP has been a focal subject in bioscience, biotechnology and medicinal chemistry, especially in neurochemistry. Up to now, many methods, such as liquid chromatography [12], capillary electrophoreses [13], chemiluminescence method [14], spectroscopy [15], fluorescence method [16] and electrochemical method [17-19] have been set up. Among of these methods, electrochemical methods appear to be the most suitable method for quantitative determination of EP because EP is easily oxidized. Additionally, the electrochemical reactions that occur at the electrode/solution interface are similar with the real reactions that take place in vivo [20]. Unfortunately, the final oxidation products (epinephrinechrome) of EP would block the electrode surface and yield poor electrochemical response when it is oxidized directly on a traditional electrode. Moreover, in brain issue, EP generally coexists with high concentration of ascorbic acid (AA) (100–1000 times the concentration of EP) which generally results in overlapped voltammetric response due to their very similar oxidation peak potentials. Therefore, to circumvent above two problems and to set up electrochemical method for detection of EP with satisfactory selectivity and low detection limit are still many research groups' endeavor goals. Recently, chemically modified electrodes have been proved to be the favorable tools for solving above problems and many materials, such as poly amino acids, carbon nanomaterials and so on, have been used in detecting EP [21-26].

Carbon nanotubes (CNTs) can promote the electron transfer between electroactive species and electrodes and have excellent electrocatalytic properties for various molecules and biomolecules [27-29]. Therefore, they have been extensively used in many research regions since they were discovered in 1991. However, the insolubility of CNTs is a major drawback limiting their use in electrochemical sensors and biosensors because they usually exist as parallel aggregated bundles in aqueous solution. Surfactants have been examined to be an effective approach for achieving the solubility without impairing their physical properties.

In this research, EP and AA were selectively determined based on the multi-wall carbon nanotubes coated with hexadecyl trimethyl ammonium bromide. At this modified electrode, both EP and AA had sensitive oxidation peaks and the peak separation between EP and AA was 256 mV, which can be used to detect the content of EP and AA and no interference between them. The proposed method was simplicity, high selectivity and sensitivity.

2. EXPERIMENTAL

2.1. Chemicals and Apparatus

Epinephrine (EP) was purchased from Shanghai Jingchun Reagent Co., Ltd (Shanghai, China). Ascorbic acid (AA) was purchased from Tianjin Kemiou Chemical reagent Co., Ltd. (Tianjin, China).

Multi-wall carbon nanotubes (MWCNTs) were purchased from Chengdu Organic Chemicals Co., LTD. Chinese Academy of Sciences (Chengdu, China); Hexadecyl trimethyl ammonium bromide (CTAB) was purchased from Tianjin Guangfu Fine Chemicals Research Institute) and used to disperse MWCNTs; The Mcllvaine buffer solution was prepared with 0.2 mol/L Na_2HPO_4-0.1 mol/L citric acid. All chemicals were of analytical reagent grade and were used as received. Double distilled water was used throughout.

Electrochemical experiments were performed with an electrochemical work station-CHI 660 C (Shanghai CH Instruments Co., China) and a conventional three-electrode system was used throughout. The working electrode was a bare or a modified GCE (3.0 mm in diameter), the auxiliary electrode was a Pt electrode and a saturated calomel reference electrode. PHS-3B (Shanghai Precision Scientific Instrument Co., Ltd., China), KQ-100 ultrasonic cleaner (Kunshan Ultrasonic Instrument Factory, China).

2.2. Procedures

Prior to modification, the GCE was pretreated to mirror-like as the procedures described in the literature 26. Then 5 mg MWCNTs were dispersed with 5mL CTAB solution of 5 mg/mL in ultrasonic bath for 30 min. 8 μL the dispersed MWCNTs was cast on the surface of the pretreated GCE and dried under the infrared light. The modified electrode was named as CTAB/MWCNTs/GCE. For comparison, the MWCNTs/GCE was prepared as above protocol except for the MWCNTs were dispersed with ultrapure water. Using the CTAB/MWCNTs/GCE as working electrode, the cyclic voltammograms were recorded in solution of EP or AA in the potential range of 0~0.6 V with the scan rate of 100 mV/s. The solution of EP or AA was maintained in nitrogen atmosphere throughout the detection.

3. RESULTS AND DISCUSSION

3.1. The electrochemical behaviors of EP

The cyclic voltammograms of EP in pH 7.0 Mcllvaine buffer were recorded on the bare GCE, multiwall carbon nanotubes modified GCE (MWCNTs/GCE) and CTAB/MCNTs/GCE in the potential range of 0.0~0.6 V with the scan rate of 100 mV/s. EP had no obvious redox peak currents on the bare GCE (curve a). When the GCE was coated with a layer of MWCNTs, an oxidation peak current of EP was found at about 210 mV and no reductive peak currents (curve b). On the CTAB/MWCNTs/GCE (curve c), the oxidative peak currents of EP increased significantly and the peak potential had almost no change compared with that on the MWCNTs/GCE, indicating that the MWCNTs had good catalytic properties for the oxidation of EP and the CTAB changed the interfacial properties of the MWCNTs.

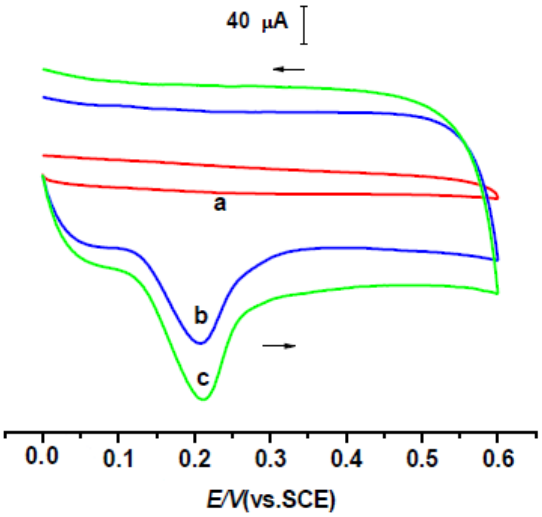


Figure 1. Cyclic voltammograms of 2.0×10^{-5} mol/L EP at bare GCE (curve a), MWCNTs/GCE (curve b) and CTAB/MWCNTs/GCE (curve c) in pH 7.0 Mcllvaine buffer with the scan rate of 100 mV/s.

3.2. The influence of pH values

In pH range of 3.0~8.0, the influence of pH values on the cyclic voltammetric behaviors of EP w investigated (Figure 2 A). The results showed that on the CTAB/MWCNTs/GCE, EP had a couple of redox peak currents at the pH 3.0. With the increase of the pH values, the redox peak currents of EP increased and simultaneously the peak potentials shift negatively. The biggest oxidation peak current was obtained at pH 7.0, and then declined as the pH value exceeded 7.0. The biggest reduction peak current was obtained at pH 4.0 and it vanished when the pH value was bigger than 5.0. So the pH 7.0 buffer solution was used.

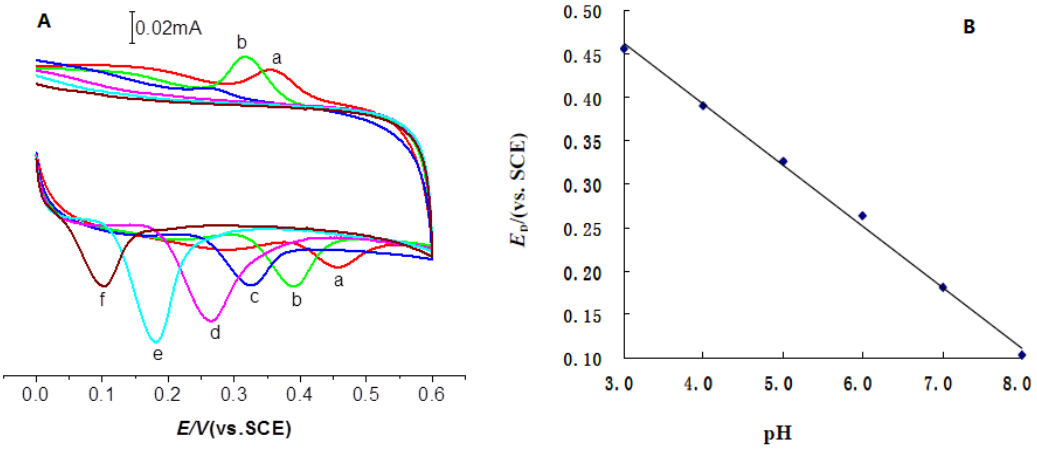
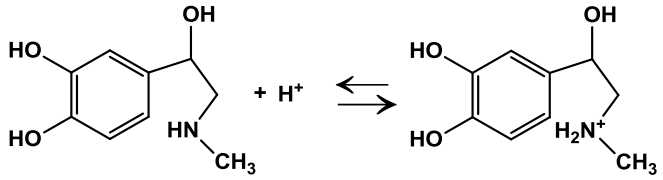


Figure 2. A: Cyclic voltammograms of 2.0×10^{-5} mol/L Ep in different pH of Mcllvaine buffer solution with the scan rate of 100 mV/s. The curves of a~f correspond to the pH values of 3.0，4.0，5.0，6.0，7.0，8.0. B: Effects of the solution pH on the oxidative peak potentials of 2.0×10^{-5} mol/L EP

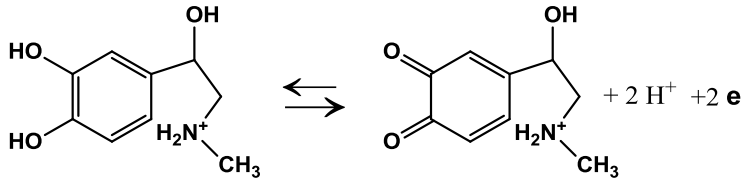
The further studies showed that the oxidation peak potentials (E_{pa}) were linear with the pH values and the regressive equation was E_{pa} (V) = -0.0701 pH + 0.6723 with r = 0.9983 (Figure 2 B), suggesting that proton take part in the electrochemical reactions of EP. The slop of 70.1 mV/pH indicate that the protons equal the electrons transferred in the electrochemical reactions. Therefore, the electrochemical reaction mechanism of EP on the modified electrode was deduced as follows:

Equation 1



epinephrine

Equation 2



epinephrinechrome

3.3. The influence of scan rates

In pH 7.0 Mcllvaine buffer, the influence of scan rates on the oxidation peak current of 2.0×10^{-5} mol/L EP was studied by cyclic voltammetry (Figure 3). It was found that the peak current was linearly increase with square root of the scan rates in the range of 20~140 mV/s, and the regressive equation was i_{pa} (1×10^{-5} A) = 2.0478 $v^{1/2}$ ((mV/s) $^{1/2}$) – 2.859 with r = 0.9994, indicating that the electrochemical reaction of EP was controlled by diffusion.

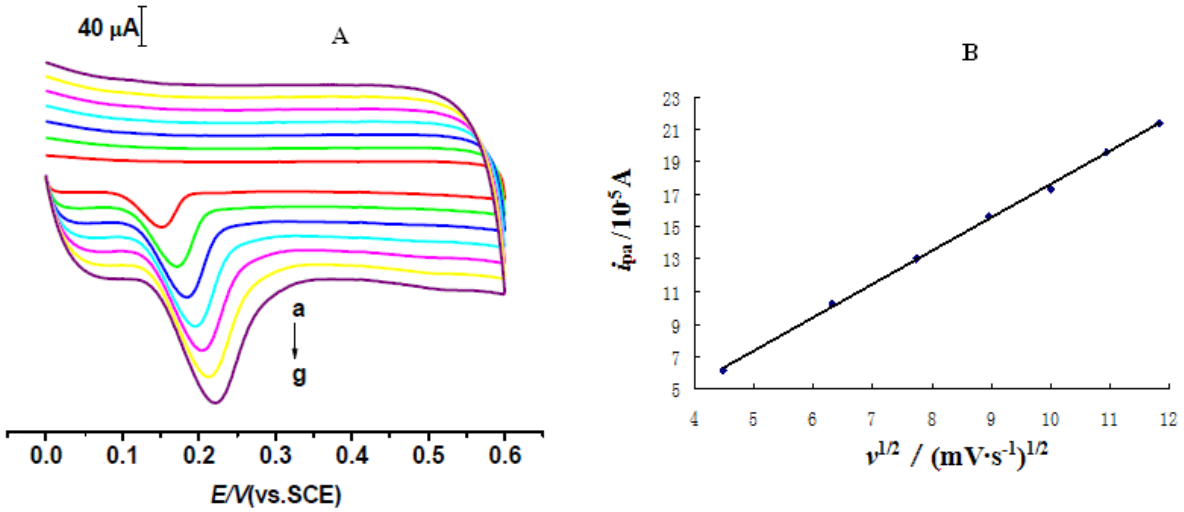


Figure 3. A: Cyclic voltammograms of 2.0×10^{-5} mol/L EP on the CTAB/MCNTs/GCE in pH 7.0 of Mcllvaine at different scan rates. Curve a to g corresponds to the scan rates : 20 mV/s; 40 mV/s; 60 mV/s; 80 mV/s; 100 mV/s; 120 mV/s; 140 mV/s, respectively. B: Relationship between oxidation peak currents and the square root of scan rate.

3.4. Interference of coexisted substances

In biological fluid, the concentration of ascorbic acid (AA) was 100–1000 times of EP. In this study, the interference of 2.0×10^{-3} mol/L AA for determination of 2.0×10^{-5} mol/L EP was investigated in different potential regions (Figure 4). In the potential range of 0 ~ 0.60 V, EP had an oxidation peak and AA had no redox peaks. When the potential region was expanded to -0.10 ~ 0.60 V, an oxidation peak of AA was obtained at -0.013 V. The peak-to-peak separation between EP and AA was 256 mV, so AA do not interfere with the determination of EP, which was probably ascribed to the electrostatic attraction between CTAB and AA, and the electrostatic repulsion between CTAB and EP (Figure 5).

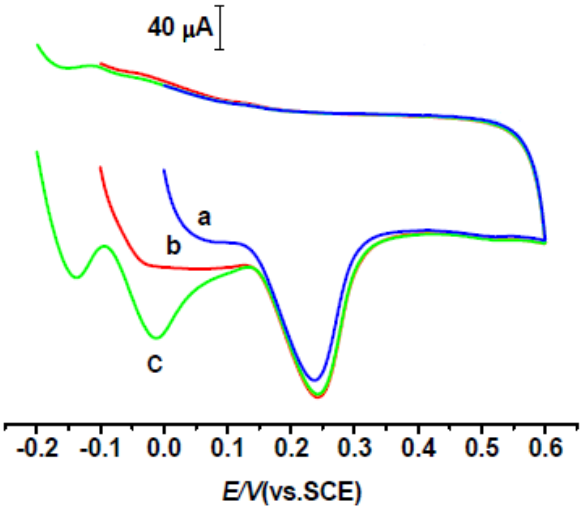


Figure 4. Cyclic voltammograms of 2.0×10^{-5} mol/L EP in the presence of 2.0×10^{-3} mol/L AA in different potential region with the scan rate of 100 mV/s.

The influence of some metal ions and some anions that usually exist in biological fluid on the determination of 5.0×10^{-5} mol/L EP was also studied. If the ±5% error is allowed, 5.0×10^{-3} mol/L K^+, Na^+, Fe^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} had no obvious interference.

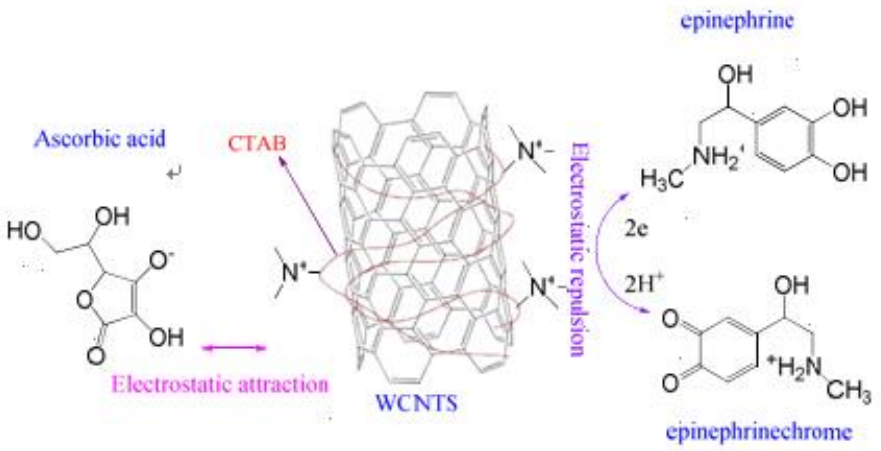


Figure 5. The electrochemistry of EP and AA on the CTAB/MCNTs/GCE.

3.5. The work curve of EP in the presence of AA

In the presence of 2.0×10^{-3} mol/L AA, the oxidative peak current of EP was linear with its concentration in two concentration regions. The linear regressive equations were i_{pa} (mA) = 0.363 C_{EP} (mol/L) + 13.452 in the range of 1.0×10^{-7} ~ 2.0×10^{-5} mol/L with r = 0.9984 and i_{pa} (mA) = 0.925 C_{EP} (mol/L) – 0.540 in the range of 1.0×10^{-3} ~ 9.0×10^{-3} mol/L with r = 0.9892, respectively (Figure 6). The detection limit was 3.0×10^{-8} (S/N=3). The linear range is much wider than the results of literatures 21-36 and the detection limit is lower than the results of literatures 31, 32, 36.

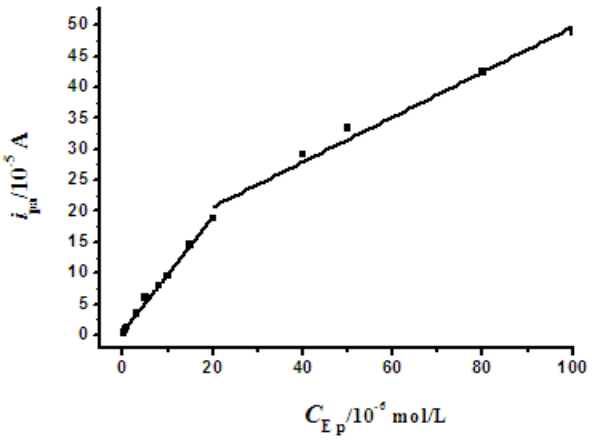


Figure 6. The work curves of EP in the presence of 2.0×10^{-3} mol/L AA

If the potential region was expanded to -0.10 ~ 0.60 V, the modified electrode can also be used to determine the concentration of AA in the presence of EP. In the presence of 2.0×10^{-5} mol/L EP, the oxidative peak current of AA was linear with its concentration in two concentration regions, too. The linear regressive equations were i_{pa} (mA) = 0.475 C_{AA} (mol/L) + 8.719 in the range of 3.0×10^{-4} ~ 1.0×10^{-3} mol/L with r = 0.9991 and i_{pa} (mA) = 1.575 C_{AA} (mol/L) – 2.533 in the range of 1.0×10^{-3} ~ 9.0×10^{-3} mol/L with r = 0.9944, respectively (Figure S1, Supporting Information).

3.6. Analytical application

Table 1. The determination results of EP and AA samples (n = 5)

	Samples	Label claimed mg/mL	Found mg/mL	RSD (%)	addition (mg)	Recovery (%)
EP	1#	1.0	0.98	1.6	0.001	99.7
	2#	1.0	1.01	2.0	0.001	101.1
AA	1#	1.0	0.99	2.8	0.001	99.9
	2#	1.0	0.97	1.1	0.001	99.6

The practical application of the proposed method was tested by measuring the concentration of EP and AA in injections which are produced in two different batches. The standard addition technique was used for recovery tests. The results were listed in Table 1.

4. CONCLUSIONS

On the CTAB/MWCNTs/GCE, the electrochemical behaviors of EP were studied and the results indicated that CTAB could expand the difference of the oxidation potential between EP and AA and further eliminated the interference of AA. The modified electrode can be used respectively to determine both EP and AA when they coexist. The application of the proposed method was verified by determination of EP and AA in real samples with simplicity, high sensitivity and selectivity.

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SUPPORTING INFORMATION
(The linear regressive curves of AA in the presence of EP).

Electronic Supporting Information
In the presence of 2.0×10^{-5} mol/L EP, the oxidative peak currents of AA were linearly with its concentration in two concentration regions. The linear regressive equations were i_{pa} (mA) = 0.475 C_{AA} (mol/L) + 8.719 in the range of 3.0×10^{-4} ~ 1.0×10^{-3} mol/L with r = 0.9991 and i_{pa} (mA) = 1.575 C_{AA} (mol/L) – 2.533 in the range of 1.0×10^{-3} ~ 9.0×10^{-3} mol/L with r = 0.9944, respectively.

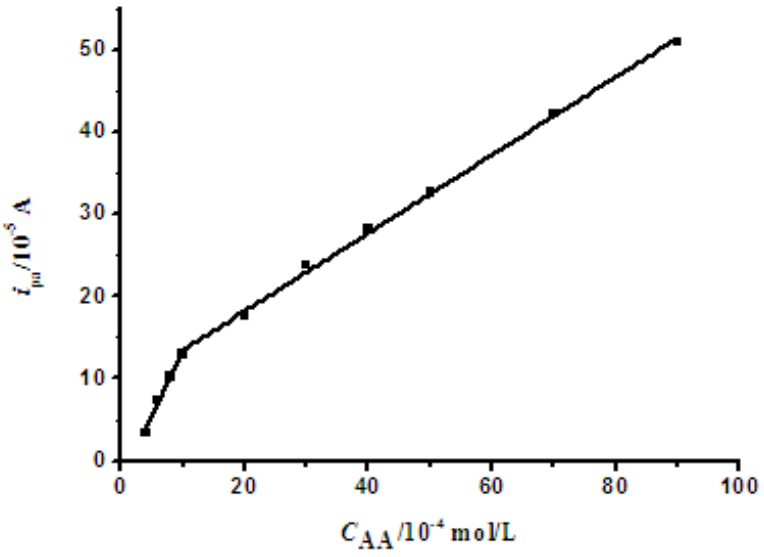

Figure S1. The linear regressive curves of AA in the presence of 2.0×10^{-5} mol/L EP

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