Int. J. Electrochem. Sci., 7 **(2012)** 991 - 1000

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Detection of Norepinephrine in the Presence of Epinephrine, Uric Acid and Ascorbic Acid Using a Graphene-modified Electrode

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Received: 28 November 2011 / *Accepted:* 4 January 2012 / *Published:* 1 February 2012

A glassy carbon electrode was modified with graphene. In phosphate buffer solution, the modified electrode showed an excellent electrocatalytical effect on the oxidation of norepinephrine (NE). This was further used to determine NE in the presence of epinephrine, uric acid and ascorbic acid by cyclic voltammetry. The reduction peak current showed a linear relationship with the concentrations of NE in the range of 6.00×10^{-7} to 1.20×10^{-4} mol·L^{-1}, with the detection limit of 4.00×10^{-7} mol·L^{-1}. The modified electrode can be used to selectively determine NE in the presence of epinephrine, uric acid and ascorbic acid by measuring reduction peak current in PBS pH 7.0. The method has high sensitivity, selectivity, stability, and has been successfully applied to analyzing injection samples of NE.

Keywords: Modified Electrode, Graphene, Norepinephrine, Reduction peak current

1. INTRODUCTION

Norepinephrine (NE), one of the important catecholamine neurotransmitters in the mammalian central nervous system, has many functions including regulation of the cardiovascular system, easing pain, sensing stress, depression and appetite. Alterations in plasma levels of NE caused by its metabolic dysfunction can lead to many pathological conditions. Various methods for determination of NE have been described, including high-performance liquid chromatography [1], gas chromatography [2], ion chromatography [3] and spectrophotometry [4, 5]. Comparing to the above methods, which usually require expensive equipments and reagents, our proposed electrochemical method is simple, rapid, low cost, and sensitive. In recent years, modified electrodes have been used for electrochemical analysis of NE [6-9], however, those methods have serious limitations due to the overlap of the

oxidation peaks from some concomitants, such as ascorbic acid (AA), epinephrine (EP) and uric acid (UA), with that of NE. Thus, developing a novel method to selectively determine NE in the presence of AA, EP and UA has attracted tremendous attention.

Graphene is one-atom-thick planar sheets of sp^2-bonded carbon atoms that are densely packed in a honeycomb crystal lattice [10]. Considering the electronic characteristics of graphene such as its large surface area, excellent conductivity, and strong mechanical strength, it has been used to produce a new generation of electrodes for electrochemical studies [11-15]. In this paper, an electrochemical sensor was fabricated with graphene-modified glassy carbon electrodes (GME), and its electrochemical properties were investigated. It can be used to selectively determine NE in the presence of EP, UA and AA by measuring reduction peak current during a cyclic voltammetry (CV) experiment. The modified electrode shows high sensitivity, selectivity, and stability. It has been successfully applied to analyzing injection samples of NE for the first time.

2. EXPERIMENTAL

2.1 Reagents and Materials

Graphite powder (<20 μm) was obtained from Qindao Graphite Corporation (Qingdao, China), sodium borohydride was from Tianjin Daofu Chemical New Technique Development Co., LTD (China), graphene was made by ourselves, norepinephrine and epinephrine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ascorbic acid and uric acid were from Beijing Chemical Factory (China). All other chemical reagents (analytical-reagent grade) were obtained from Beijing Chemical Reagent Company (Beijing China). Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.2 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} citric acid. All aqueous solutions were prepared in double distilled water.

2.2 Apparatus

CHI 660C Electrochemical Workstation (CH Instruments, Shanghai Chenhua Instrument Corporation, China), was used for electrochemical measurements. Varian660-IR (Agilent Technologies, America), was used for Characterization of graphene. A conventional three-electrode system was employed with a bare or graphene-modified glassy carbon electrode (3 mm in diameter) as the working electrode, a platinum wire electrode as the counter electrode, and an Ag / AgCl electrode as reference electrode. All potentials reported in this paper were referenced to the Ag / AgCl electrode. PHS-3B was applied by Shanghai Precision Scientific Instrument Co., Ltd (China), and KQ-100 ultrasonic cleaner was from Kunshan Ultrasonic Instrument Factory (China).

2.3 Preparation of the Nano-graphene

Graphene oxide (GO) was made by a modified Hummers method [16] using expandable graphite flake as starting material. Briefly, graphite flake (4 g) was stirred in 98% H_2SO_4 (92 mL) for 8

h. $KMnO_4$ (12 g) was gradually added while keeping the temperature under 36 ºC for 0.5 h. The mixture was stirred at 80 ºC for 45 min, and then 184 mL of water was added and heated at 95-105 ºC for 30 min. The reaction was terminated by adding distilled water (184 mL) and 30% H_2O_2 solution (40 mL). The mixture was washed by repeated centrifugation and filtration, first with 5% HCl aqueous solution, and then distilled water until no SO_4^{2-} was detected by $BaCl_2$. The final product was dried at 50 ºC for 24 h and stored for further use.

A suspension [17-19] was obtained in a jacketed vessel by dispersing the treated GO (0.5 g) into 500 mL of distilled water (final concentration: 1 $mg \cdot mL^{-1}$) with the aid of intensive sonication (100 W, 40 KHz, 1 h). To prepare an aqueous suspension of GO at pH 10, sodium carbonate was added to the jacketed vessel. The temperature was strictly controlled by a constant temperature circulator at 80 ºC while sodium borohydride (5 g) was added, and then the mixture was stirred at 80 ºC for 1.5 h. The powder of graphene was obtained by filtration and drying in vacuum, and characterized by IR and SEM.

2.4 Preparation of Graphene Modified Electrode

Graphene (7mg) was dispersed in doubly distilled water (10 mL) at a concentration of 0.7 $mg \cdot mL^{-1}$. Glassy carbon electrode (GCE, ∅ = 3 mm) was polished before each experiment with gold sand paper and 0.05 μm alumina powder, respectively. After rinsed thoroughly with doubly distilled water between each polishing step, the electrode was subjected successively with 50% nitric acid, ethanol and doubly distilled water in ultrasonic bath, and dried in air. Graphene modified electrode (GME) was prepared by casting 4 μL of graphene suspension on the GCE and dried under an infrared lamp.

2.5 Determination of NE

Under optimal conditions, a series of different concentrations of NE in PBS (pH 7.0) with or without concomitants (AA, EP and UA) were investigated by CV with a three-electrode system including a GCE modified with graphene as working electrode, a platinum electrode as counter electrode, and Ag / AgCl (1 $mol \cdot L^{-1}$ KCl) as reference electrode. Cyclic voltammograms of NE were recorded at a scan rate of 120 $mV \cdot s^{-1}$ between 0.0 V and 0.6 V. After each experiment, the modified electrode was restored by repeatedly running the scan in PBS until no peak being detected.

3. RESULTS AND DISCUSSION

3.1 Characterization of Graphene by IR

Fig. 1 shows the image of IR of graphite and graphene. Functional groups and wave numbers of graphene are listed in table 1.These results show that the graphene was synthesized successfully. Graphene sheets contain abundant C–O–C (epoxide) and C–OH groups, while the sheets are

terminated with C–OH groups. The functionalized and defective graphene sheets are more hydrophilic and can be easily dispersed in solvents with long-term stability. Fig. 2 shows the SEM image of graphene film on the surface of the GCE, revealing the typical crumpled and wrinkled graphene sheet structure on the rough surface of the film.

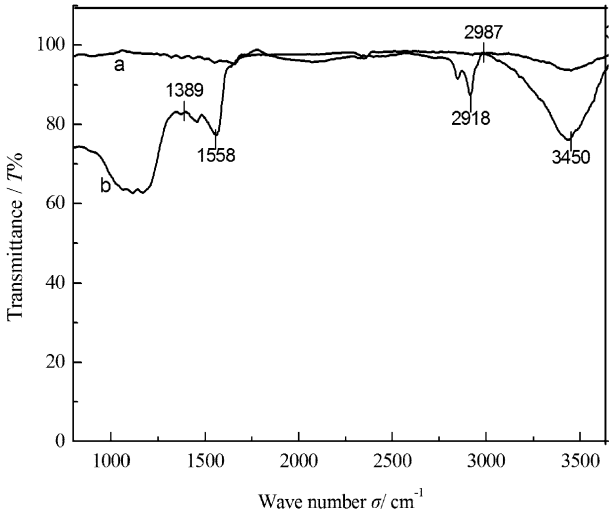

Figure 1. IR of the graphite (a) and graphene (b)

Table 1. Functional groups and wave numbers of graphene

Functional group	Wave number σ /cm^{-1}
-OH	3450
C=C	1558
phenyl	2800-3000
C-O-C	1110-1200

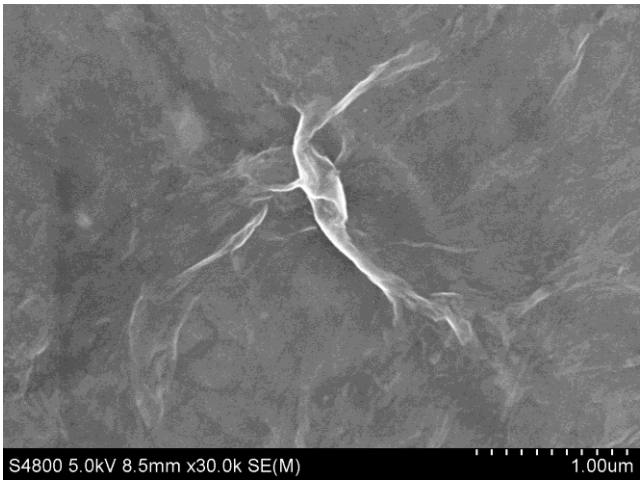

Figure 2. SEM image of graphene-film modified GCE

3.2 Optimization of GME

The amount of absorbed graphene on GCE strongly affects the intensity of the redox peak current. To find the optimal amount, GME was prepared in different concentrations of graphene suspension and used for the analysis of NE, as described under *Experiment Section*. The peak current clearly increased with the increase of graphene concentration from 0.3 $mg \cdot mL^{-1}$ to 0.5 $mg \cdot mL^{-1}$, and then the peak current increased slightly until it reached the maximum value at 0.7 $mg \cdot mL^{-1}$ graphene. The peak current decreased when the amount of graphene exceeded 0.7 $mg \cdot mL^{-1}$, which may be ascribed to the fact that the excessive deposition of graphene on the surface of GME causes the decrease of electrical conductivity and inhibits the electrical catalyst on the electrode surface. Meanwhile, if the graphene film is too thick it will block substrates spreading to the electrode surface. Therefore, we use 4 μL of 0.7 $mg \cdot mL^{-1}$ graphene to prepare the GME as a working electrode in our experiments.

3.3 Cyclic Voltammograms (CVs) of NE at Bare and Graphene Modified Electrode

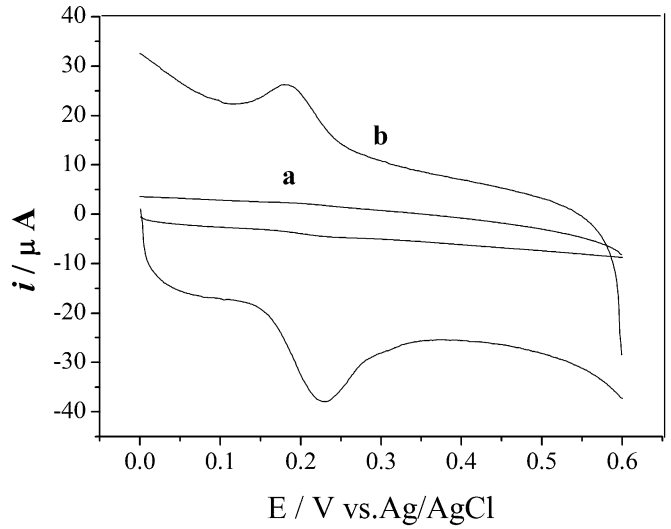

Figure 3. Cyclic voltammograms of the bare electrode (a) and the graphene modified electrode (b) in PBS (pH 7.0) and NE (1.0×10^{-5} $mol \cdot L^{-1}$). Scan rate is 100 $mV \cdot s^{-1}$.

From Figure 3, we can see that the intensity of redox peak current at GME (Fig. 2b) was sharply increased comparing to almost no response at GCE (Fig. 2a), suggesting that the graphene-thin-film deposited on electrode has a high electrocatalytic activity to NE. Such electrocatalytic behavior of graphene is attributed to its unique physical and chemical properties. In general, graphene has a large surface area, excellent conductivity, and strong mechanical strength. Furthermore, the functionalized and defective graphene sheets prepared by the redox process in this study contain abundant hydrophilic groups such as C–O–C (epoxide) and C–OH groups in oxidized rings and C–OH and –COOH at the terminals. All the unique features of functionalized and defective graphene may

effectively enhance the access and electron transfer between NE and surface of GME. The redox peak with E_{pa} = 0.232 V, E_{pc} = 0.174 V, i_{pa} = -18.82 μA, i_{pc} = 12.45 μA, i_{pa} / i_{pc} > 1 suggests that the reaction of NE at GME is a half-irreversible process. This unique and excellent performance shows that GME is a simple, sensitive, and quantitative electrochemical sensor for detecting NE.

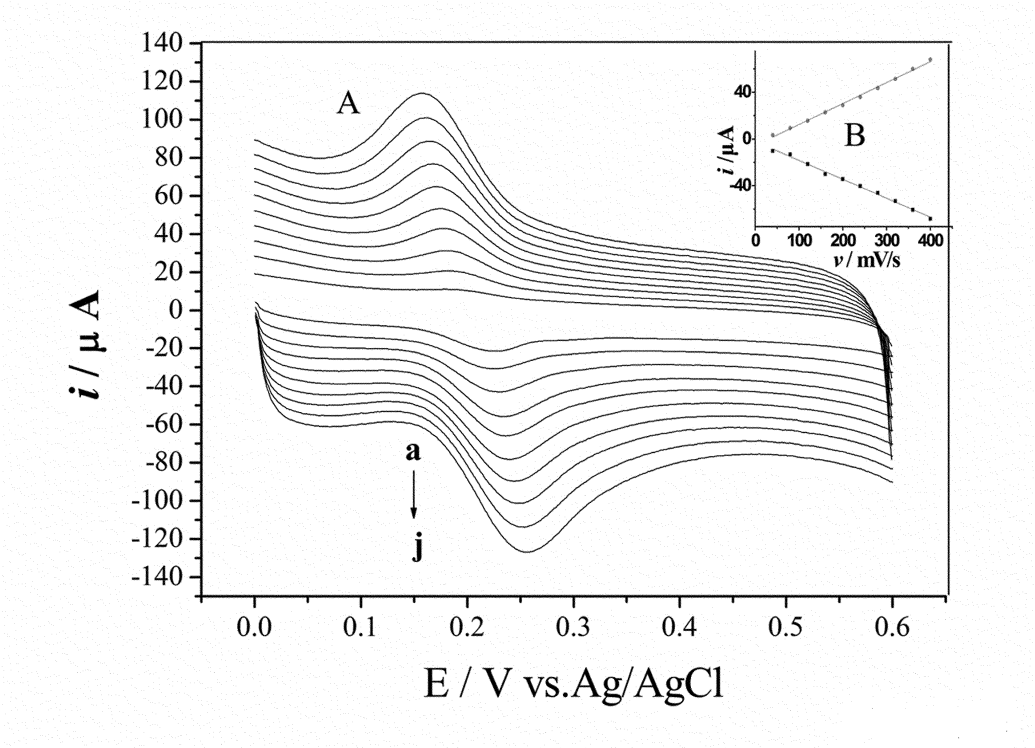

Figure 4. Cyclic voltammograms of NE (1.0×10^{-5} mol·L^{-1}) at the graphene modified electrode（A）and its variation with scan rates (B). v (mV s^{-1}): a. 40; b. 80; c.120; d.160; e. 200; f. 240; g. 280; h. 320; i. 360; j. 400.

Fig. 4 is the CVs of NE at GME with different scan rates. We can see that the oxidation potential slightly shifts in the positive direction with the increase of scan rate (Fig. 4A). The redox peak currents linearly increased with the scan rates ranging from 40 to 400mV.s^{-1} (Fig. 4B). The linear regression equations of the i_{pa} and i_{pc} for the scan rates are expressed as i_{Pa} (A) = -2.44×10^{-6} - $1.61\times10^{-7}v$ (mV), r = 0.9987, and i_{Pc} (A) = -5.15×10^{-6}-1.78×10^{-7} v (mV), r = 0.9978 respectively, suggesting that the electrochemical behaviors of NE on GME is an adsorption process. In transferring GME from NE solution to PBS solution, the redox peak currents can be still detected but sharply decrease until disappearing with repeated scanning, which further confirms the adsorption process of NE on GME.

3.4 Effect of Solution pH

The effect of solution pH on the electrochemical signal was analyzed in PBS. Fig. 5 shows the important influence of pH on the redox reaction of NE at GME, the redox peak negatively shifts with increasing the pH value of solution. The relationship between E_p [E_p =（E_{pa} + E_{pc}）/2] and pH can be

described using the following equation: E_p=0.62 –0.059pH, r = 0.9991, with a slope of 0.059, which indicates that the redox reaction involves a transfer process of two protons. The effect of solution pH on the electrochemical signal is similar to the effect of solution pH on carbon-loated nickel magnetic nanoparticles modified electrode. [20]. When the potential range was 0.0~0.6V and the solution pH were changed from pH 2.2~8.0, respectively, the redox peak currents increased as the pH changed from pH 2.2~5.0, and then decreased after pH > 5.0. This indicates that protons are involved in the redox reactions. A buffer solution of pH 7.0 was chosen as the supporting electrolyte because NE could be selectively detected in the presence of AA, EP and UA, as shown in following study.

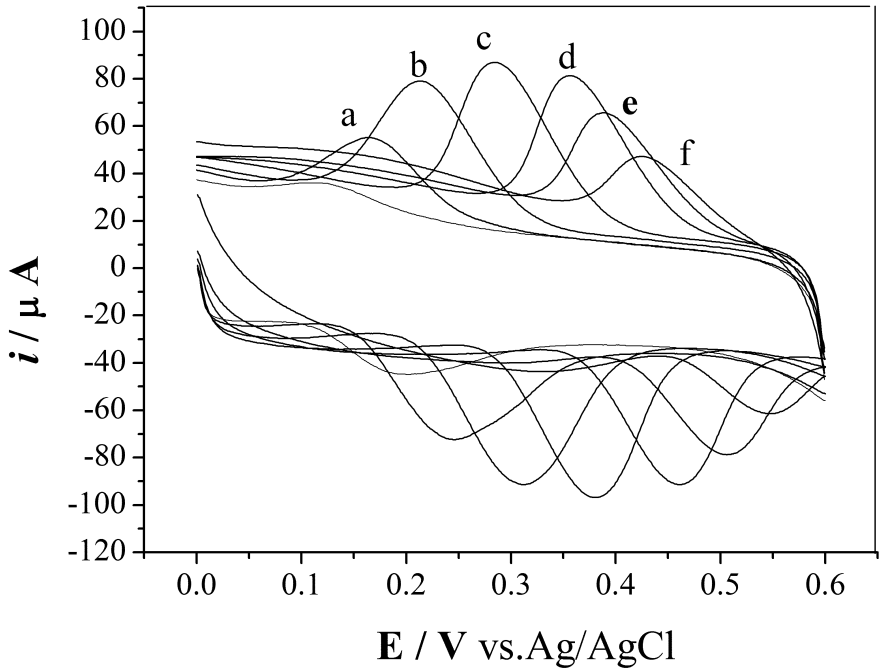

Figure 5. Cyclic voltammograms of NE (1.0×10^{-5} mol·L^{-1}) at different pH levels. pH（a-f）: 2.2, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0.

3.5 Effect of Stirring Time

Due to the adsorption process, stirring time should be an important factor for the accumulation of NE at GME. To study the effect of stirring time on the level of NE adsorption at the electrode surface, we detected 2.0×10^{-5} mol·L^{-1} of NE by varying the accumulation time between 20 and 140 s. Results showed that the sensitivity of detection was significantly improved by stirring. The peak current increases as stirring time increases, and reaches its maximum value at 120 s. Therefore, 120 s was used as the accumulation time in this study.

3.6 Effect of NE Concentration and Detection Limit

Fig. 6 shows the reductive peak current at GME was proportional to the concentration of NE in PBS (pH = 7.0), which is 6.00×10^{-7} to 1.20×10^{-4} mol· L^{-1}, with the detection limit of 4.00×10^{-7} mol· L^{-}

[1]. The linear regression equation is i_{Pc} (A) = 4.72×10^{-6} + 1.58*c* (mol· L^{-1}), with a correlation coefficient of *r* = 0.9995. The linear range is wider by one order of magnitude than that of in the some literature [20-21].

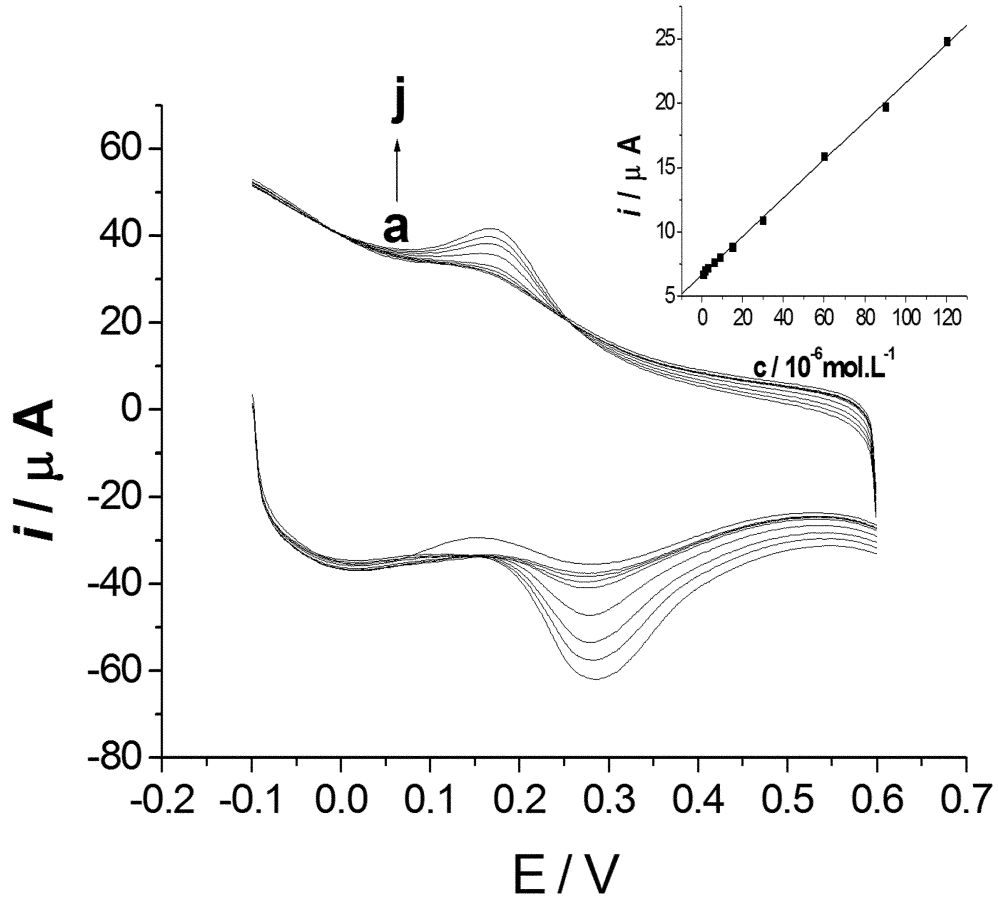

Figure 6 CVs on graphene/GCE for different norepinephrine concentrations (a–j): 6.00, 1.50, 3.00, 6.00, 9.00, 15.00, 30.00, 60.00, 90.00 and 120.00 μmol·L^{-1} in PBS pH7.0. Inset is the relationship of current responses to norepinephrine concentration.

3.7 Interference Studies

Selective determination of NE can be realized by selecting pH. It was found that the interference problem could be resolved by using the GME in PBS (pH 7.0). As shown in Fig. 7, in pH 7.0 PBS NE has a pair of redox peaks but AA, EP and UA have no reductive peaks at GME from 0.0 to 0.6 V.

Thus, it provides a method for the selective determination of NE by reduction peak current of CV in the presence of EP, UA and AA. The effect of dopamine (DA) brings serious interference, and the redox peak of NE and DA are partially overlapping. If there is DA in the sample, it can be removed by paper chromatography before the determination of NE. Some methods [20-23] for the selective determination of NE only related to UA and AA, but blenched the interference of EP and DA. In

addition, no interference has been found when including up to 100 times of K^+, Na^+, Ca^+, NH_4^+, Mg^{2+}, Cl^-, alanine, and 10 times of glucose and tartaric acid also.

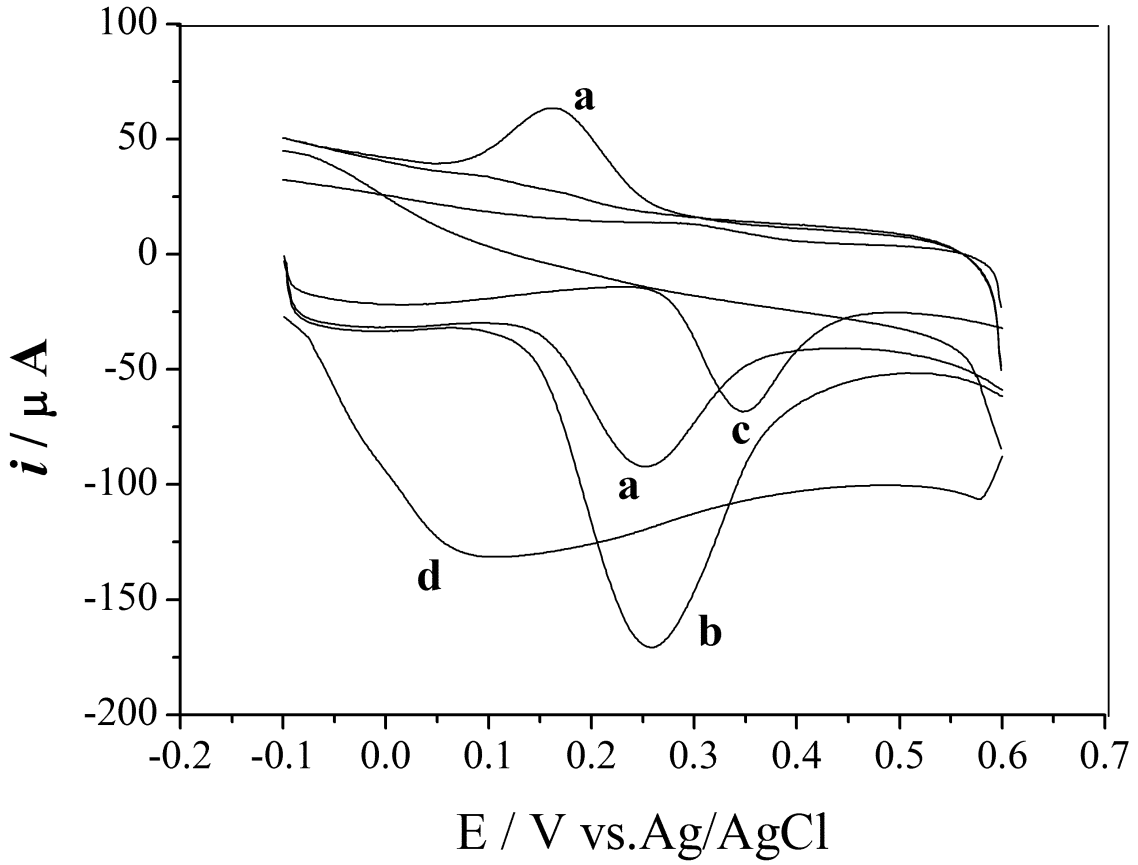

Figure 7. Cyclic voltammograms of 1.0×10^{-6} mol·L^{-1} norepinephrine (a), 2.0×10^{-4} mol·L^{-1} epinephrine (b), 2.0×10^{-4} mol·L^{-1}uric acid (c) and 2.0×10^{-3} mol·L^{-1} ascorbic acid (d) in PBS (pH 7.0).

4. ANALYTICAL APPLICATION

The present method was used for the determination of NE in three pharmaceutical injections from different batches. These samples were diluted in the capacity of 50 mL brown volumetric flask separately. NE in the diluted solution was determined by CV at the GME, and the recoveries were calculated according to the reductive peak currents. The results are listed in Table 2.

Table 2. Determination results of NE in injection (n=6)

Sample	Content ($\times10^{-5}$ mol·L$^-$)	R.S.D (%)	Added ($\times10^{-5}$ mol·L^{-1})	Found ($\times10^{-5}$ mol·L^{-1})	Recovery (%)
1	3.79	2.1	3.00	6.85	102.0
2	3.98	1.9	3.00	7.05	102.3
3	3.89	2.4	3.00	6.98	103.0

5. CONCLUSION

The graphene modified glassy carbon electrode exhibit highly electrocatalytic activity to NE oxidation. The major difficulty from the interference of EP, UA and AA can be efficiently overcome by using GME in PBS 7.0 and measuring the reduction peak current of NE. The proposed method can be applied for the detection of NE in Pharmaceutical injections samples.

ACKNOWLEDGEMENTS
This work was financially supported from a grant from the National Natural Science Foundation of Shan Dong Province (No.2R2009BM003) and the Shandong City High School Science and Technology Fund Planning Project of (J10LB64).

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