[Frontiers in Bioscience 10, 23-29, January 1, 2005]

POLY (*O*-AMINOBENZOIC ACID) MODIFIED GLASSY CARBON ELECTRODE FOR ELECTROCHEMICAL DETECTION OF DOPAMINE IN THE PRESENCE OF ASCORBIC ACID

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3.Experimental
3.1. Apparatus and reagents
3.2. Procedures
4. Result and discussion
4.1. Electropolymerization of o-aminobenzoic acid at the GCE surface
4.2. Cyclic voltammograms of DA at bare and modified electrodes
4.3. Effect of ph
4.4. Determination of DA
4.5. Interferences
4.6. Analytical applications
5. Conclusions
6. Acknowledgements
7. References

1. ABSTRACT

o-aminobenzoic acid (*o*-ABA) film is deposited on glassy carbon electrode (GCE) by electropolymerization in pH 7.0 phosphate buffer solution (PBS). The polymeric film shows an excellent electrocatalytical activity on the oxidation of dopamine (DA). Difference pulse voltammetry (DPV) was performed to determine DA in an excess of ascorbic acid (AA). The oxidation peak potentials of DA and AA recorded are 144 mV and –52 mV, respectively. In pH 7.0 PBS, the anodic peak current of DA increases linearly over two concentration intervals, *viz.,* 1.0×10^{-7} ~ 1.0×10^{-5} mol L^{-1} and 1.0×10^{-5} ~ 2.0×10^{-4} mol L^{-1}, with correlation coefficient, 0.9966 and 0.9960, respectively. The relative standard deviation of 10 successive scans is 2.8 % for 1.0×10^{-6} mol L^{-1} DA and the recovery is 96 % ~ 101 %. The interference of AA and DOPAC with the determination of DA could be eliminated because of the very distinct attracting interaction between DA cations and the negatively poly (*o*-ABA) film in pH 7.0 PBS. The proposed method exhibits good recovery and reproducibility.

2. INTRODUCTION

Dopamine (DA), one of the most significant catecholamine, belongs to the family of excitatory chemical neurotransmitter (1, 2). It plays a very important role in the function of central nervous, renal, hormonal and cardiovascular system (3, 4). Because extreme abnormalities of DA levels are symptoms of several diseases such as Schizophrenia and Parkinsonism (5), the determination of such compounds in real biological samples and identify changes in neurotransmission that correlate with the behavioral state of animal are an obvious target in neurochemical studies. Generally, the determination of DA is performed with high performance liquid chromatography (HPLC) (6), ion chromatography (7) and spectrophotometry (8). DA can be determined by electrochemical methods because it is an electrochemically active compound. The development of voltammeteric sensors for detection of neurotransmitters in the extracelluar fluid of the central nervous system has received much interest in the past few decades (9-10). The electrochemical methods have more advantages over other methods because the electrodes can be made conveniently to sense the neurotransmitters in the living organism (9). Wightman and co-workers have done a lot of merits work on behavior of animal and changes in neurotransmitter in rat by fast-scan voltammery (FSCV) (11-14). Ewing and co-workers (15-17) adopt advance capillary electrophoretic (CE) separation neurotransmitter *in vivo*, and determination neurotransmitter in single cell cytoplasm using Nafion-coated carbon-fiber microelectrodes as electrochemical detection. Ewing research group have used this methodology to examine the transient chemical events happening in the nanometer environment of the synapse. The major problem in the detection of DA is the presence of a high concentration of ascorbic acid (AA), which is oxidized at almost the same potential as DA on bare electrodes, the bare electrodes very often suffers from a fouling effect due to the accumulation of oxidized products on the electrode surface, which results in rather poor selectivity and sensitivity. The homogeneous catalytic oxidation of AA by oxidized DA is another major interference in the measurement of DA. So it is very difficult to determine DA directly (18). In order to resolve these problems, some modified electrodes have been used to determine DA (19-22).

As well known, DA exists as a cation and AA exists as an anion at physiological pH 7.0 (23). A possible way to overcome this problem is coating the electrodes with a thin film of Nafion (24-26). The kind of electrode usually

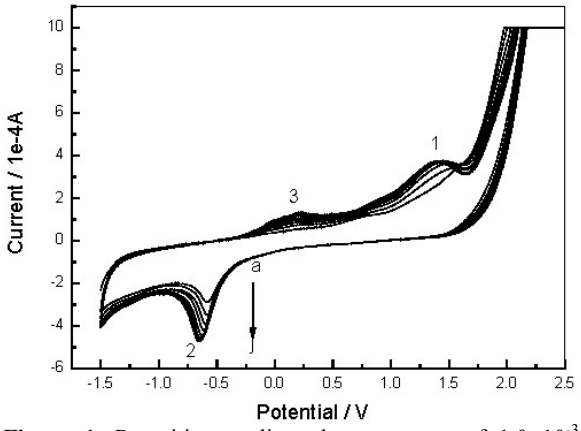

Figure 1. Repetitive cyclic voltammograms of 1.0×10^{-3} mol L^{-1} *o*-ABA in pH 7.0 PBS solution; Terminal potential: +2.5 V; Initial potential: –1.5 V; Sensitivity: 1.0×10^{-4} A/V; Scan rate: 100 mV/s.

suffers from slow response due to low diffusion coefficients (27-28) of analytes in the films. In order to overcome this problem, double-layer film (29-31) modified electrodes are used, which are coated first with an electroactive material having a catalytic effect on the oxidation of DA and then with a Nafion layer.

In recent years, polymer film modified electrodes have attracted great attention, since polymer film has good stability and reproducibility. A number of researchers have employed polymer film modified electrode to detect DA (32-40). Jin and co-workers (41) have ever applied poly (*p*-ABA) modified electrode to an experimental Parkinsonian animal model. Their works are main preparation of poly (*p*-ABA) modified electrode by cyclic voltammetry, and employ it for electrochemistry detection of chromatogry. The method is adopted to investigate Parkinsonian animal model. But the method hadn't discussed electrochemical behavior of poly (*p*-ABA) modified electrode and interference of AA and DOPAC for DA detect when they co-exist. In present paper, we use of *o*-Aminobenzoic acid (*o*-ABA) as a modifier to fabricate modified electrode for selective detection of DA in the presence of AA. In molecule of *o*-ABA, it has electron-rich N atom and high electron density of carbonyl group. The polymer film is negatively charged, which attracts DA cation and repels AA and DOPAC anion, so the interference of AA and DOPAC are eliminated. So the polymer film modified electrode can be used to determine DA at physiological pH in presence of AA and DOPAC.

3. EXPERIMENTAL

3.1. Apparatus and reagents

Electrochemical experiments were performed using a CHI 660A electrochemical workstation (CH Instruments, Chenhua Corp, Shanghai, China). A conventional three-electrode system was employed with a bare or poly (*o*-ABA) modified electrode (3.0 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum electrode as the counter electrode. All the potentials quoted in this paper were referred to the SCE.

o-Aminobenzoic acid *(o*-ABA)was obtained from Guangyao Reagent Factory (Jiangsu, China). Dopamine, 3,4-Dyhyroxyphenyl acetic acid and Ascorbic acid were purchased from ICN Biomedicals Inc (USA). Dopamine hydrochloride injections were purchased from Shanghai Harvest Pharmaceutical CO., LTD. They and all the other chemical reagents were of analytical reagent grade and were used without further purification. Phosphate buffer solutions (PBS) were prepared by 0.1 mol L^{-1} KH_2PO_4-K_2HPO_4, and by adjusting the pH with 0.1 mol L^{-1} H_3PO_4 and 0.1 mol L^{-1} NaOH. All aqueous solutions were prepared with double distilled, de-ionized water.

3.2. Procedures

The bare glassy carbon electrode (GCE) was polished successively with 0.3 and 0.05 μm Al_2O_3 slurry on emery paper before modification. Then it was rinsed with double distilled water and sonicated in 1:1 nitric acid, acetone and double distilled water for 10 min, respectively. After cleaning, electrode was activated by 20 circle sweepings from –1.2 to +1.2 V in pH 7.0 PBS. Finally, the electrode was deposited by cyclic sweeping from –1.5 to +2.5 V at 100 mV s^{-1} for 10 circles in pH 7.0 PBS containing 1.0×10^{-3} mol L^{-1} *o*-ABA.

Sample Preparation. The DPV was used in the determination of DA in pharmarceutial products, commercially available as injection (2.0 mL in dosages 20 mg). 2.00 mL of this product was transferred to a 50.0 mL volumetric flask and diluted to volume with pH 7.0 PBS.

Electrochemical measurement. A 5.00 mL volume of PBS containing a suitable amount of special substance was added to the 10.0 mL voltammetry cell. The solution was purged with nitrogen for 10 min, and the flow of nitrogen over the cell was maintained throughout the experiment. In DPV measurement, potential scanning was performed in the range of - 0.6 to + 0.6 V. The pulse height is 50 mV and scan rate is 5 mV/s. The injections were analyzed by the standard–addition method. All experiments were conducted at room temperature.

4. RESULT AND DISCUSSION

4.1. Electropolymerization of *o*-aminobenzoic acid at the GCE surface

Figure 1 show cyclic voltammograms of 1.0×10^{-3} mol L^{-1} *o*-ABA in pH 7.0 PBS at the GCE .In the first scan, anodic peak 1 and cathodic peak 2 were observed with peak potential value at +1.4 V and -0.6 V, respectively. From the second cycle onwards, a new anodic peaks 3 appeared at +0.2 V. Then larger peaks were observed upon continuous scanning, reflecting the continuous growth of the film. It also can be observed that the film growth was faster during the first six cycles than during the other cycles. From the seventh cycle, the film hardly grew. These facts indicated that *o*-ABA was deposited on the surface of GCE by electropolymerization mode. After modification, the poly (*o*-ABA) film electrode was carefully rinsed with doubly distilled water, then stored in air and prepared for use later on.

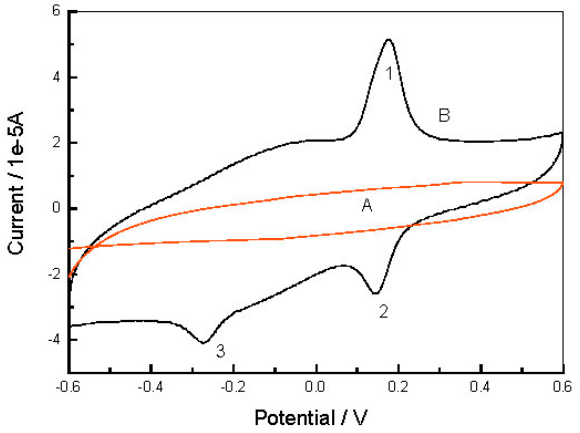

Figure 2. Cyclic voltammograms of 1.0×10^{-4} mol L^{-1} DA in pH 7.0 PBS at the bare electrode (A) and the modified electrode (B). Sensitivity: 1.0×10^{-4} A/V. Scan rate: 100 mV/s.

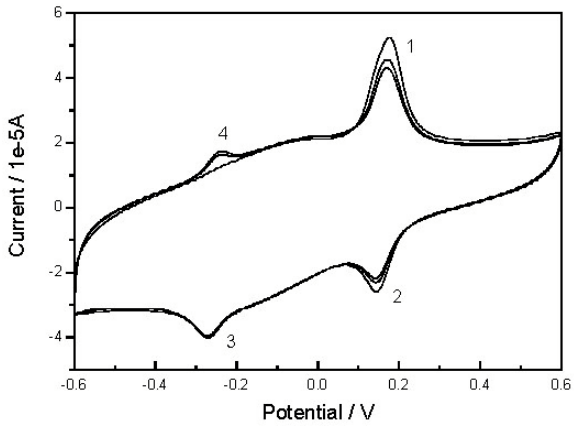

Figure 3. Repetitive cyclic votammograms (6 scans) of 1.0×10^{-4} mol L^{-1} DA in PBS of PH 7.0 at modified electrode. Sensitivity: 1.0×10^{-4} A/V. Scan rate: 100 mV/s.

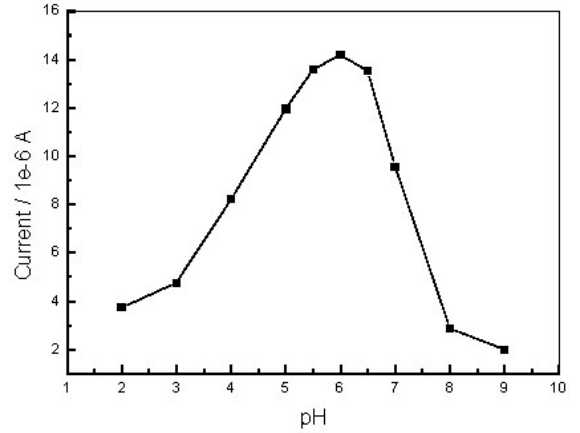

Figure 4. Effects of solution pH on the cyclic voltammetric response of 1.0×10^{-5} mol L^{-1} DA. Sensitivity: 1.0×10^{-4} A/V. Scan rate: 100 mV/s.

4.2. Cyclic voltammograms of DA at bare and modified electrodes

Figure 2 shows cyclic voltammograms of 1.0 × 10^{-4} mol L^{-1}DA in pH 7.0 PBS at modified or bare GCE. DA exhibited poor electrochemical response at the bare GCE (Figure 2A). The peak potential separation value (ΔE_p) was 465 mV between the anodic peak potential (E_{pa} = 0.365 V) and cathodic potential (E_{pc} = -0.100 V). But at the modified GCE (Figure 2B), the peak current of DA was increased and the peak potential was shifted negatively. Peak 1 and peak 2 was a couple of redox peaks with the anodic and the cathodic peak potential at 0.177 V and 0.144 V, respectively. So the peak potential separation (ΔEp) was 33 mV. It also can be seen that DA oxidation peak potential shifted negatively from +0.365 V (bare GCE) to +0.177 V (modified GCE) and the overpotential decrease 178 mV. The oxidation peak current of DA (peak 1) at the modified electrode was more 100 times than that of DA at the bare GCE. It may be due to DA accumulation because the modified film is negatively charged and DA is cationic at pH 7.0.

To further investigate the DA electrochemical behavior at the modified electrode, repetitive CVs of DA was obtained in pH 7.0 PBS (Figure 3). From the second circle onwards, an obvious oxidation peak 4 can be observed. The electrochemical behavior of DA at the poly(*o*-ABA) modified electrode was similar to the result of references reported (38-39). Peak 1 was corresponding to oxidation of dopamine to dopaminequinone while peak 2 was corresponding to reduction of dopaminequinone to dopamine. Peak 3 was the reduction of dopachrome to leucodopachrome while peak 4 was oxidation of leucodopachrome to dopachrome.

In addition, the effect of the scan rate on the peak current of DA was investigated. The anodic peak current was proportional to the square root of scan rate over the range of 20 ~ 300 mV s^{-1}. The linear regression equation was i_p (μA) = -0.0557 + 0.9777 v $^{1/2}$ (mV s^{-1}) $^{1/2}$, with a correlation coefficient of R = 0.9965. Therefore, the contribution of diffusion played a more important role in the electrode process.

4.3. Effect of pH

The effect of the solution pH on the response of DA was investigated over the range of 2 ~ 9. Figure 4 shows that the anodic peak current increased with the increasing of solution pH until it reached 6.Then the anodic peak current decreased with increasing pH over the range of 6 – 9. The reason may be as follows: the negative carboxylate group on electrode surface is increased with pH increasing, so more DA cations were attracted to electrode surface. The DA anodic peak current increased with the increase of pH over the range of 2-6. Then the protonated degree of DA decreased with the increasing of pH, because hydroxy group of DA is easily oxidated in alkali. When pH of solution is higher 6, the negative carboxylate group of electrode surface is increased, but DA cations decease more, therefore, the static attraction interaction between DA and poly (*o*-ABA) decreased. The anodic peak current of DA decreased with the increase of pH over the range of 6 – 9. Since pH 7.0 is the physiological pH value, it was chosen for the support electrolyte in the electrochemical detection of DA

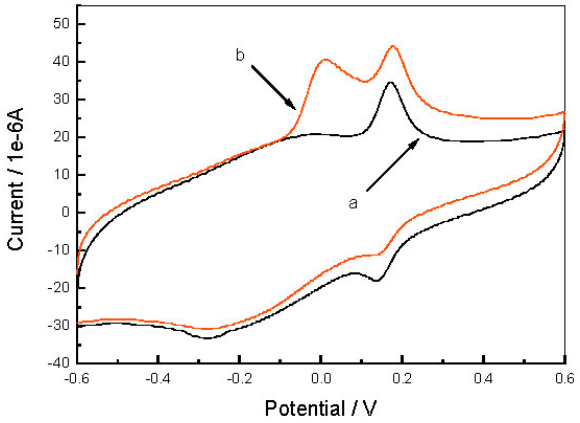

Figure 5. Cyclic voltammetric recordings at the modified electrode in pH 7.0 PBS. (a) 1.0×10^{-5} mol L^{-1} DA. (b) 1.0×10^{-5} mol L^{-1} DA + 1.0×10^{-3} mol L^{-1} AA.

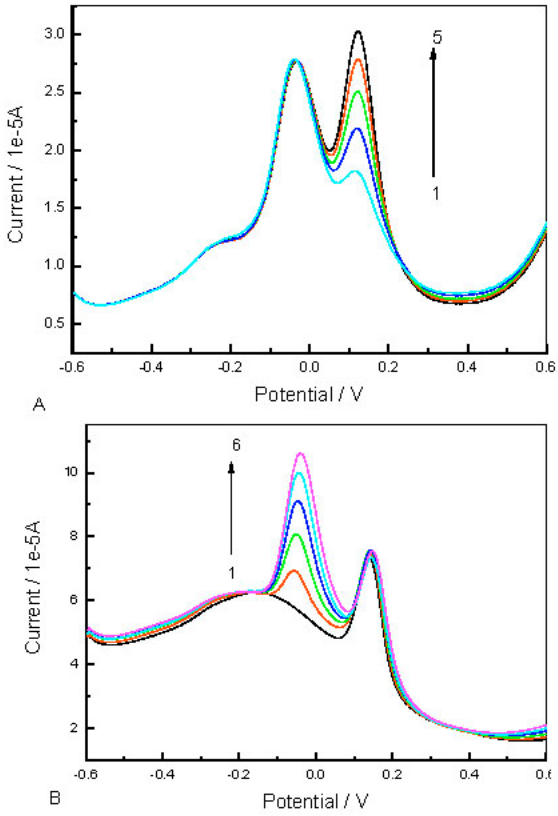

Figure 6. (A) DPV recordings of DA at modified electrode and in the presence of 2.0×10^{-3} mol L^{-1} AA in pH 7.0 PBS. DA concentration (10^{-6} mol L^{-1}): (1) 1.0; (2) 2.0; (3) 3.0; (4) 4.0; (5) 5.0 (B) DPV recordings of AA in the presence of 1.0×10^{-5} mol L^{-1} DA. AA concentration (10^{-3} mol L^{-1}): (1) 0; (2) 1.0; (3) 2.0; (4) 3.0; (5) 4.0; (6) 5.0.

In addition, we studied the relationship between DA anodic peak potential (E_{pa}) and pH. It can be found that the anodic peak potential shifted negatively with the increasing of solution pH, indicating protons take part in electrode process. The linear regression equation was E_{pa} (V) = 0.5306 - 0.0564 pH, with a correlation coefficient of r = - 0.9994. The slope of the plots ΔE_p /ΔpH was about 56 mV per pH unit. This suggested that equal amounts of proton and electron were involved in the oxidation of DA.

4.4. Determination of DA

To estimate the linear calibration curves, correlation coefficient and detection limit of DA, a series of standard DA solutions were tested by DPV with the concentrations ranging from 1.0×10^{-7} ~ 2.0×10^{-4} mol L^{-1}. The results showed that anodic peak current was proportional to the concentration of DA over two concentration intervals of DA, viz., 1.0×10^{-7} ~ 1.0×10^{-5} mol L^{-1} and 1.0×10^{-5} ~2.0×10^{-4} mol L^{-1}. The linear regression equation was ip (μA) = 0.8980 + 0.4993 C (μmol L^{-1}) and ip (μA) = 4.2272 + 0.3241 C(μmol L^{-1}), with a correlation coefficient of r = 0.9966 and r = 0.9960, respectively. The detection limit (S/N = 3) was 1.0×10^{-8} mol L^{-1}. The relative standard deviation of 10 successive scans was 2.8 % for 1.0×10^{-6} mol L^{-1} DA, indicating excellent reproducibility of modified electrode.

Furthermore, the stability of the modified electrode has been investigated. The peak current could hardly change after storage in air for at least one month.

4.5. Interferences

The electrochemical behavior of 1.0×10^{-5} mol L^{-1} DA and mixture of 1.0×10^{-5} mol L^{-1} DA + 1.0×10^{-3} mol L^{-1} AA at modified electrode were studied (Figure 5). The electrochemical signal of DA and AA can be clearly observed. The anodic peak potential for DA and AA at modified electrode was +0.177 V and +0.010 V, respectively. The separate value was 167 mV. So AA has no interference with DA detection. The reason may be as follows: electrostatic repulsion between the AA anions and the negative carboxyl groups at the modified electrode retarded the electron transfer and shifted the potential of AA toward more negative value so that the peak of DA could be separate from that of AA.

Figure 6A shows DPVs recordings at various DA concentrations in the presence of AA at the modified electrode. Curve (1) in Figure 6A was recorded in 1.0×10^{-6} mol L^{-1} DA and 2.0×10^{-3} mol L^{-1} AA. Curve (2-4) showed that the anodic peak current of DA increased when its concentration increased in the presence of AA. But the anodic peak current of AA kept almost constant. We can observe that even a 2000-fold excess of AA does not interfere with the determination of DA.

Figure 6B shows DPVs of various AA concentrations in the presence of DA. Curve (1) in Figure 6B was recorded in 1.0×10^{-5} mol L^{-1} DA. Curve (2-4) in Figure 6b shows DPVs of various AA concentrations in the presence of DA. It can be seen that the anodic peak current of AA also increases continually with increasing AA concentration. So electrochemical response to DA and AA at modified electrode alone exists when they co-exist in pH 7.0 PBS. It is therefore possible to simultaneously determine DA and AA in sample at a poly (*o*-ABA) modified electrode.

Table 1. Determination results of DA in injections (n =10)

Sample	Content (mg/mL)	Found (mg/mL)	RSD (%)	Recovery (%)	Pharnacopeia Method (mg/mL)
1	2.0	1.92	3.0	96	2.03
2	2.0	1.98	2.8	99	2.05
3	2.0	2.01	3.1	101	2.06

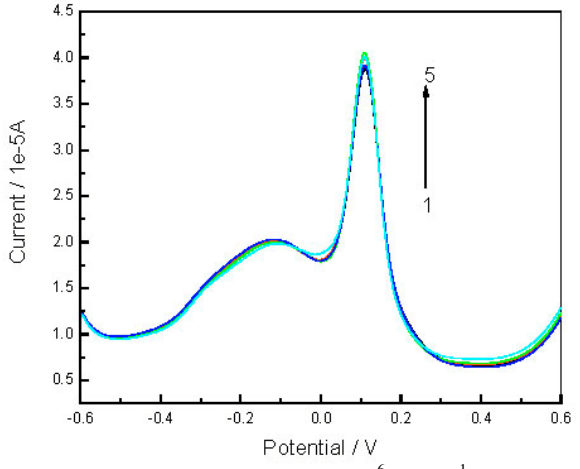

Figure 7. DPV recordings of 5.0×10^{-6} mol L^{-1} DA in the presence of various DOPAC concentration in pH 7.0 PBS. DOPAC concentration (1.0×10^{-5} mol L^{-1}): (1) 0; (2) 2.0; (3) 5.0; (4) 8.0; (5) 10.0.

Figure 7 shows DPVs recording with 5.0×10^{-6} mol L^{-1} DA in the presence of various DOPAC concentrations. When DOPAC concentration is 2.0×10^{-5}, 5.0×10^{-5}, 8.0×10^{-5} and 1.0×10^{-4} mol L^{-1}, respectively, the DA anodic peak current keeps almost constant (Relative Error < ±5%). We can observe that 20-fold excess of DOPAC does not interfere with the determination of DA.

Interference studies were also carried out with other compounds. No interference could be observed for the following compounds: hippuric acid (200), citric acid (100), cysteine (50), glucose (50), NaCl (400), KCl (400), $CaCl_2$ (200), where the data in brackets were the concentration ratios to 1.0×10^{-5} mol L^{-1} DA.

4.6. Analytical applications

The injection of DA was analyzed by the standard addition method. The results are shown in Table 1. It is accordant with the result by the China Pharmacopoeia Method (42), showing that the proposed methods could be efficiently used for the determination of DA in injection.

5. CONCLUSIONS

Poly (*o*-Aminobenzoic acid) film modified electrode exhibits highly electrocatalytic activity to DA oxidation. The major difficulty from the overlapped oxidation potential of AA can be efficiently overcome through the distinct ability of DA to be attracted by the negatively charged poly (*o*-Aminobenzoic acid) film. In addition, the interference of DOPAC has been eliminated at this polymeric film modified electrode. The determination of DA in the presence of an excess of AA is possible with the present polymeric film modified electrode.

6. ACKNOWLEDGEMENTS

This work was supported by a grant from the Nature Science Foundation of Anhui Province Ministry of Education (No. 2004Kj 168), the Doctoral Start Foundation of Anhui Normal University, and key teacher Foundation of Universities in Anhui province.

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Key Words: Chemically modified electrode, *o*-Aminobenzoic acid, Dopamine, Ascorbic acid, 3,4-Dyhyroxyphenyl acetic acid, Electropolymeriation, Electrocatalysis, Review

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