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Determination of Dopamine and Ascorbic Acid in Hypoxanthine System with Mercury Film Electrode Acid

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In this article, based on the analyses of former research (determination of dopamine in hypoxanthine system with glassy carbon mercury film electrode). The electrocatalytic oxidation for both dopamine and ascorbic acid in this system were also studied and the interference of ascorbic acid and dopamine in different pH values was discussed in detail. It indicated that at pH 1.81 buffer solution, the reduction peak of dopamine was almost unchanged when ascorbic acid coexisted. The reduction peak current of dopamine was proportional to its concentration in the range of 1.5×10^{-5}-5.6×10^{-4} mol L^{-1} and the detect limit could reach 2×10^{-6} mol L^{-1}. The oxidation peak current of ascorbic acid was linear with its concentration from 5×10^{-5}-2.4×10^{-3} mol L^{-1}. Detect limit of it reached 8×10^{-6} mol L^{-1}. At pH 4.10 buffer solution, the oxidation peak of dopamine could be separated from that of ascorbic acid about 200 mV and the presence of dopamine had little effect on the oxidation peak of ascorbic acid. The method could obtain better result when it applied to the determination dopamine and ascorbic acid in medicament sample and synthetic sample.

Key Words: Glassy carbon mercury film electrode, Hypoxanthine, Dopamine, Ascorbic acid.

INTRODUCTION

Ascorbic acid (vitamin C) is used clinically in the treatment and prevention of scurvy. Ascorbic acid/ascorbate is a vital component in the diet of humans. Ascorbate is known to take part in several biologicalreactions and is present in mammalian brain. Similarly, dopamine, the most significant catecholamine, belongs to the family of inhibitory neurotransmitters and plays a very important role in the functioning of central nervous, renal, hormonal and cardiovascular system. Extreme abnormalities of dopamine concentration levels may lead to several diseases such as Parkinson's[1]. Thus simultaneous determination of dopamine and ascorbic acid is a problem of critical importance in the field of neurochemistry and biomedical chemistry. Electro-chemical sensors attracted wide attention due to their advantages of simple, inexpensive and fast analysis in combination with high sensitivity and selectivity. Both dopamine and ascorbic acid are oxidizable compounds makes their detection

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possible by electro-chemical methods based on anodic oxidation. However, a major problem is that both ascorbic acid and dopamine are oxidized at nearly same potential with poor sensitivity at solid electrodes, which resulted in overlapped voltammeter responses making their discrimination highly difficult. Most of the studies on these compounds demonstrated the separate determination of either AA or dopamine by eliminating the other using different membranes or selecting particular potentials. However, it is most important to develop a sensor, which can determine both ascorbic acid and dopamine. One possibility for alleviating this problem is the use of chemically modified electrodes (CMEs), some efforts have been taken to separate the oxidation signals by chemically modified electrodes, such as a cetylpyridine bromide/chitosan composite film[2], ferrocene derivative mediators[3], poly(phenosafranine)[4] and poly (3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphthalene-2,7-disulfonic acid) film[5] were used to modify the different electrode for the detection of determine dopamine and ascorbic acid. The other possibility is activating electrode by the physical pretreating method, various methods have been developed to remove polishing contaminants from carbon electrodes. These include ambient or vacuum heat treatment, removes polishing contaminants[6], exposure to UV-generated ozone[7], laser irradiation[8], exposure to radio frequency[9] and ultrasonic vibrating[10]. As a result, significant improve-ments in reproducibility and electrode kinetics for certain redox systems have been reported. In this article, based on the analyses of former research, we describe herein a method to increase the available potential range of mercury film and its electrocatalytic oxidation dopamine and ascorbic acid. Present results showed that adding certain amount of hypoxanthine in electrolyte solution, it had good inhibition on the stripping peak of mercury at higher potentials and the glassy carbon mercury film electrode (GCMFE) showed a better stability in the solution. Furthermore, the electrochemical behaviours of dopamine and ascorbic acid in hypoxanthine system with the GCMFE were examined. Thus, dopamine and ascorbic acid concentration can be determined, respectively when dopamine coexist with ascorbic acid. The proposed method has been applied for the determination of dopamine and ascorbic acid in synthetic sample with satisfactory result.

EXPERIMENTAL

Cyclic voltammetry (CV) measurements were carried out using a MEC-12B multi-function electrochemical analyzer (Jiangsu Jiangfen Instrument Int., China). All the cyclic voltammograms were made using a three-electrode system with a glassy carbon electrode as a working electrode, an Ag/AgCl (saturated with KCl) as a reference electrode and a platinum wire electrode as a counter electrode. The KQ218 ultrasonic instrument (Kunshan Ultrasonic Instrument Factory, China) was used.

Dopamine (Shanghai Hefeng Chemicals, China) and ascorbic acid (Tianjin Chemicals, China) were used as received. Hypoxanthine was analytical grade and purchased from Sigma (USA). They and all other chemical reagents were used

without further purification. All other chemicals used in this investigation were of analytical grade. 0.05 mol L^{-1} H_3PO_4 + 0.05 mol L^{-1} CH_3COOH + 0.05 mol L^{-1} H_3BO_3-0.2 mol L^{-1} NaOH buffer solutions of various pHs were prepared by mixing two stock solutions, ascorbic acid and dopamine were prepared daily. All solutions were prepared with double-distilled water.

Preparation of the glassy carbon mercury film electrode (GCMFE): According to the reference[11].

Procedure: The electrochemical behaviours of GCMFE in hypoxanthine solution cycled at pH 1.81 buffer solution in the potential range between -0.3 and +0.7 V. Application of the electrode for the determination of ascorbic acid and dopamine was evaluated by running the CVs at pH 1.81 buffer solution (containing 0.1 g L^{-1} hypoxanthine in order to prevent the stripping of the mercury film). The potential range between -0.3 and +0.7 V and the scan rate was 100 mV s^{-1}. The effect of pH can be studied by running the experiments in electrolytes of different pH values. At the same time cyclic voltammograms was applied for investigating the cyclic voltammetric behaviours of dopamine and ascorbic acid on GCMFE. The experimental results were obtained at room temperature.

RESULTS AND DISCUSSION

Electrochemical behaviours of GCMFE in hypoxanthine solution: The electrochemical behaviours of GCMFE in hypoxanthine solution were investigated according to the report of Lian *et al.*[11].

Electrochemical behaviours of ascorbic acid (AA) and dopamine (DA) at the GCMFE: Fig. 1 showed the cyclic voltammograms of different concentration ascorbic acid at the GCMFE at pH 1.81 buffer solution (containing 0.1 g L^{-1} hypoxanthine). It can be seen, the oxidation peaks of ascorbic acid were observed at 0.25 V, but the corresponding reduction peaks were not observed, it showed that the redox process of ascorbic acid on the GCMFE was irreversible. The oxidation peak of ascorbic acid was linear with its concentration from 5×10^{-5}-2.4×10^{-3} mol L^{-1} with the linear regression equation was i_{Pa} (μA) = 0.8488 + 0.2120c (R = 0.9995). Detect limit of it reaches 8×10^{-6} mol L^{-1}. the cyclic voltammograms of different concentration dopamine at the GCMFE according to the reference[11]. The oxidation peaks of dopamine were observed at 0.28 V and more sensitive reduction peaks were observed at 0.07 V. The reduction peak of dopamine was linear with its concentration from 1.5×10^{-5}-5.6×10^{-4} mol L^{-1} with the linear regression equation was i_{Pc} (μA) = 0.2403 + 0.0983c (R = 0.9994). Detect limit of it reached 2×10^{-6} mol L^{-1}.

Interference of ascorbic acid and dopamine in different pH values: The oxidation and reduction peaks of GCMFE in the blank solution, ascorbic acid solution, dopamine solution and the mixture solution of ascorbic acid and dopamine at different pH values had been investigated.

All solutions contained 0.1 g L^{-1} hypoxanthine: In the blank solution, the oxidation peak (1) current ($I_{Pa,1}$) decreased rapidly and the oxidation peak potential

($E_{Pa,1}$) negative shifted with increasing solution pH value, at the same time, the reduction peak (2) current ($I_{Pc,2}$) decreased and the reduction peak potential ($E_{Pc,2}$) negative shifted with increasing solution pH value too.

In 1×10^{-3} mol L^{-1} ascorbic acid solution, the oxidation peak current of ascorbic acid ($I_{Pa,AA}$) decreased slightly and the oxidation peak potential ascorbic acid ($E_{Pa,AA}$) negative shifted with increasing solution pH value. The oxidation peak potential difference($E_{Pa,1}$-$E_{Pa,AA}$) increased with increasing solution pH value, the difference was from 208-262 mV. Therefore, there did not exist interference.

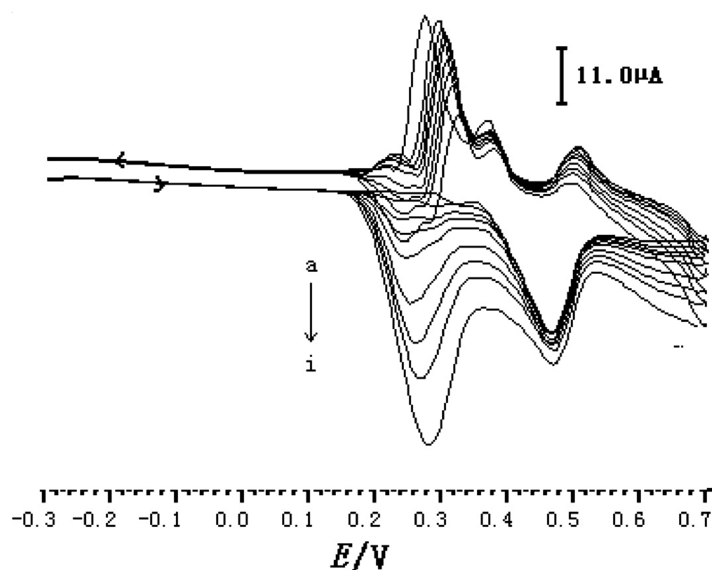

Fig. 1. Cyclic voltammograms of different concentrations of ascorbic acid (pH 1.81). Concen-trations of ascorbic acid (10-5 mol L^{-1}): a, 0; b, 5; c, 20; d, 40; e, 60; f, 100; g, 140; h, 180; I, 240

In 2×10^{-4} mol L^{-1} dopamine solution, the changes of the dopamine oxidation and reduction peak currents ($I_{Pa,DA}$ and $I_{Pc,DA}$) were irregular with increasing solution pH value, which might be that the electrode process was comparatively complicated. The reduction peak potential of dopamine ($E_{Pc,DA}$) was not significant change here, the reduction peak potential difference ($E_{Pc,2}$-$E_{Pc,DA}$) decreased with increasing solution pH value because of the reduction peak potential ($E_{Pc,2}$) negative shifted, there would exist a overlapped peak (I) due to the reduction peak of dopamine overlapped the reduction peak (1) when the pH value > 4.65.

In the the mixture solution of 1×10^{-3} mol L^{-1} ascorbic acid and 2×10^{-4} mol L^{-1} dopamine, the oxidation peak potential difference between dopamine and ascorbic acid ($E_{Pc,DA}$-$E_{Pc,AA}$) was small at 1.81-2.21, there would exist another overlapped peak (II) due to the oxidation peak of dopamine overlapped the oxidation peak of ascorbic acid. But the reduction peak current and potential were almost unchanged

compared to when dopamine existed alone at pH 1.81-1.98 (Fig. 2), the determination of dopamine reduction peak was not influenced by ascorbic acid in this case. The oxidation peak potential difference between dopamine and ascorbic acid ($E_{Pc, DA}$-$E_{Pc, AA}$) increased with increasing solution pH value, the potential difference was about 200 mV when pH > 3.29 and the oxidation peak current and potential of ascorbic acid were almost unchanged compared to when ascorbic acid existed alone (Fig. 3), the determination of ascorbic acid oxidation peak was not influenced by dopamine in this case. Furthermore, at pH 4.10 buffer solution, the oxidation peak of ascorbic acid was linear with its concentration from 6×10^{-5}-2.4×10^{-3} mol L^{-1} with the linear regression equation was i_{Pa} (μA) = 0.5997 + 4.9268c (R = 0.9995). But the reduction peak current of dopamine was reduced compared to when dopamine existed alone at pH 4.10.

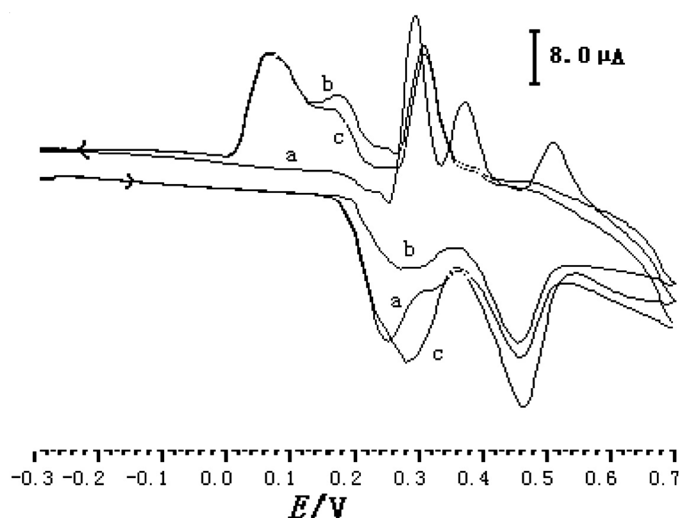

Fig. 2. Cyclic voltammograms of ascorbic acid and dopamine at glassy carbon mercury film electrode (pH 1.81). a. 1×10^{-3} mol L^{-1} ascorbic acid; b. 2×10^{-4} mol L^{-1} dopamine; c. 1×10^{-3} mol L^{-1} dopamine + 2×10^{-4} mol L^{-1} ascorbic acid

As per discussion, no matter whether existed singly or jointly coexisted with ascorbic acid, the determination of dopamine could be carry out at pH 1.81 buffer solution, because in this condition, the determination of dopamine reduction peak was not influenced by ascorbic acid and the peak urgent was more sensitive, the quantification of dopamine were estimated by the work curve of the relationship between dopamine reduction peak current and concentration. For the determination of ascorbic acid, if ascorbic acid existed singly, the determination could be carry out at pH 1.81 buffer solution because of the ascorbic acid peak current was more sensitive in this case. If ascorbic acid coexisted with dopamine, the determination could be carry out in pH 4.10 buffer solution because of ascorbic acid oxidation peak was not influenced by dopamine in this case.

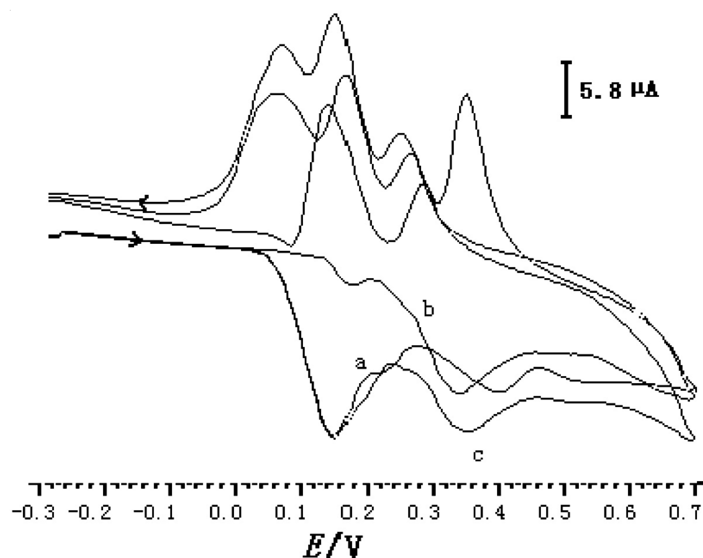

Fig. 3. Cyclic voltammograms of ascorbic acid and dopamine at glassy carbon mercury film electrode (pH 4.10). a. 1 × 10^{-3} mol L^{-1} ascorbic acid; b. 2 × 10^{-4} mol L^{-1} dopamine; c. 1 × 10^{-3} mol L^{-1} dopamine +2 × 10^{-4} mol L^{-1} ascorbic acid

Sample analysis

Analysis of ascorbic acid in vitamin C tablets: Five vitamin C tablets were ground into fine powder. Then dissolved in water and filtered before making a volume of 100 mL. the sample solution was 7.10 × 10^{-4} mol L^{-1} which prepared by diluting the 250 μL solution mentioned above to 10 mL with pH 1.81 buffer solution (containing 0.1 g L^{-1} hypoxanthine). The sample of vitamin C determined 7 times by the cyclic voltametric method. The results were 101.2-104.5 mg/tablet (the reference content is 100 mg/tablet) with RSD of 1.3 %.

TABLE-1
DETERMINATION RESULTS OF ASCORBIC ACID (AA) AND DOPAMINE (DA) IN SYNTHETIC SAMPLE

No.	Quantity of AA in sample(× 10^{-4} mol L^{-1})	Quantity of DA in sample (× 10^{-4} mol L^{-1})	Detect quantity of AA (× 10^{-3} mol L^{-1})	Detect quantity of DA (× 10^{-4} mol L^{-1})
1	2	5	2.04	5.02
2	2	5	2.04	4.95
3	2	5	1.97	4.98
4	2	5	1.96	4.97
5	2	5	2.01	99.6
Average	2	5	2.01	4.98
Recovery (%)			100.5	99.6
RSD (%)			1.9	0.5

Analysis of ascorbic acid and dopamine in synthetic sample: The mixture of ascorbic acid and dopamine had been prepared with pH 1.81 and 4.10 buffer solution (containing 0.1 g L^{-1} hypoxanthine). As discussed above, the determination of ascorbic acid and dopamine carried out under the selection conditions. The determination results were shown in Table-1.

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