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Poly(3-thiophenemalonic acid) Modified Glassy Carbon Electrode for Selective Determination of Dopamine and Urine Acid in the Presence of Ascorbic Acid

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A polymerized film of 3-thiophenemalonic acid (3-TPA) was prepared on the surface of glassy carbon electrode (GCE) in neutral solution by cyclic voltammetry (CV). The poly(3-TPA) film-coated GCE exhibited excellent electrocatalytic activity toward the oxidation of dopamine (DA) and uric acid (UA). This work provides a simple and easy approach to selective detection of DA and UA in the presence of AA. The detection limits of 2.60×10^{-7} and 5.20×10^{-7} M for DA and UA, respectively, were obtained by DPV method in pH 6.86 PBS. Additionally, the proposed methods can be successfully applied to the detection of DA in human urine samples.

Keywords: polymerized film, 3-thiophenemalonic acid, selective detection, dopamine, uric acid,

1. INTRODUCTION

Dopamine (DA) is an important catecholamine neurotransmitter in a mammalian central nerve system and functions as one of the most important biochemical messengers. Low levels of DA are related to neurological disorders, such as schizophrenia, Parkinson's disease, and to HIV infection. Besides, uric acid (UA) is the primary end product of purine metabolism. Abnormal levels of UA are symptoms of several diseases, such as gout, hyperuricemia, and Lesch-Nyan disease. In general, ascorbic acid (AA), an essential vitamin with a recommended daily intake of about 70 mg, coexists with DA and UA in biological fluids, such as blood and urine, therefore, it is important to develop a technique to selectively detect DA and UA in the presence of AA conveniently in a routine assay.

Earlier attempts by fluorometric methods required a large volume of samples, but still lacking selectivity and sensitivity [1]. Later, methods based on chromatography combined with spectrometry

have been developed such as mass spectrometry has been combined with GC[2], HPLC[3,4], and capillary electrophoresis [5]. Although these methods are highly specific and sensitive, they require sophisticated and expensive instrumentation, and are time-consuming. As an alternative to mass spectrometry, electrochemical method have also been introduced originally by Kissinger [6] which is also widely used in liquid chromatography and capillary electrophoresis [7–11] with respect to their simplicity, immediacy, and high sensitivity of detecting analytes. However, the voltammetric responses of them are not resolved enough, at ordinary electrodes, due to the very close electrochemical redox potentials. Various approaches on the modification of electrode by polymer film [12], nano materials [13], covalent modification [14], self-assembled monolayers [15], as well as the use of carbon paste electrode [16] and the electrochemical pretreatment of the electrode [17] have been developed to distinguish them selectively from each other. Among them, the polymer modified electrodes prepared by the electropolymerization have received extensive interests in the detection of analytes because of its enhanced selectivity and sensitivity in addition to the homogeneity and the strong adherence to the electrode surface along with the excellent chemical stability of the film. Here, we present the preliminary results of simultaneous determination of the DA and UA in the presence of AA by using the Poly(3-thiophenemalonic acid) modified glassy carbon electrode(3-TPA/GC).

2. EXPERIMENTAL

2.1. Reagents

3-thiophenemalonic acid and ascorbic acid were purchased from ACROS ORGANICS (USA), Phosphate buffered solution (PBS) was from Tianjin Kemiou chemical reagent Co.Ltd (China), Dopamine was from MERCK-schuchardt (Germany), Urine acid was from Shanghai WeiFang Fine Chemical Co.Ltd (China). All of them were analytical grade purity and used without further purification. Distilled water (Zhengzhou Xuefeng Water Treatment Company, China) was used for preparation of all solutions.

2.2. Apparatus

Electrochemical measurements were performed at room temperature with an electrochemical workstation, CHI760C. GC and 3-TPA/GC were used as working electrodes, SCE, and Pt wire were used as reference, and auxiliary electrodes, respectively.

2.3. Electrode preparation

GC electrode was consecutively polished with 1.0, 0.3, and 0.05μm α-Al_2O_3 slurries in turn until mirror finish and rinsed with copious amount of water in each polishing step then ultrasonicated in ethanol and water for 10mins each. After dried at room temperature, GC was polarized at 1.8 V for 400 s in pH 6.86 PBS. The electropolymerization was conducted in pH 6.86 PBS containing 1.0 mM

3-thiophenemalonic acid between +0.5 and -1.0V in Cyclic Voltammetry for 20 circles. The obtained electrode was subsequently cycled in PBS (pH=6.86) between -0.5 and 1.0 V (vs. SCE) for 10 circles and referred as 3-TPA/GC.

3. RESULTS AND DISCUSSION

3.1. Effect of scan rate on the oxidation of DA

Figure 1 shows the scan rate dependency of peak current of DA (1.0 mM) at 3-TPA/GC in a pH 6.86 buffer solution. The oxidation current peak increases linearly with the square root of scan rates in the range from 10 to 300 mV/s, indicating that the DA oxidation is diffusion-controlled. In addition, the anodic peak potential moved positively and the cathodic peak potential shifted negatively indicating that the electrons transfer slowed down in the film because polymer film got thicker with the increase of scan rate. The fact that the peak potential separation increases with scan rate tells that the redox process is quasireversible [18].

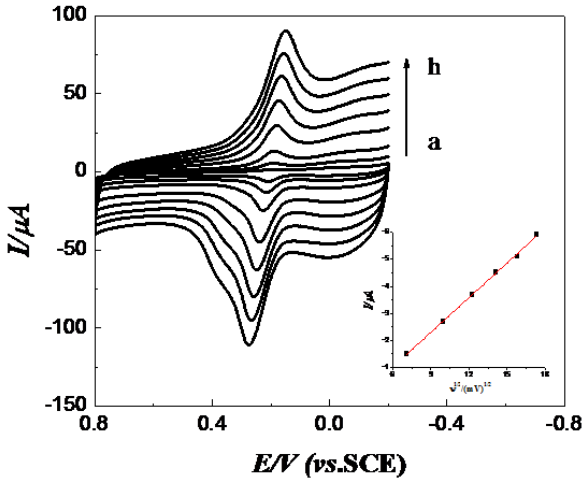

Figure 1. Cyclic voltammograms of 1.0 mM DA at 3-TPA/GC in pH=6.86 PBS. Curves (a) to (h) correspond to 10, 25, 50, 100, 150, 200, 250 and 300 mVs^{-1}, respectively. *Inset*: plot of peak current *vs.* square root of scan rate

3.2. Effect of pH on the oxidation of DA

Effect of pH on peak current and potential for the oxidation of 1.0 mM DA at 3-TPA/GC is displayed in Figure 2. The oxidation peak current of DA increased first with the increase of pH and reached the maximum at pH 6.86 then declined. Besides, with the pH value increasing, the oxidation shifted negatively. The oxidation peak potentials were linearly proportional to the pH with a slope of 52.8 mV/pH indicating the oxidation of DA involves two electrons coupled with two protons [19].

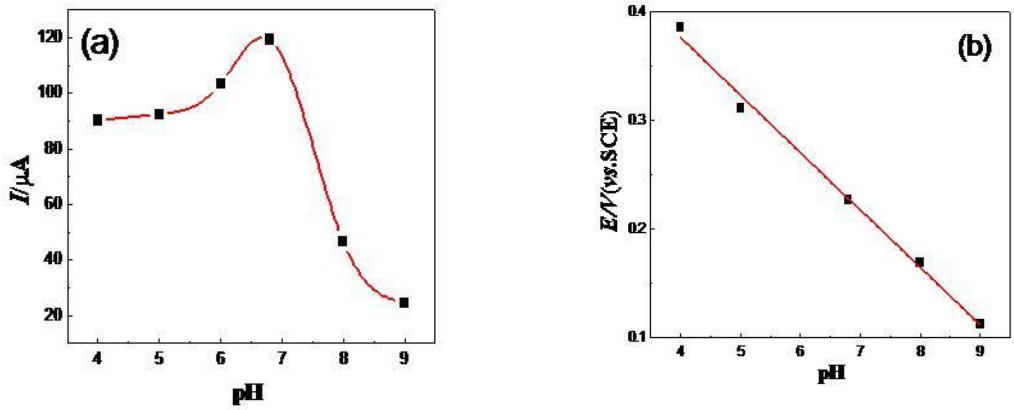

Figure 2. Effect of pH on peak current (a) and peak potential (b) for the oxidation of 1.0 mM DA at 3-TPA/GC.

3.3. Electrocatalytic oxidation of AA, DA, UA at a bare GC and 3-TPA/GC

Figure 3 shows cyclic voltammograms of AA, DA, UA individually and a DA/UA/AA mixture at a bare GC and 3-TPA/GC. At a bare GC (Fig. 3(a)), the oxidation potentials of AA, DA and UA were 0.25, 0.16, 0.35 V, respectively. The oxidation of the DA/UA/AA mixture is a broad peak at about 0.25 V resulting a failure of selective determination of DA, UA and AA at a bare GC. However, at 3-TPA/GC (Fig. 3(b)), the anodic peak potentials of AA, DA and UA were -0.01, 0.22 and 0.38 V, respectively. For the mixture of DA, UA and AA, the anodic peak separations (ΔE_{pa}) were 0.16 V (between UA and DA), 0.23 V (between DA and AA), and 0.39 V (between UA and AA) which were large enough to detect DA, UA and AA separately. Moreover, when 3-TPA/GC was immersed in water for a week or used for several times, we found that the oxidation peaks of DA and UA were almost not changed, indicating that 3-TPA/GC has good stability.

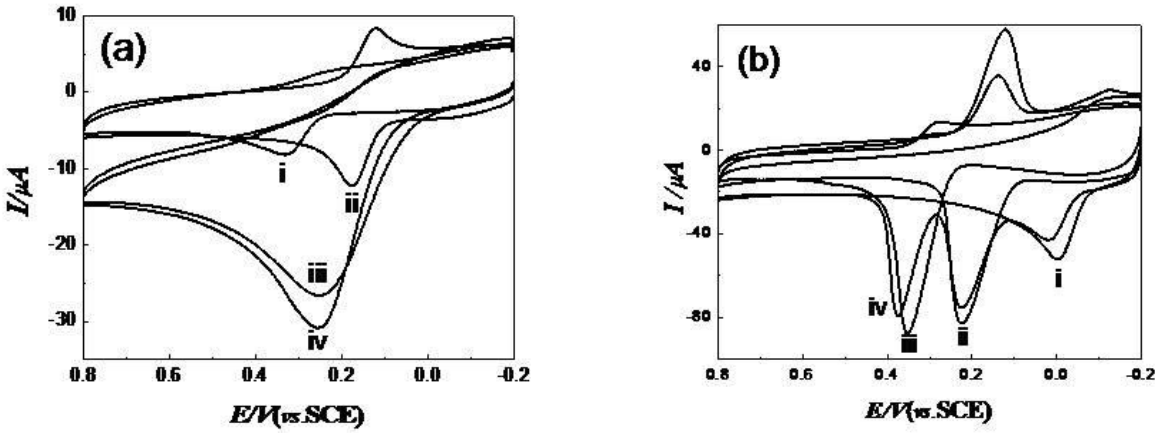

Figure 3. Cyclic voltammograms of AA(i), DA(ii), UA(iii), and a DA/UA/AA mixture(iv) at a bare GC (a) and 3-TPA/GC (b) in pH 6.86 PBS.

3.4. Simultaneous detection of DA and UA

When DA and UA mixtures were simultaneously determined, the oxidation potentials of DA and UA were 0.16 and 0.31 V, respectively. In other words, 3-TPA/GC could detect DA and UA selectively with the method of differential pulse voltammetry. As shown in figure 4, various concentrations of DA and UA exhibited good DPV response, indicating that the responses of DA and UA at 3-TPA/GC were relatively concentration-independent. The dependences of the peak current measured by employing DPV in pH 6.86 PBS were in a linear range from 5.2×10^{-7} M to 8.35×10^{-6} M for DA and from 1.04×10^{-6} M to 1.67×10^{-5} M for UA, respectively (inset of Fig.4). The detection limits (S/N=3) of 2.6×10^{-7} M and 5.2×10^{-7} M were obtained for DA and UA, respectively. This value is favorably compared to 0.2 mM by Protiva *et al.* [20] who used GC electrodes modified with N,N-dimethylaniline by electropolymerization.

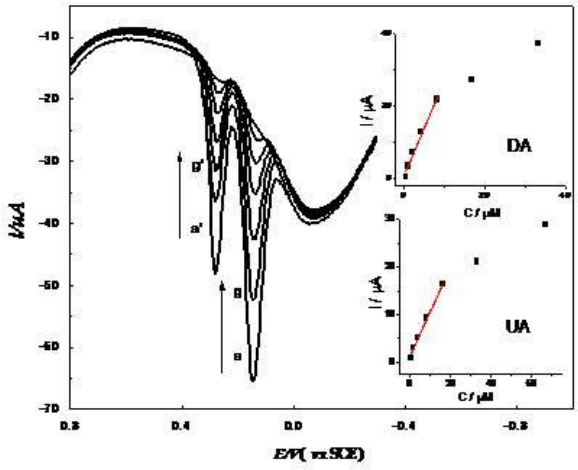

Figure 4. Differential pulse voltammograms of different concentrations of DA/UA mixtures at 3-TPA/GC in pH 6.86 PBS. DA: (a)3.33×10^{-5}M, (b)1.67×10^{-5}M, (c)8.35×10^{-6}M, (d)4.17×10^{-6}M, (e)2.08×10^{-6}M, (f)1.04×10^{-6}M, (g)5.2×10^{-7}M. UA: (a')6.67×10^{-5}M, (b')3.33×10^{-5}M, (c')1.67×10^{-5}M, (d')8.35×10^{-6}M, (e')4.17×10^{-6}M, (f')2.08×10^{-6}M, (g')1.04×10^{-6}M.

3.5. Differential pulse voltammograms of DA and UA in the presence of AA at 3-TPA/GC

We also carefully examined the oxidation currents of unitary (DA), binary (DA, UA) and ternary (DA, UA, AA) analytes at 3-TPA/GC (Fig.5). From Fig.5(b), we discovered that with the increase in concentration of DA, the current peak of DA increased, however, the current peak of UA decreased. That's because the surface of the electrode and the space for reactivity were certain. Besides, as the role of electrostatic interaction, when the concentration of DA increasing, the diffusion velocity of UA to the surface of the electrode decreased. The greater the concentration of DA, the more impact to UA, so the current peak of UA dropped. The oxidation current peak (I_{pa}) was linearly proportional to the correspond concentration of DA. Moreover, as we can see in Fig.5(c), the oxidations of UA, DA and AA were 0.31, 0.16 and -0.01 V, respectively, indicating that 3-TPA/GC

could detect DA and UA simultaneously in the presence of AA. At pH 6.86, because of the existing of electrostastics interaction, 3-TPA/GC attracted DA, and excluded AA and UA [21].

Fig.5(d) shows the comparison of calibration curves of different detecting systems. At the same concentration, the oxidation current of DA in curve (i) was higher than that in curve (ii). At optimal experiment conditions, DA was cationic, and UA, AA were anionic, these ions interacted each other because of electrostatic interaction. This caused the diffusion velocity of DA decreasing, so the oxidation current of DA in curve (ii) was lower than that in curve (i). The oxidation current in curve (ii) was higher than that in curve (iii). Compared the solution in curve (ii) and (iii), we had added AA in the solution of curve (ii), and AA played the role of reduction to the oxidation products of DA, making the conentration of DA on the electrode surface higher than that in curve (iii). So the oxidation current of DA in curve (ii) was higher than that in curve (iii).

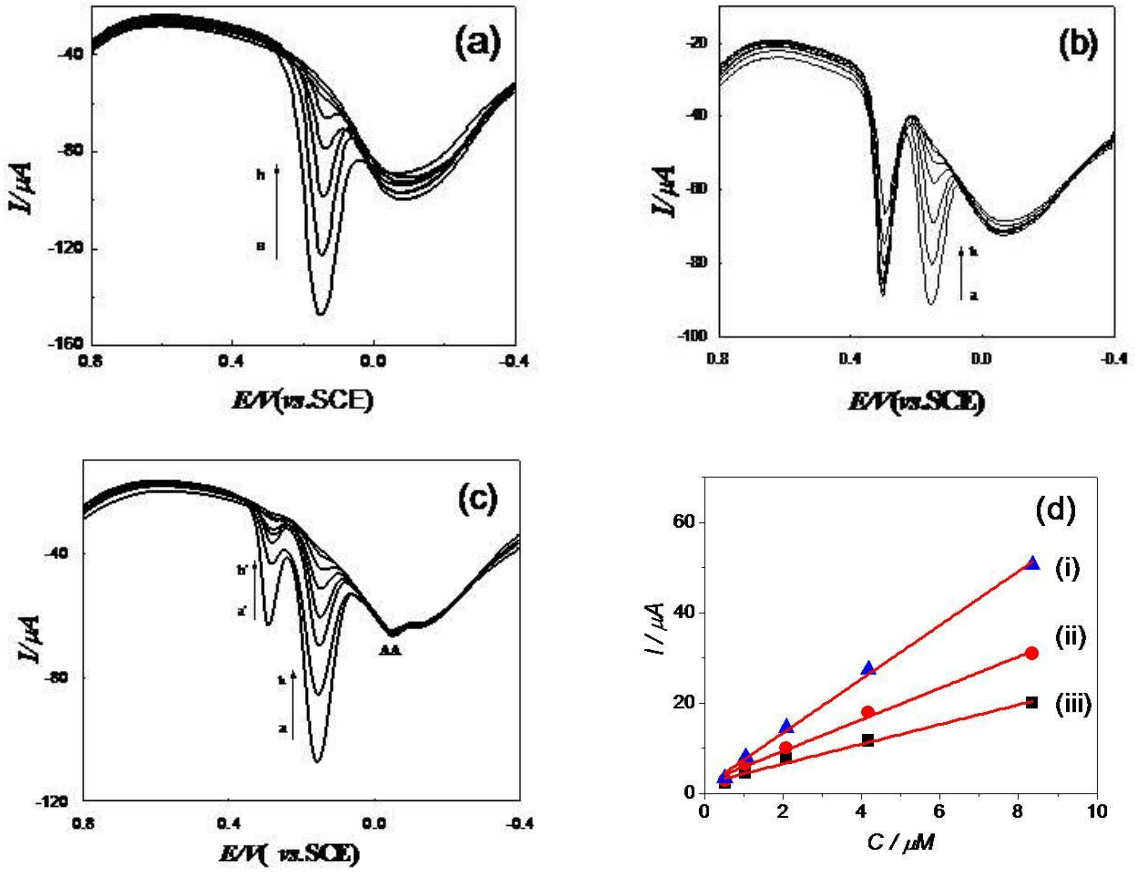

Figure 5. (a) DPVs of different concentrations of DA at 3-TPA/GC in pH 6.86 PBS. DA: (i)3.33×10^{-5}M, (ii)1.67×10^{-5}M, (iii)8.35×10^{-6}M, (iv)4.17×10^{-6}M, (v)2.08×10^{-6}M, (vi)1.04×10^{-6}M, (vii)5.2×10^{-7}M, (viii)2.6×10^{-7}M. (b) DPVs of different concentrations of DA with constant 5.0×10^{-6}M UA at3-TPA/GC in pH 6.86 PBS. DA: (i)5×10^{-5}M, (ii)2.5×10^{-5}M, (iii)1.25×10^{-5}M, (iv)6.25×10^{-6}M, (v)3.12×10^{-6}M, (vi)1.56×10^{-6}M, (vii)7.8×10^{-7}M, (viii)3.9×10^{-7}M. (c) DPVs of different concentrations of DA/UA mixtures with constant 1.0mM AA at 3-TPA/GC in pH 6.86 PBS. DA/UA: (i)5×10^{-5}M/6.67×10^{-5}M, (ii)2.5×10^{-5}M/3.33×10^{-5}M, (iii)1.25×10^{-5}M/1.67×10^{-5}M, (iv)6.25×10^{-6}M/8.35×10^{-6}M, (v)3.12×10^{-6}M/4.17×10^{-6}M, (vi)1.56×10^{-6}M/2.08×10^{-6}M, (vii)7.8×10^{-7}M/1.04×10^{-6}M, (viii)3.9×10^{-7}M/5.2×10^{-7}M. (d) Calibration curves of DA by DPV at a 3-TPA/GC electrode obtained from panel a, b and c.

3.6. Recovery

Table 1. Recovery of DA in spiked human urine samples determined by DPV.

Number	[DA] added (μM)	[DA] founded (μM)	Recovery (R.S.D)
1	3.13	3.45	110.2
2		3.16	101.0
3		2.90	92.7
Mean		3.17	101.3 (8.70)

The detection of DA in spiked human urine samples was conducted by DPV method. The results are shown in Table 1. The recovery and relative standard deviation (R.S.D) values were acceptable, showing that the proposed methods could be efficiently used for the determination of DA in real samples.

4. CONCLUSION

In this work, we had shown that poly(3-Thiophenemalonic acid) film of negatively charged compound formed on an electrode surface could act both as an electrocatalyst for the DA and UA oxidation and as a discriminating layer for DA against AA and UA. Therefore, DA, UA and AA could be determined simultaneously by 3-TPA/GC. Moreover, compared with bare GC, the oxidation current of DA and UA increased significantly at 3-TPA/GC with good reproducibility and stability. The detection limits of 2.60×10^{-7} and 5.20×10^{-7} M for DA and UA, respectively, were obtained by DPV method in pH6.86 PBS. The proposed methods can be applied to the detection of DA in human urine samples.

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