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ORIGINAL PAPER

Selective determination of uric acid in the presence of ascorbic acid at poly(*p*-aminobenzene sulfonic acid)-modified glassy carbon electrode

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Abstract The electrocatalytic behavior of uric acid has been investigated with a glassy carbon electrode modified with *p*-aminobenzene sulfonic acid through electrochemical polymerization. This resulting electrode shows an excellent electrocatalytic response to uric acid and ascorbic acid, with a peak-to-peak separation of 0.267 V in a 0.1 mol L^{-1} phosphate buffer solution (PBS) at pH 7.0. These results indicate that the proposed electrode can eliminate the serious interference of ascorbic acid, which coexists with uric acid in body fluids. Differential pulse voltammetry (DPV) was used for detecting uric acid with selectivity and sensitivity. The anodic peak current of uric acid was proportional to its concentration in the range of 1.2×10^{-7}–8.0×10^{-4} mol L^{-1}, with a detection limit of 4.0×10^{-8} mol L^{-1}. The proposed method has been applied with satisfactory results to the determination of uric acid in human urine without any pretreatment.

Keywords Glassy carbon electrode · Electro-polymerization · Uric acid · Ascorbic acid · Selective determination

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1 Introduction

Uric acid (2, 6, 8,-trihydroxypurine, UA) is a principal final product of purine metabolism in the human body. Abnormal levels of UA are symptoms of several diseases such as gout, hyperuricemia, and Lesch-Nyan disease [1, 2]; therefore, an accurate determination of its concentration in the human body is very important for patients' health. Usually colorimetric, enzymatic, and electrochemical techniques have been used to determine the concentration of UA. However, the colorimetric method cannot accurately determine the concentration of UA [3], and the enzymatic method is very expensive and no relatively high detection limit can be achieved using this method [4]. Electrochemical analytical methods for UA have received much interest because they are more selective, less expensive, less time consuming and can potentially be applied to real-time determinations in vivo [5–7]. However, the major obstacle in the determination of this compound is the interference from other electroactive constituents, such as ascorbic acid (AA), which oxidizes at the same potential as UA on different types of carbon electrodes. Various functionalized electrodes have been used to separate the overlapping anodic peak of UA and AA, such as cobalt (II) tetrakisphenylporphyrin (Co(II)TPP) film [8], enzyme-based electrode [9], nanoparticle-modified electrode [10], and ion-exchange membranes that are widely used to coat electrodes like Nafion film [11]. Although these voltammetric techniques are more selective, less costly and less time consuming than those based on colorimetry or spectrophotometry, a sensitive and selective determination of UA is still needed because of its clinical significance.

Due to their good stability, reproducibility, homogeneity in electrochemical deposition, strong adherence to electrode surfaces, and their many active sites, polymer-modified electrodes have attracted much attention in the recent

years [12–16]. Electropolymerization is a good approach to immobilize polymers onto electrodes by adjusting the electrochemical parameters that control the film thickness, permeation, and charge-transport characteristics. As compared with the metal electrodes, glassy carbon electrodes (GCE) have been widely used because of their biocompatibility with tissue, low residual current over a wide potential range, and minimal propensity to show deteriorated response as a result of electrode fouling [17, 18]. A poly(*p*-aminobenzene sulfonic acid)-modified GCE (ABSA/GCE) has been prepared to detect dopamine [19]. The selective determination of UA in the presence of AA with this kind of modified electrode has not been reported.

In this article, we applied an ABSA/GCE to investigate the electrochemical behavior of UA and AA. The proposed electrode showed well-defined electrocatalytic response to UA and AA in a 0.10 mol L^{-1} phosphate buffer solution (PBS) at pH 7.0. The proposed method has been applied to the selective detection of UA in human urine with satisfactory results.

2 Experimental

2.1 Apparatus and chemicals

CHI660C electrochemical workstation (CHI, China) was used for the electrochemical measurements. A conventional three-electrode cell was employed with a bare GCE or ABSA/GCE (3.0 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode.

Uric acid and ascorbic acid were purchased from Alfa Aesar and used without further purification. Para-aminobenzene sulfonic acid was purchased from Wulian Chemical Industry Factory (Shanghai, China). Other chemicals were of analytical grade. All the solutions were prepared from redistilled water. Prior to each experiment, solutions were purged with purified nitrogen for 15 min to remove oxygen. All the experiments were carried out at room temperature.

2.2 Preparation of ABSA/GCE

The preparation of a *p*-aminobenzene sulfonic acid-modified glassy carbon electrode is based on reference [19]: The bare GCE was polished with 0.3 and 0.05 μm Al_2O_3 slurry on emery paper and chamois leather, respectively, rinsed with redistilled water, and ultrasonicated in nitric acid (1:1), acetone, and redistilled water, each for 10 min, respectively. After being cleaned, the electrode was immersed in a 0.1 mol L^{-1} PBS containing 1.0×10^{-3} mol L^{-1} ABSA and was conditioned by cyclic sweeping between −1.5 and +2.5 V at 100 mV s^{-1} for 10 scans (when the polymerization cycles exceed 10 scans, the electrochemical response of UA will decrease at the ABSA/GCE). Finally, the modified electrode was activated by cyclic voltammetry from −1.0 to +1.0 V in a 0.1 mol L^{-1} PBS at pH 7.0.

3 Results and discussion

3.1 Characterization of ABSA/GCE

The electrochemical impedance spectra (EIS) of the electrodes can provide information on the impedance changes of the electrode surface during the modification process. In EIS, the radius of EIS equals the electron transfer resistance. This resistance controls the electron transfer kinetics of the redox probe at the electrode interface. Figure 1 depicts the EIS of the change of the electrode surface status with the formation procedure, shown as the Nyquist plot (Z"im versus Z're). Curve a is the EIS of the bare GCE revealing an almost straight line characteristic of diffusion controlled by the electrochemical process. On the modified electrode, a semicircular arch appears at the high-frequency region, while a straight remains at the low-frequency region (Fig. 1, curve b), indicating that the formed ABSA film introduced a barrier to the interfacial electron transfer. Due to the pinhole lacuna of the film, the probe can touch the electrode surface. Thus, the electrochemical reaction of $[Fe(CN)_6]^{4-/3-}$ probed at the modified electrode is controlled by both diffusion and electrochemical reactions. The impedance change of the modification

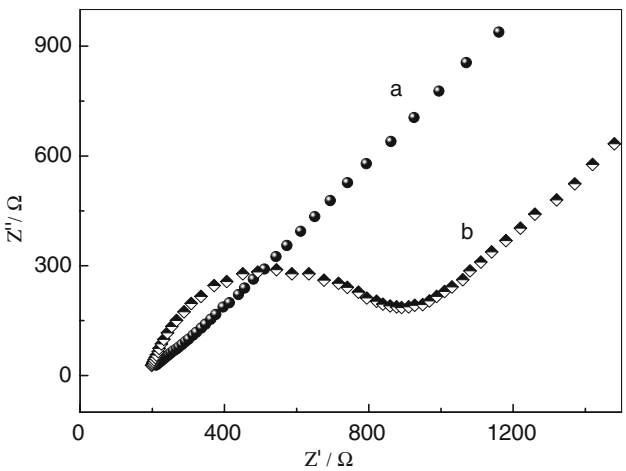


Fig. 1 Nyquist plots of bare GCE (**a**) and ABSA/GCE (**b**) in 5.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ + 0.1 mol L^{-1} KNO_3, with an AC voltage of 5 mV amplitude in a frequency range from 0.001 Hz to 100 kHz under a 0.04 V open circuit potential

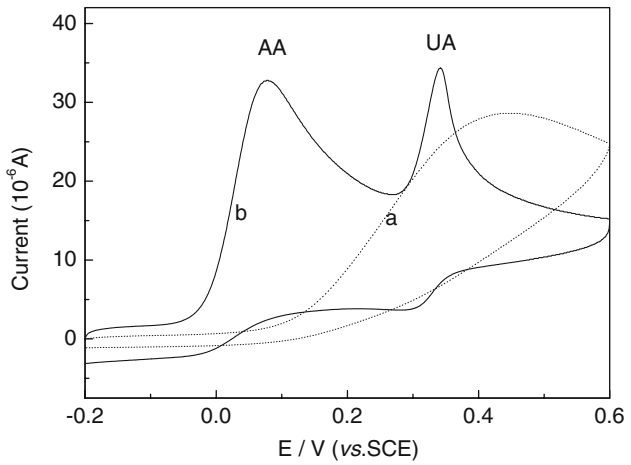


Fig. 2 CV curves of 2.0×10^{-5} mol L^{-1} UA and 1.0×10^{-3} mol L^{-1} AA in 0.1 mol L^{-1} PBS on bare GCE (**a**) and ABSA/GCE (**b**), scan rate: 100 mV s^{-1}, rest time: 2 s

process demonstrates that ABSA has been attached to the electrode surface.

3.2 Electrocatalytic oxidation of UA and AA

The cyclic voltammetry (CV) study of UA and AA on the modified electrode was performed in a 0.1 mol L^{-1} PBS at pH 7.0 (shown in Fig. 2). Curve (a) shows the CV record of the bare GCE in a 0.1 mol L^{-1} PBS containing a mixture of 2.0×10^{-5} mol L^{-1} UA and 1.0×10^{-3} mol L^{-1} AA. UA and AA exhibit an overlapped and broad anodic peak with a peak potential at 0.419 V, which indicates that the oxidation of UA and AA at GCE is irreversible and undergoes a sluggish electron-transfer kinetic. However, on ABSA/GCE, two anodic peaks at 0.341 and 0.074 V appear, which are attributed to the oxidation of UA and AA, respectively (Fig. 2, curve b), and the anodic currents of UA and AA at the modified electrode are 8- and 2.5-fold larger than that of the bare GCE, respectively. This modified electrode exhibits potent and persistent electron-mediating behavior followed by well-separated oxidation peaks with a separation of 0.267 V toward UA and AA, which is good enough for accurate and selective determination of UA in the presence of AA.

The probable electrocatalytic oxidation of UA at ABSA/GCE [20] is

The relationship between the anodic peak current and the scan rate was investigated. The anodic peak current of UA is proportional to the square root of the scan rate in the range of 20 to 200 mV s^{-1}, and the linear progress equation is obtained as

$$Ipa(\mu A) = (0.089 \pm 0.0012) + (2.24 \pm 0.053)v^{1/2}(\text{mV s}^{-1}), \quad (2)$$

with a correlation coefficient of 0.998, which indicates that the oxidation process of UA at the ABSA/GCE is controlled by diffusion [21].

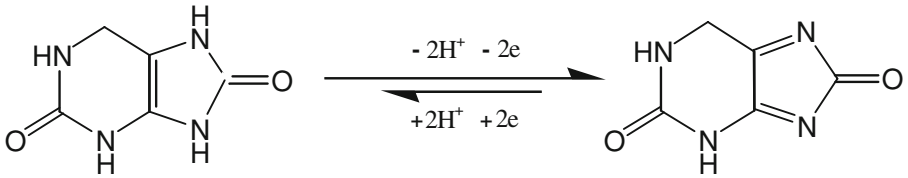
3.3 Effect of pH and stability of ABSA/GCE

Acidity of the solution is a very important impact factor on the electrochemical reaction. Cyclic voltammetry was carried out to characterize the effect of the pH value of the solution on the electrochemical behavior of UA and AA at the ABSA/GCE. Figure 3a shows the relationship between anodic peak potentials of the two acids and pH of the solution. The anodic peak potential (E_{pa}) is proportional to the pH of the solution in the range of 5.0 to 9.0. The linear regression equation of UA is thus obtained as

$$E_{pa}(\text{V}) = (0.748 \pm 0.014) - (0.0567 \pm 0.0019)\text{pH}, \quad (3)$$

with a correlation coefficient of 0.997, indicating that the electrode process is an equal proton–electron transfer. Figure 3b shows that the anodic peak current increases with the increase in the pH of the solution until it reaches 7, and then, it decreases rapidly with the increase in the pH of the solution. Hence, pH 7.0 was chosen for the electrochemical detection of UA and AA.

The stability and reproducibility of the ABSA/GCE was also examined. A series of 50 differential pulse voltammetry (DPV) measurements was examined using 2.0×10^{-5} mol L^{-1} UA. The relative standard deviation was found to be less than 2%, indicating that the modified electrode had good repeatability. The CV experiments were carried out using the modified electrode once a day under the same operation conditions, and the modified electrode was stored in the 0.1 mol L^{-1} PBS at pH 7.0 after every experiment. In one month, the anodic peak current of UA scarcely changed, and after 4 weeks, the anodic current of UA at the ABSA/GCE reached 91% of the current initial



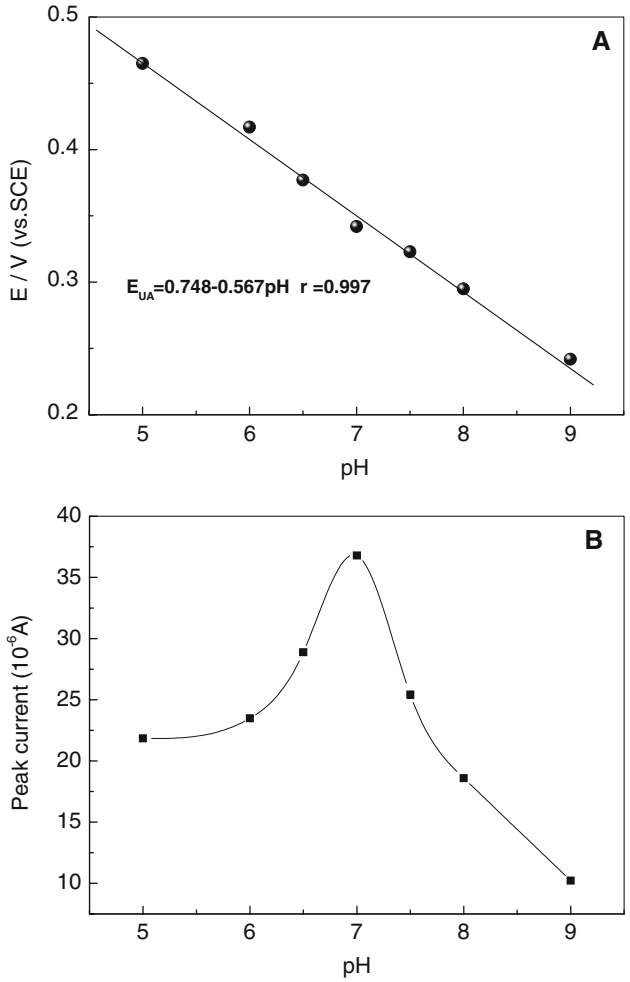


Fig. 3 Effect of pH of the solution on peak potential (**a**) and peak current (**b**) of UA (concentration: 2.0×10^{-5} mol L^{-1})

value, showing that the modified electrode had good stability.

3.4 DPV determination of UA and AA

The DPV method is normally used for the determination of samples because of its high sensitivity [22, 23]. The determination of UA or AA was also performed with the DPV method using the ABSA/GCE. In order to test the versatility of the ABSA/GCE electrode, DPV curves were recorded at different concentrations of UA at the ABSA/GCE. Figure 4a shows the DPV curves at different concentrations of UA at the ABSA/GCE. Clearly, the anodic peak current increases linearly with UA concentration ranging from 1.2×10^{-7} to 8.0×10^{-4} mol L^{-1}. The linear regression equation was

$$I_{UA}(\mu A) = (2.033 \pm 0.043) + (0.487 \pm 0.016) C_{UA}(\mu \text{mol } L^{-1}), \quad (4)$$

with a detection limit of 4.0×10^{-8} mol L^{-1} (S/N = 3) and a correlation coefficient of 0.998. In order to test the interference of AA with the DPV method, DPV curves are recorded at the ABSA/GCE at different concentrations of AA with a constant concentration of 5.0×10^{-5} mol L^{-1} UA (Fig. 4b). Data show that with the increase in AA concentration, the oxidation current of UA remains constant, demonstrating that AA does not affect the accurate determination of UA. The oxidation current of AA is linear with its concentration in the range of 6.0×10^{-6} to 9.0×10^{-3} mol L^{-1}, and the linear equation was obtained as

$$I_{AA}(\mu A) = (6.38 \pm 0.113) + (12.06 \pm 0.62) C_{AA}(\text{mmol } L^{-1}) \quad (5)$$

with a detection limit of 2.0×10^{-6} mol L^{-1} (S/N = 3), and the correlation factor of the straight line is 0.998. These

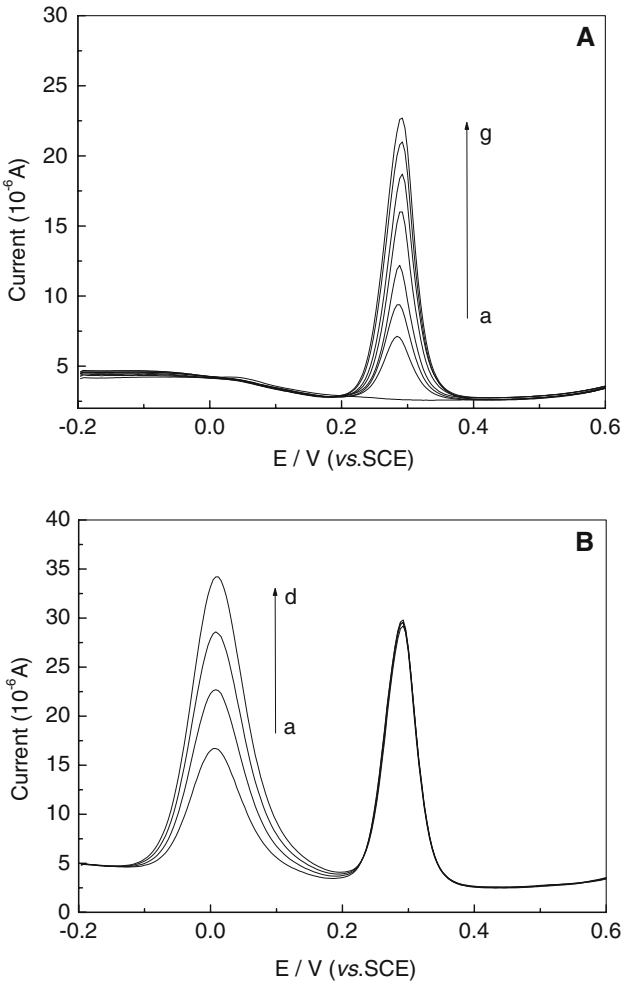


Fig. 4 DPV curves of ABSA/GCE in 0.10 mol L^{-1} PBS (pH 7.0) containing: (**a**) different concentrations of UA (a → g: 0, 5, 10, 15, 25, 40, 50, 60 × 10^{-6} mol L^{-1}); (**b**) 5.0×10^{-5} mol L^{-1} UA with different concentrations of AA (a → h: 0.5, 1.0, 1.5, 2.0 × 10^{-3} mol L^{-1})

results illustrate that the proposed ABSA/GCE can also be applied to the selective determination of AA in the presence of UA. We have also studied the interference of dopamine, which has a similar oxidation potential at the bare GCE. Data showed that dopamine has a peak-to-peak separation of 106 mV with UA, indicating that the proposed electrode can also avoid the interference of dopamine in the physiological system.

3.5 Analysis of urine samples

The proposed ABSA/GCE was applied for the determination of UA and AA in human urine. Figure 5 shows the DPV curves of the ABSA/GCE in the absence (Fig. 5, curve a) and presence (Fig. 5, curve b) of human urine without any pretreatment in a 0.1 mol L^{-1} PBS at pH 7.0. It is found that only the anodic peak of UA is observed, with an estimated concentration of 8.7×10^{-6} mol L^{-1}. A standard addition method was used for the calculation of UA concentration [24]. Table 1 presents the results obtained from five parallel measurements. The results confirm that the proposed method can be used efficiently for the quantitative analysis of UA. A comparison of the proposed method with the recently reported methods listed in Table 2 indicates that ABSA/GCE is superior to the existing electrodes in its working concentration range, detection limit, stability, and ability to effectively determine UA in human urine.

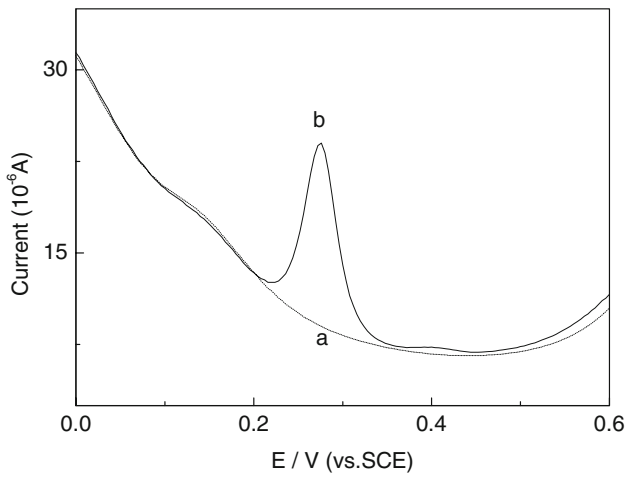


Fig. 5 DPV curves of ABSA/GCE in 10 mL PBS (pH 7.0) in the absence (**a**) and presence (**b**) of 50 μL of human urine without any pretreatment

4 Conclusion

Sulfonic acid was polymerized onto a glassy carbon electrode to fabricate a poly (*p*-aminobenzene sulfonic acid)-modified glassy carbon electrode. A peak-to-peak separation of uric acid and ascorbic acid oxidation potentials was obtained, which was about 0.267 V at the proposed electrode, illustrating that the proposed electrode can be used to accurately determine uric acid in the presence of high concentration of ascorbic acid. This modified electrode shows satisfactory selectivity, good sensitivity, and stability for the electrocatalytic oxidation of biomolecular uric acid. The proposed method has been applied to the selective determination of uric acid in human urine of healthy

Table 1 UA in human urine ($n = 5$)

Sample	UA detected (μmol L^{-1})	UA added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	RSD (%)
Urine 1	8.8 ± 0.11	10	10.1 ± 0.23	101	1.7
Urine 2	8.7 ± 0.13	20	19.6 ± 0.35	98	1.4
Urine 3	8.7 ± 0.16	30	30.4 ± 0.41	101	1.6

Table 2 Comparison of working range and detection limit of ABSA/GCE with reported methods

Serial	Reference	Detection method	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	Stability (day)	Analysis of real sample
1	[25]	Amperommetry	5.0×10^{-6}–1.5×10^{-4}	2.0×10^{-6}	–	No
2	[26]	CV	1.97×10^{-5}–4.0×10^{-3}	–	30	No
3	[27]	Amperommetry	1.0×10^{-6}–1.0×10^{-4}	5.0×10^{-8}	–	Urine and blood
4	[28]	DPV	8.0×10^{-7}–6.0×10^{-4}	5.0×10^{-7}	7	No
5	[29]	DPV	2.0×10^{-5}–6.6×10^{-4}	5.0×10^{-7}	21	No
6	This work	DPV	1.2×10^{-7}–8.0×10^{-4}	4.0×10^{-8}	30	Urine

people with satisfactory results. The reliability and stability of the modified electrode are favorable for its application to the routine analysis of uric acid and ascorbic acid in clinical test.

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