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ORIGINAL PAPER

Simultaneous determination of uric acid and ascorbic acid at the film of chitosan incorporating cetylpyridine bromide modified glassy carbon electrode

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Abstract A glassy carbon electrode (GCE) modified with the film composed of chitosan incorporating cetylpyridine bromide is constructed and used to determine uric acid (UA) and ascorbic acid (AA) by differential pulse voltammetry (DPV). This modified electrode shows efficient electrocatalytic activity and fairly selective separation for oxidation of AA and UA in mixture solution. UA is catalyzed by this modified electrode in phosphate buffer solution (pH 4.0) with a decrease of 80 mV, while AA is catalyzed with a decrease of 200 mV in overpotential compared to GCE, and the peak separation of oxidation between AA and UA is 260 mV, which is large enough to allow the determination of one in presence of the other. Under the optimum conditions, the anodic peak currents (I_{pa}) of DPV are proportional to the concentration of UA in the range of 2.0×10^{-6} to 6.0×10^{-4} M, with the detection limit of 5.0×10^{-7} M at a signal-to-noise ratio of 3 (S/N=3) and to that of AA in the range of 4.0×10^{-6} to 1.0×10^{-3} M, with the detection limit of 8.0×10^{-7} M (S/N=3).

Keywords Cetylpyridine bromide · Chitosan · Simultaneous determination · Ascorbic acid · Uric acid

Introduction

As the primary end product of purine metabolism, uric acid (UA) is a greatly important analyte in clinical field. In a healthy human being, the typical concentration of UA in urine is in the millimolar range (around 2 mM), whereas that in blood is in the micromolar range (120–450 μM) [1, 2]. Abnormal levels of UA are associated with a number of clinical situations such as gout, hyperuricemia, Lesch–Nyhan syndrome, cardiovascular and kidney diseases, etc. [3, 4]. Therefore, the determination of UA with a simple method is essential because it serves a marker for the detection of the above diseases [5]. Ascorbic acid (AA) is a vital vitamin in the diet of humans and is present in mammalian brain along with several neurotransmitter amines. AA has been used for the prevention and treatment of common cold, mental illness, infertility, cancer, and AIDS [6]. As UA and AA coexist in biological fluids such as blood and urine, it is important to selectively detect UA in the presence of AA conveniently in routine assay [7]. However, not only the electrooxidation of UA and AA at inert metal electrodes requires high overpotentials but also the oxidation potentials of UA and AA are too close to be separated at common electrodes [8, 9]. To solve this problem, various approaches such as colorimetric, enzymatic, and electrochemical techniques have been widely used to determine the concentration of UA, with reducing the interference of AA [1, 2, 10], but they also have some shortcomings such as complicated operation, high cost, higher determination limit, and low selectivity. The application of chemically modified electrodes (CMEs) in electroanalysis offers many advantages such as lowering redox overpotentials, strengthening electron transfer rate, enhancing detection sensitivity, and improving selectivity [7, 11, 12]. Thus, a number of CMEs have been developed to separate the electrochemical response of AA and UA [7, 11–24]. Until now, there is still an expanding demand for the development of simple, reliable, and efficient sensors with enhanced characteristics for effective sensing of AA and UA simultaneously.

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Surfactants, a kind of amphiphilic molecules with a hydrophilic head on one side and a long hydrophobic tail on the other side, can significantly improve the electrochemical response to the determining substance, leading to improve its analysis of sensitivity and selectivity. In addition, surfactant film electrodes similar to the structure of the lipid membrane with bi-layer structure have been widely applied in electrochemistry. Peng at el. have reported that cetylpyridine bromide has been used to determine AA and UA in the micelles [25]. Han et al. have studied the electrochemical behaviors of AA and UA at CPB in situ modified carbon paste electrodes [26].

Chitosan has also attracted much attention due to its non-toxic nature, antibacterial and anti-oxidative activity, film-forming property, biocompatibility, and biodegradability [27]. In our previous work, chitosan has been used to prepare CPB/chitosan composite film on the GCE to determine simultaneously dopamine and AA [28], and the results are satisfactory.

In this paper, a chitosan/CPB film modified electrode has been constructed for effective separation of AA and UA in 0.1 M phosphate buffer solution (PBS, pH 4.0). The modified electrode has exhibited high electrocatalytic activities toward the oxidation of AA and UA and displayed good voltammetric peak separation between AA and UA. Based on the different electrocatalytical activity of the modified electrode toward UA and AA, simultaneous determination of them in routine analysis has been achieved.

Experimental

All reagents such as UA (Sigma-Aldrich), AA (Sigma-Aldrich), CPB (SCRC), and chitosan (SCRC) were used as received without further purification. All chemicals were of analytical grade. The PBS (0.10 M) with various pH values were prepared by mixing stock standard solution of KH_2PO_4 and $K_2HPO_4{\cdot}3H_2O$. Freshly prepared solution of UA and AA were used in all experiments. All solutions were prepared with doubly distilled water. The experiment was carried out at room temperature.

Various concentrations of CPB together with 0.5% (*w*/*w*) of chitosan according to CPB/chitosan =2:1 (*v*/*v*) were prepared. Then, 5 μl of the mixed solution was dropped on the electrode surface and dried at room temperature.

Electrochemical experiments were carried out using a CHI-660B electrochemical workstation (CHI, China). A conventional three-electrode system, consisting of chitosan/CPB film modified glassy carbon working electrode (3.0 mm in diameter), a saturated calomel reference electrode, and a platinum wire counter electrode, was employed. All experimental solutions were deoxygenated by purging with nitrogen gas prior to the start of each experiment.

The chitosan/CPB/GCE, CPB/GCE, and bare GCE were characterized by JEOL Field Emission Scanning Electron Microscope Instrument (JSM-6700F, 15 kV).

Results and discussion

Characterization of chitosan/CPB modified electrode

The morphology of the chitosan/CPB/GCE, CPB/GCE, and bare GCE can be obviously observed by scanning electron microscopy (SEM; Fig. 1). Figure 1b shows that CPB was dispersed randomly without uniform on the GCE, and the bare GCE can be seen uncovered compared to Fig. 1a. However, when using chitosan/CPB to modify GCE (Fig. 1c), there is a uniform film composed of many beads (about 1–2 μm diameter) dispersed regularly on GCE due to the good film-forming property of chitosan [27].

Optimization of CPB concentration for differential pulse voltammetry response of UA and AA oxidation

The variation of the peak potentials (E_{pa}) and variation of the peak currents (I_{pa}) of both AA (2.0×10^{-4} M) and UA (1.0×10^{-4} M) have been examined at the chitosan/CPB film modified electrode with various concentrations of CPB in 0.10 M PBS (pH 4.0) supporting electrolyte. The results have shown that the E_{pa} of both AA and UA shifted toward less positive and the *I*pa values both increased abruptly when the concentrations of CPB changed from 0.0 to 5.0×10^{-3} M, then changed smoothly in the concentration range of 5.0 to 8.0×10^{-3} M. Obviously, CPB can effectively catalyze the oxidation of AA and UA. When CPB concentration is 5.0×10^{-3} M, the I_{pa} of both of them reached maximal values, and at the same time, the anodic peaks of UA and AA can be well separated. Hence, 5.0×10^{-3} M CPB was chosen for further experiments.

Oxidation of AA and UA at the chitosan/CPB film modified GCE

Figure 2 depicts the oxidation of AA at chitosan/CPB/GCE (c), chitosan/GCE (b), and bare glassy carbon electrode (GCE, a) in 0.10 M PBS (pH 4.0). It shows that the oxidation of AA took place at 0.39 V at bare GCE, the oxidation overpotential of AA is 0.30 V at chitosan/GCE, while at the chitosan/CPB film modified GCE, the oxidation of AA is at 0.19 V. AA oxidation occurs with a large overpotential at bare GCE, indicating that slow electron transfer rate happens at bare GCE. Such a sluggish

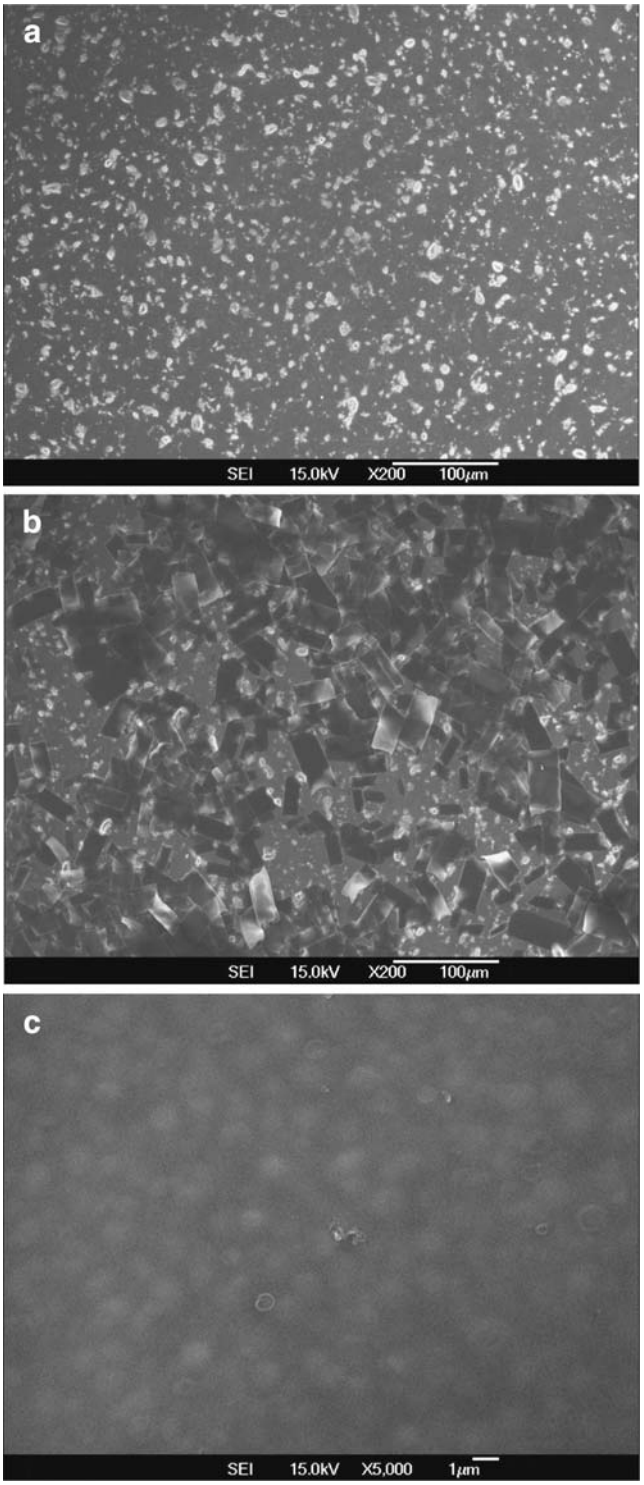

Fig. 1 SEM images of the electrodes: **a** bare GCE, **b** CPB/GCE, and **c** chitosan/CPB/GCE

electron transfer kinetic is mainly due to the electrode fouling caused by the deposition of oxidation of AA [7]. The anodic peak current at the chitosan/CPB film modified GCE is about five times higher than that at bare GCE. The

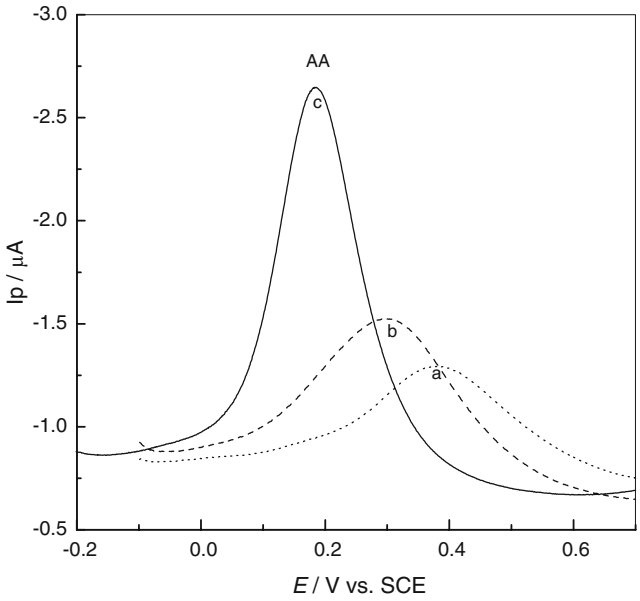

Fig. 2 DPVs of 0.10 M PBS (pH 4.0) in the presence of 2.0×10^{-4} M (*AA*) at different electrodes: *a* bare GCE, *b* chitosan/GCE, *c* chitosan/CPB/GCE

great increment of the currents and a large negative shift (by 200 mV) of the potential is mainly ascribed to the catalytical effect of CPB. It is believed that the electrostatic interaction between the cation surfactant CPB and anionic AA essentially facilitates the oxidation of AA.

The oxidation of UA at bare GCE (a), chitosan/GCE (b), and chitosan/CPB/GCE (c) in 0.10 M PBS (pH 4.0) is shown in Fig. 3. UA has a pK_a of 5.75 and in the

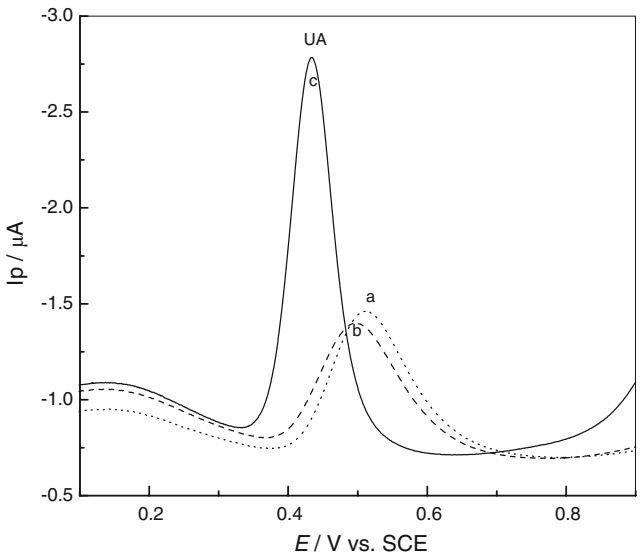

Fig. 3 DPVs of 0.10 M PBS (pH 4.0) in the presence of 1.0×10^{-4} M UA at different electrodes: *a* bare GCE, *b* chitosan/GCE, *c* chitosan/CPB/GCE

environment of blood and normal tissues with pH 7.34. UA exists in its anionic form (urate) [29]. At bare GCE, the oxidation of UA occurs at 0.51 V and the oxidation of UA at 0.49 V at chitosan/GCE, while at the chitosan/CPB/GCE, the oxidation of UA occurs at 0.43 V. The enhanced current and the negative shift (80 mV) of oxidation potential may be due to a favorable electrostatic attraction between anionic UA and CPB on the electrode surface, which changes the overpotential of the electrode and alters the electron transfer rate as well. It is evident that the electron transfer rate is enhanced, and the modified electrode shows effective electrocatalysis for the oxidation of UA.

Effect of pH on the oxidation of AA and UA in mixture

For UA and AA determination, the pH effect on DPV signals at the chitosan/CPB/GCE has been examined. The anodic peak potential for the oxidation of AA and UA shift toward negative direction with the increase of pH, showing that the protons have taken part in electrode processes. And, the peak currents of both AA and UA increase with the solution pH increase until pH 4.0.

The effect of pH on the response AA and UA mixture at the chitosan/CPB film modified GCE has been investigated using DPV technique in various pH of PBS (3.0, 4.0, 5.0, and 6.0) containing AA (2.0×10^{-4} M) and UA (1.0×10^{-4} M). The results are shown in Fig. 4. It can be seen that both of the peak currents of DPV for AA and UA increase when pH of the PBS vary from 3.0 to 4.0, while the peak current weaken and weaken when the pH vary from 4.0 to 6.0, respectively. Especially, the peak is apparently separated between AA and UA ($\Delta E_p = E_{p_{UA}} - E_{p_{AA}}$) when the peak current of AA and UA obtained their maximum at pH 4.0. Thus, 0.10 M PBS (pH 4.0) was chosen as the supporting electrolyte in following experiments.

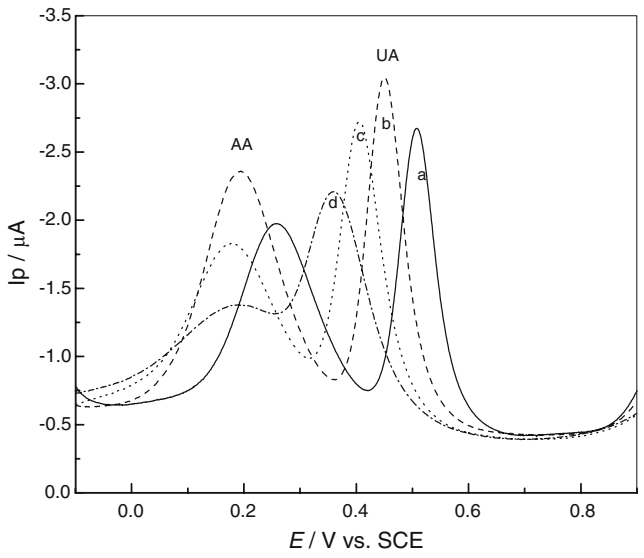

Fig. 4 DPVs of the chitosan/CPB film modified electrode in PBS containing 2.0×10^{-4} M AA and 1.0×10^{-4} M UA with different pH values: *a* 3.0, *b* 4.0, *c* 5.0, *d* 6.0

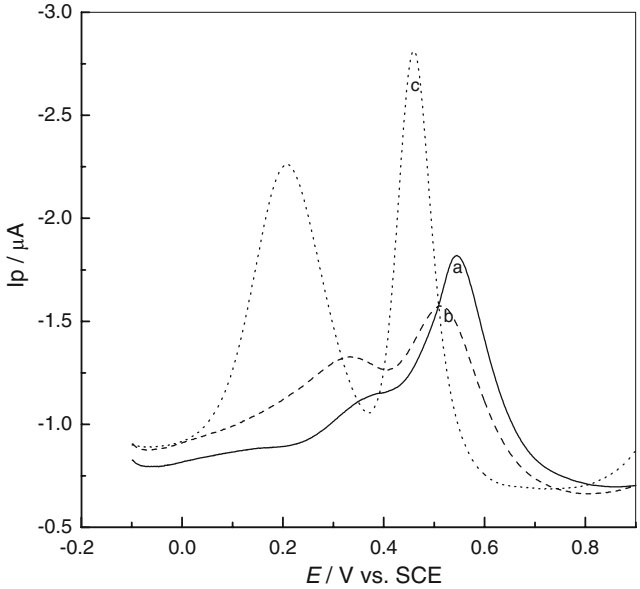

Fig. 5 DPVs of 0.10 M PBS (pH 4.0) in the presence of (1.0×10^{-4} M) UA and (2.0×10^{-4} M) AA at different electrodes: *a* bare GCE, *b* chitosan/GCE, *c* chitosan/CPB/GCE

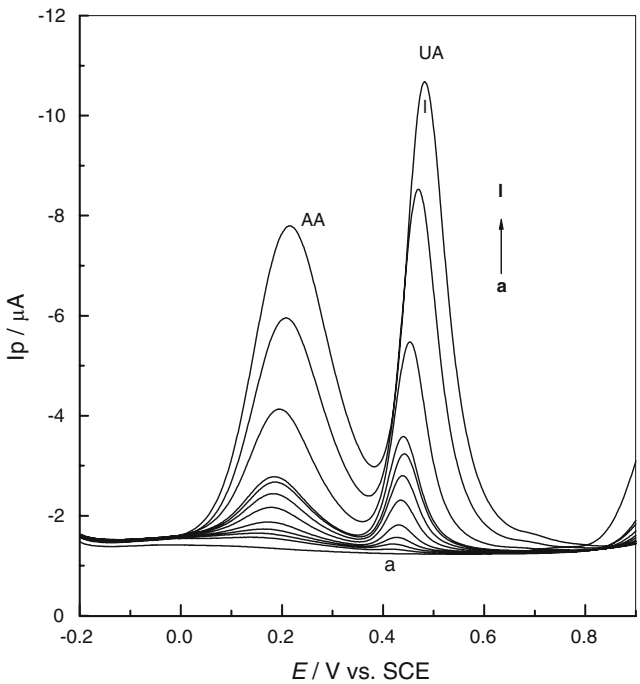

Fig. 6 DPVs of the chitosan/CPB film modified electrode in 0.10 M PBS (pH 4.0) with different concentrations of UA: (from the bottom to the top) *a* 0.0, *b* 2.0×10^{-6}, *c* 5.0×10^{-6}, *d* 1.0×10^{-5}, *e* 2.0×10^{-5}, *f* 4.0×10^{-5}, *g* 6.0×10^{-5}, *h* 8.0×10^{-5}, *i* 1.0×10^{-4}, *j* 2.0×10^{-4}, *k* 4.0×10^{-4}, *l* 6.0×10^{-4} M, and the AA concentrations were double of concentrations of UA

DPV technique for simultaneous determination of AA and UA

UA and AA coexist in many samples. In order to detect them simultaneously, the electrochemical behaviors of mixture of UA and AA at the chitosan/CPB/GCE has been investigated.

Figure 5 shows the DPV of AA and UA coexisting in 0.10 M PBS at the bare (a), chitosan (b), and chitosan/CPB (c) film modified GCEs. As shown, the bare electrode could not separate the responses of UA and AA and a broad single peak occurs at a potential different from the individual oxidation potentials of AA and UA (Fig. 5 a). At chitosan modified electrode, DPV exhibits two ill-defined oxidation peaks corresponding to UA and AA (Fig. 5 b). However, at chitosan/CPB film modified electrode, two well-defined oxidation peaks are obtained corresponding to AA and UA (Fig. 5 c), and the oxidation peaks potential were 0.20 and 0.46 V, respectively, with a peak potential separation of 0.26 V. In this case, the peak potential separation is large enough to determine AA and UA simultaneously.

The electrooxidation processes of AA and UA in the mixture have been investigated when the concentrations of AA and UA were both changed and the DPV of two species were recorded at the chitosan/CPB modified electrode (Fig. 6). The results show that the peak currents of AA and UA increased linearly with the concentration of their own in the range of 4.0×10^{-6} to 1.0×10^{-3} M (I_p=0.7034c+0.0765) for AA and 1.0×10^{-6} to 6.0×10^{-4} M (I_p=1.487c+0.3731) for UA, with correlation coefficients of 0.9969 and 0.9844, respectively.

Stability of the modified electrode

The stability and lifetime of the electrode are also important but for its sensitivity and selectivity in research and application. The stability of the chitosan/CPB modified electrode was assessed after being stored in air at room temperature for some time. The results showed that there was no apparent decrease in current response to AA and UA after almost 15 days. The modified electrode has shown high stability and a long lifetime, which were due to the regular and uniform film of chitosan/CPB at the GCE (Fig. 1c).

Conclusions

The chitosan/CPB film modified GCE has been prepared and applied to simultaneous determination of AA and UA. This modified electrode has exhibited strong electrocatalytic activity toward AA and UA with obvious reduction of overpotential and displayed good voltammetric peak separation between AA and UA. In DPV, the anodic peak potential difference between AA and UA can reach 0.26 V. The chitosan/CPB film modified electrode is suitable and effective for simultaneous detection of AA and UA with good sensitivity and selectivity. Furthermore, the modified electrode has a long lifetime, which is of potential application.

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