Review Article

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Electrochemical Behaviors of Baicalin at an Electrochemically Activated Glassy Carbon Electrode and Its Determination in Human Blood Serum

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Electrochemical behavior of baicalin (BA) at an electrochemically activated glassy carbon electrode (GCE) had been investigated in Britton-Robinson (B-R) buffer solution (pH = 2.87) by cyclic voltammetry (CV) and square wave voltammetry (SWV). The experimental results suggested that the electrode exhibited an electrocatalytic activity toward the redox of BA. The electron transfer coefficient (α) and the standard rate constant (k_s) of BA at the electrochemically activated glassy carbon electrode were calculated. The reaction mechanism was proposed and discussed in this work. Under the selected conditions, the reduction peak current was linearly dependent on the concentration of BA in the range of 5.0×10^{-8} to 3.0×10^{-6} mol L^{-1} ($r = 0.9990$), with a detection limit of 2.0×10^{-8} mol L^{-1}. The relative standard deviation (R.S.D.) for five times successful determination of 1.0×10^{-6} mol L^{-1} baicalin were 2.9%. The proposed method was also successfully applied for the determination of BA in human blood serum.

Keywords: Baicalin; Square wave voltammetry; Adsorptive voltammetric behaviors; Blood serum samples.

INTRODUCTION

Surface treatment of a glassy carbon electrode has been used extensively to improve the electrochemical performance of the electrode. Especially, electrochemical pretreatment has been established as one of the widely used pretreatment techniques to clean and activate electrode surfaces.[1-4] The surfaces of glassy carbon electrodes (GCE) can be oxidized, and thus various kinds of oxygenous groups, such as phenolic, quinoidal, and carboxyl functionalities, can be added on the surfaces. The existences of these active groups can adsorbe aromatic cations and neutral species and catalyze some reactions.[5-7]

Baicalin (7-glucuronic acid-5,6-dihydroxy-flavone, BA, structure shown in Fig. 1), is predominant flavone glucuronide of the commonly used traditional Chinese medicine (TCM) Scutellariae radix. A mass of work indicated that BA exhibited various pharmaceutical effects such as anti-viral,[8] anti-inflammation,[9] anti-oxidation[10,11] and anticancer activities.[12-14] Studies have also revealed that BA can act on the dopamine system, influence cerebral function and also can relieve fever by affecting the central nervous system (CNS).[15,16] To quantify BA in biological samples, in addition to analytical methods based on UV-vis spectrophotometry,[17] thin layer chromatography (TLC),[18] high performance liquid chromatography (HPLC)[19-25] and capillary electrophoresis (CE)[26-28] have been developed. However, these measurements are hindered by low sensitivity, a long retention time, low throughput or require expensive devices. Electrochemical detection of analyte is a very elegant method in analytical chemistry.[29,30] Additional application of electrochemistry includes the determination of electrode mechanisms. And redox properties of organic molecules can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity. To our knowledge, by far a few literatures have been published concerning electrochemical behavior of BA at solid electrode. Recently, Zi-yi Sun and his co-workers investigated BA-DNA interaction through electrochemical and spectroscopic techniques, but did not establish relevant method to determine the BA concentration.[31]

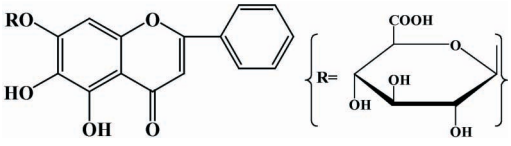

Fig. 1. The chemical structure of BA.

In this approach, a new, simple, fully validated, rapid and selective voltammetric method to directly determine the BA concentration in blood serum samples without any separation steps prior to drug assay is developed. At the same time, our aim of this study was also to establish the

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experimental conditions, to investigate the adsorptive voltammetric behavior and redox mechanism of BA on the surface of an electrochemically activated glassy carbon electrode using cyclic voltammetric techniques.

RESULTS AND DISCUSSION

1. Characterization of the slurry polished and activated GCE

Potassium ferricyanide was selected as a probe to evaluate the performance of an alumina slurry freshly polished GCE and the GCE(ox). Fig. 2 showed the cyclic voltammograms of the alumina slurry freshly polished GCE and the GCE(ox) in 1.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ + 0.1 mol L^{-1} KCl solution, respectively. At the alumina slurry freshly polished GCE, the peak-to-peak potential separation (ΔE_p) was about 0.103 V (Fig. 2a). While at the GCE(ox), the ΔE_p was decreased to 0.085 V, indicating the quick charge transfer kinetics of the electrons on the activated electrode.[32] On the other hand, the peak currents of $K_3[Fe(CN)_6]$ at the GCE(ox) were much bigger than that at the alumina slurry freshly polished GCE. This phenomenon illuminates that $Fe(CN)_6^{3+}/Fe(CN)_6^{2+}$ can reach the surface of the activated electrode more easily than that of the alumina slurry freshly polished GCE.

2. Electrochemical behavior of BA at the GCE(ox)

Fig. 3 showed electrochemical responses of the alumina slurry freshly polished GCE and the GCE(ox) in 2.0×10^{-6} mol L^{-1} BA + Britton-Robinson (B-R) solutions (pH = 2.87), respectively. At the alumina slurry freshly polished GCE and the GCE(ox), BA showed a pair of redox peaks, with oxidation peak potential (E_{pa}) of 0.525 V and reduction peak potential (E_{pc}) of 0.489 V. However, the peak current of BA at the GCE(ox) was much larger than that at the alumina slurry freshly polished GCE, and it was about 10 times of that at the alumina slurry freshly polished GCE. This result testified the GCE(ox) exhibited an electrocatalytic activity toward the redox of BA, which is beneficial to the determination. Thus, in the following discussion, the GCE(ox) is used.

To elucidate the electrode reaction of BA, the repetitive cyclic voltammograms of BA in B-R buffer (pH = 2.87) supporting electrolyte were recorded, see Fig. 4. The peak current decreases with increasing number of cycles, and after the second cyclic sweep, the peak current decreases slightly. This phenomenon may be caused by the adsorption of BA at the GCE(ox).

Effect of scan rate

To further estimate the electrode reaction of BA, the influence of potential scan rate (v) on i_p of 2.0×10^{-6} mol L^{-1} BA at the GCE(ox) was studied by CV at various sweep rates. As shown in Fig. 5A, the peak currents of BA grow with the increasing of scan rates and there are good linear relationships between i_p and v (Fig. 5B). The regression equation is i_{pc} (μA) = 0.456 + 11.074v (r = 0.9997); i_{pa} (μA) = -1.852 – 20.803v (r = 0.9963), indicating the redox process of 2.0×10^{-6} mol L^{-1} BA at the GCE(ox) was adsorption-controlled. According to the above results, it could be

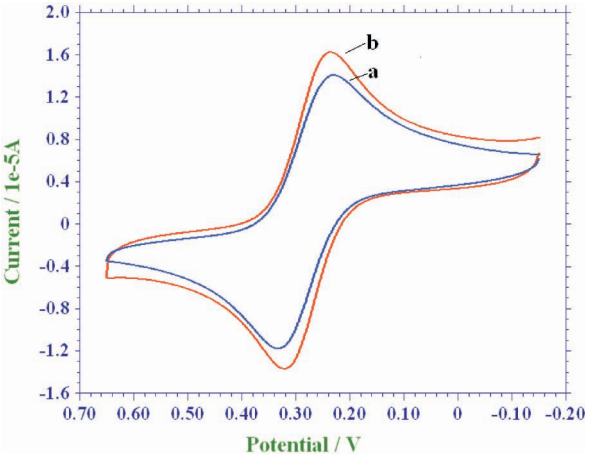


Fig. 2. CV curves of a alumina slurry freshly polished GCE (a) and the GCE(ox) (b) in 1.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ + 0.1mol L^{-1} KCl solution, scan rate 0.1 V s^{-1}.

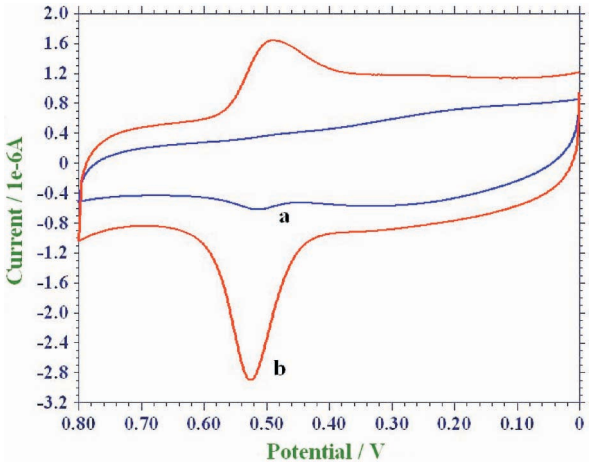


Fig. 3. CV curves of 2.0×10^{-6} mol L^{-1} BA in B-R buffer solution (pH = 2.87) at the alumina slurry freshly polished GCE (a) and the GCE(ox) (b); the accumulation step under open circuit for 300 s, and scan rate 0.05 V s^{-1}.

deduced reasonably that BA was firstly adsorbed on the surface of the electrode, and then the electrode reaction was followed.

The influence of v on the peak potentials of BA was also investigated. With the increase of v the oxidation peak potential was positively shifted, and the reduction peak potential was negatively shifted, indicating that the redox reversibility of BA was impaired. The relationship of the peak potentials with a scan rate was further constructed, which could be used for the calculation of the electrochemical parameters of BA. According to the Laviron's equation:[33]

$$E_{pc} = E^0 - \frac{RT}{\alpha nF}\ln \nu \quad (1)$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF}\ln \nu \quad (2)$$

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nF\nu} - \frac{(1-\alpha)\alpha nF\Delta E_p}{2.3RT} \quad (3)$$

where α is the charge transfer coefficient, n the number of electron transfer, k_s the electrode reaction constant, v the scan rate, E^0 the formal potentials and F the Faraday's constant. A linear relationship between the E_p with the ln$v$ was established, and two straight lines were gotten with two linear regression equations as E_{pa} (V) = 0.03246 ln$v$ + 0.5469 (r = 0.9902) and E_{pc} (V) = -0.03389 ln$v$ + 0.4502 (r = 0.9992), see Fig. 6. According to the Eqs. (1) and (2) the values of α and n were calculated to be 0.49 and 2, respectively. Based on the Eq. (3), the value of k_s was further calculated to be 3.37 s^{-1}, which is much higher than the reported value for baicalein in the DNA Langmuir–Blodgett film modified glassy carbon electrode (0.246 s^{-1}).[34] Baicalein is an also kind of flavonoid, and is similar structure and character with BA. This result indicated that the GCE(ox) possessed a high electrocatalytic activity toward the redox of baicalin.

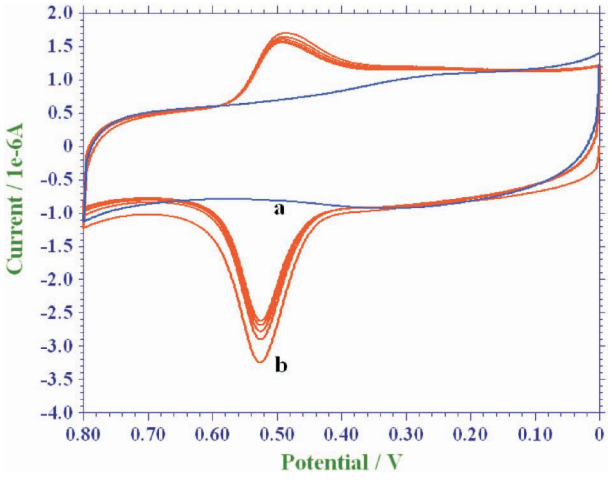


Fig. 4. The voltammograms of the background (a) and the solution (b) containing 2.0 × 10^{-6} mol L^{-1} BA in B-R buffer solution (pH = 2.87) for multi-cycles with scan rate of 0.05 V s^{-1} at the GCE(ox), other experimental conditions are the same as those described in Fig. 3.

Effect of supporting electrolyte and pH value

The types of supporting electrolytes played a key role in the voltammetric responses of BA. The voltammetric behavior of BA at the GCE(ox) was investigated over a wide pH range (2.0-10.0) in HCl, H_2SO_4, phosphate, NaOH, acetate, B-R and borate buffer solution by using cyclic voltammetry. Experiments indicated that only the measurements in acidic media produced stable responses, and the best de-

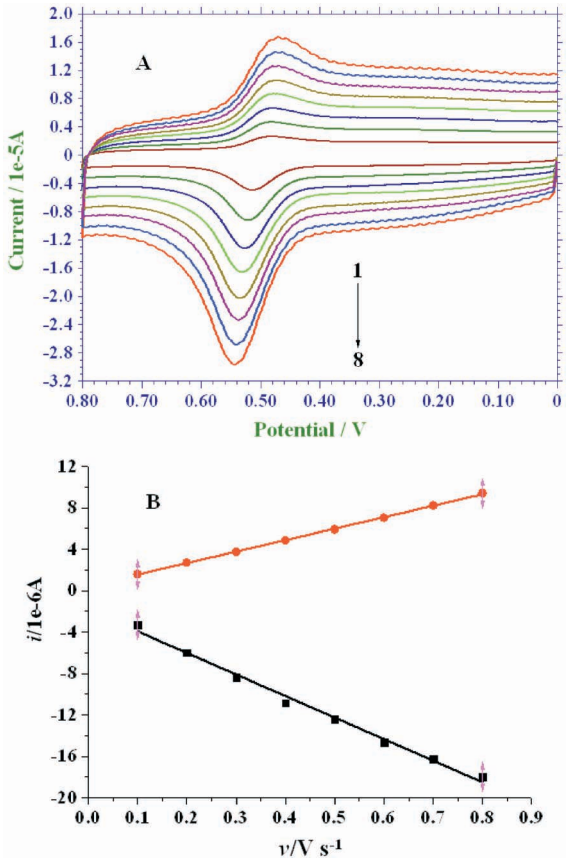


Fig. 5. CV curves (A) and linear relationship of i_p vs. v (B) of 2.0 × 10^{-6} mol L^{-1} BA in B-R buffer solution (pH = 2.87) at the GCE(ox). Scan rate (inner to outer): 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80 V s^{-1}.

fined peak was obtained in B-R buffer. Therefore, in this work B-R buffer was chosen as the supporting electrolyte.

The effect of the buffer pH on the formal peak potential (E^0) was also investigated, see Fig. 7. In the pH range from 2.0 to 7.0, the value of E^0 shifted to the negative direction with the increase of the buffer pH. A linear regression equation was obtained as E^0 (V) = -0.057 pH + 0.615 (r = 0.9988). The slope of -0.057 was close to the theoretical value of -0.059 V/pH at 25 °C. According to the equation: -0.054 m/n = -0.059, where n is the electron transfer number and m is the number of hydrogen ion participating in the reaction, so the uptaking of electron was accompanied by an equal number of hydrogen ion and m = n = 2.

Reaction mechanism of BA

According to the above results, the electro-oxidation reaction of BA at the GCE(ox) was a two-electron two-proton process, and the possible electrode reaction mechanism was expressed as shown in Scheme I.

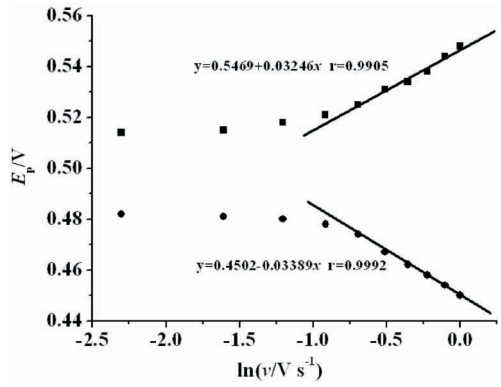


Fig. 6. Relationship between E_p and logarithm of potential scan rate.

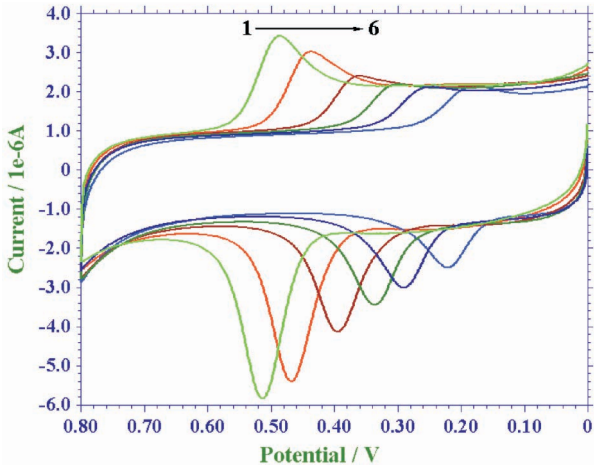
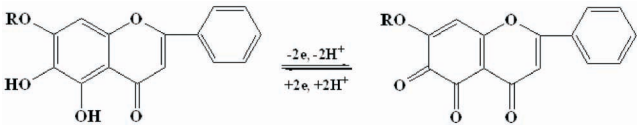


Fig. 7. The cyclic voltammograms of 2.0 × 10^{-6} mol L^{-1} BA at different pH values with scan rate of 0.10 V s^{-1}, pH: (1) 1.98, (2) 2.87, (3) 4.10, (4) 5.02, (5) 6.09, (6) 7.00.

Scheme I The electrochemical redox reaction mechanism of BA



3. Analytical applications and methods validation

Square wave voltammetry (SWV) is effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection limits.[35] Therefore, in this study SWV was used as a determinate technique. At the same time, for the analytical determination of BA, the redution peak was chosen, since it is much smaller background currents and better stable responses.

Effect of instrumental parameters

The SWV was used for the determination of BA. In order to obtain a much more sensitive peak current, the optimum instrumental conditions (pulse-amplitude E_{sw}, frequency f) were studied using anodic square wave voltammetry for 2.0 × 10^{-6} mol L^{-1} BA solution following t_{acc} of 300 s and open circuit. The results indicated that the peak current i_p increased with the increasing of square wave amplitude from 5 to 50 mV or square wave frequency in the range of 5-45 Hz, but the peak potential shifted to more positive values, and the peak changed unshapely. So 35 mV was chosen as the optimum amplitude and 25 Hz was chosen as the optimum frequency.

Effect of accumulation potential and accumulation time

Accumulation step is usually a simple and effective way to enhance the determining sensitivity. Accumulation potential (E_{acc}) and time (t_{acc}) are two crucial parameters for the accumulation step. The reduction peak current of 2.0 × 10^{-6} mol L^{-1} BA was compared after 300 s accumulation at different potential and open circuit by SWV. The peak current almost does not vary, revealing that the accumulation potential has no influence on the reduction peak current of BA at the GCE(ox). Thus, the accumulation of BA was carried out under open circuit.

Unlike the accumulation potential, accumulation time severely influences the oxidation peak current responses. The reduction peak current increases significantly

in the first 210 s, and then levels off. This may be caused by the fact that the adsorption of BA on the electrode surface becomes saturated. Thus, the accumulation step in this study was performed under open circuit for 210 s.

Calibration curve

Series concentrations of standard solutions of BA were detected under the optimized working conditions described above. Fig. 8 showed that the peak currents increased linearly with increasing concentration of BA. The inserted calibration plot highlights a linear relationship between peak currents and increasing concentration with a regression coefficient of 0.9990. The peak currents responded were linearly relationships with BA concentrations in the range of 5.0×10^{-8} to 3.0×10^{-6} mol L^{-1}. The linear equation was:

$$i_p(\mu A) = 1.588 + 1.597 \times 10^7 c \ (r = 0.998)$$

Based on the signal-to-noise ratio of 3 (S/N),[36] the detection limit was obtained as 2.0×10^{-8} mol L^{-1}. These values confirmed the sensitivity of the proposed method for the determination of BA. To estimate the repeatability of the proposed method, the R.S.D. of five times successful measurement of peak current of 2.0×10^{-6} mol L^{-1} BA at the GCE(ox) was calculated to be 2.9%, which demonstrates the good repeatability of the method.

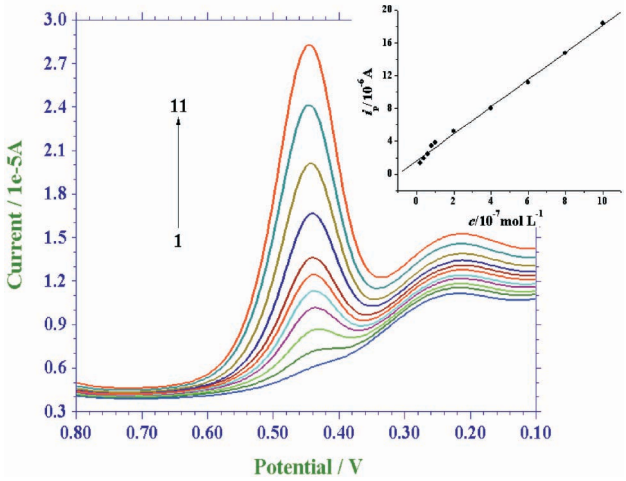


Fig. 8. Square wave anodic stripping voltammograms and their associated calibration plot (insert) for increasing concentrations of BA at the GCE(ox) under optimum conditions; BA concentration: 1) 0 mol L^{-1}, 2) 5.0×10^{-8} mol L^{-1}, 3) 6.0×10^{-8} mol L^{-1}, 4) 8.0×10^{-8} mol L^{-1}, 5) 1.0×10^{-7} mol L^{-1}, 6) 2.0×10^{-7} mol L^{-1}, 7) 4.0×10^{-7} mol L^{-1}, 8) 6.0×10^{-7} mol L^{-1}, 9) 8.0×10^{-7} mol L^{-1} , 10) 1.0×10^{-6} mol L^{-1}, 11) 2.0×10^{-6} mol L^{-1}.

Table 1. Determination results of BA in human blood serum samples

No	Standard solution added (10^{-7} mol L^{-1})	Standard solution found (10^{-7} mol L^{-1})	Recovery [a] (%)	RSD (%)
1	1.00	0.98	98.0	2.5
2	2.00	2.05	102.5	1.9
3	4.00	4.10	102.5	1.7
4	6.00	5.89	98.2	2.3
5	8.00	7.83	97.9	3.0
6	10.00	9.68	96.8	2.8

[a] Average of five determinations

Interference studies

For the possible analytical application of the proposed method, the effect of various species that likely to be in biological samples were evaluated by analyzing sample solutions containing a fixed amount of BA (2.0×10^{-6} mol L^{-1}) spiked with various excess amounts of the species under the same experimental conditions. The tolerance limit for a foreign species was taken as the largest amount yielding a relative error $< \pm 5\%$ for the determination of BA. The results show that no serious interference occurred from the classical additives tested. The influence of ascorbic acid, which is a potentially interfering compound present in biological samples, was investigated. It was found that an equimolar concentration or even at higher molar excess (2:1) of ascorbic acid had no distinct effect on the peak response of BA. Also interference of some metal ions was tested under the same conditions. It was observed that 100-fold excess of Al(III), Cu(II), Fe(II), Zn(II) and Mg(II) metal ions had no effects on BA determination. Therefore, the proposed method can be used as a selective method.

Determination of BA in human blood serum

The applicability of the SWV to the determination of BA in spiked urine was investigated. The direct determination of BA in human blood serum samples was found to be possible by employing a high dilution of the sample with the supporting electrolyte. The repeatability of a total analytical process was determined from multiple measurements at each of the human blood serum samples (Table 1). An average deviation of 2.4% was obtained.

EXPERIMENTAL

Apparatus and Reagents

Model 650A electrochemical system (CHI Instrument Company, Shanghai, China) was employed for elec-

trochemical techniques. A standard three-electrode electrochemical cell was used for all electrochemical experiments with a bare GCE or activated GCE (d = 3 mm) as working electrode, a platinum (Pt) wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. All the pH measurements were carried out with a pHS-2C Digital pH meter (Shanghai Lida, Shanghai, China) equipped with a combined glass electrode, which was calibrated regularly with standard buffer solutions (pH 4.00 and 6.86) at 25 ± 0.1 °C.

All reagents were of analytical grade and were used as received. BA was purchased from National Institute for the control of pharmaceutical and biological products (Beijing, China). Double distilled water was used for all preparations. Stock solutions (5.0×10^{-4} mol L^{-1}) of BA were prepared with ethanol and stored at 4 °C in the dark. Dilutions were done just prior to use. Each assay was performed at room temperature.

Working voltammetric procedure

GCE was polished progressively with finer emery-paper, then thoroughly with 0.5 μm Al_2O_3 (CHI Company) on a polishing cloth. The working electrode was cleaned in an ultrasonicating bath for 1 min, and then the electrode was activated by performing 50 cycles from 0.5 to 1.3 V in 0.2 mol L^{-1} NaOH with a scan rate of 100 mV s^{-1} before use. This electrochemically activated glassy carbon electrode was named GCE(ox).

About 10 mL of the electrolyte solution containing an appropriate amount of BA standard solution or sample was added to the electrolytic cell. Then the electrodes were immersed and cyclic voltammograms (CV) or square wave voltammetry (SWV) were recorded between 0.0 and 0.8 V or 0.1 and 0.8 V.

Analysis of spiked serum samples

Serum samples of healthy individuals (after having obtained their written consent) were stored frozen until assay. Acetonitrile removes serum proteins more effectively, as the addition of 1-1.5 volumes of serum is sufficient to remove the proteins. After vortexing for 30 s, the mixture was then centrifuged for 30 min at 5000 rpm for getting rid of serum protein residues and the supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into the volumetric flask and diluted up to the volume with ethanol to achieve final concentration of 5×10^{-4} mol L^{-1}. These solutions were analyzed in the voltammetric cell containing B-R buffer solution at pH 2.87. Quantifications were performed by means of the calibration curve method from the related calibration equation.

CONCLUSIONS

The electrochemical voltammetric behaviour of BA at the GCE(ox) in B-R buffer (pH = 2.87) solution was investigated. The experimental results suggested that the process at the GCE(ox) was adsorption controlled and involved two-electron two-proton transfer. An analytical method for the determination of BA in human blood serum samples was successfully developed on the basis of the voltammetric studies. Under the selected conditions, the reduction peak current was linearly dependent on the concentration of baicalin in the range of 5.0×10^{-8} to 3.0×10^{-6} mol L^{-1} (r = 0.9990), with a detection limit of 2.0×10^{-8} mol L^{-1}. The method is highly sensitive and has a reasonable selectivity and good precision. Additional, the oxidation mechanism was also proposed and discussed in this work.

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