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# Study of the voltammetric behavior of jatrorrhizine and its sensitive determination at electrochemical pretreatment glassy carbon electrode

Zhuo Ye [a], Yinfeng Li [a,b], Jianguo Wen [a,*], Kunjing Li [a], Baoxian Ye [a,*]

[a] The First Affiliated Hospital and Department of Chemistry, Zhengzhou University, Zhengzhou 450001, China
[b] School of Chemical and Material Engineering, Henan University of Urban Construction, Pingdingshan 467036, China

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ABSTRACT

A simple, inexpensive and highly sensitive electrochemical method for the determination of jatrorrhizine was developed using an electrochemically pretreated glassy carbon electrode (EPGCE). The electrochemical behavior of jatrorrhizine was systematically investigated in detail and some kinetic parameters were calculated for the first time. A reasonable reaction mechanism of jatrorrhizine on the EPGCE was also discussed and proposed, which could be a reference for the pharmacological action of jatrorrhizine in clinical study. And the first electroanalytical method of jatrorrhizine was established with a wide linear range from $7.0\times10^{-8}$ to $2.0\times10^{-5}$ mol $L^{-1}$ and a low detection limit of $5.0\times10^{-8}$ mol $L^{-1}$. The proposed method was successfully applied in determination of jatrorrhizine in pharmaceutical sample, *Tinospora capillipes* Gagnep (a traditional Chinese medicine), with satisfactory results.



## 1. Introduction

Jatrorrhizine is one of the major chemical constituents of *Tinospora capillipes* Gagnep and is distributed in many medicinal plants, especially abundant in the plant families of *Menispermaceae*, *Ranunculaceae*, *Berberidaceae*, *Menispermaceae*, *Rutaceae*, *Papaveraceae* and *Leguminosae* [1]. Jatrorrhizine is a kind of protoberberine alkaloid and Scheme 1a illustrates its molecular structure. Jatrorrhizine has important and comprehensive development values due to its abundant resources and various biological activities such as antimicrobial activity [1], anti-protozoal activity [2], anti-phototoxicity [3], antiradical and antioxidant activities [4], antifungal activity [5], the neuroprotective effects against $A\beta_{25-35}$-induced injury [6], the treatment of gastroenteritis and diarrhoea [7], and hepatoprotective effects [8]. Jatrorrhizine has shown the functions of decreasing the blood glucose level in alloxan-diabetic mice, inhibiting cytochrome P450 3A4 (CYP3A4), and has also shown an acetylcholinesterase inhibitory property [9–11]. Consequently, developing a sensitive analytical method for jatrorrhizine is highly needed. A variety of separation techniques have been proposed for assay of jatrorrhizine, including high performance liquid chromatography (HPLC) [12–15], quantitative nuclear magnetic resonance (qNMR) [16], and capillary zone electrophoresis (CZE) [17]. To the best of our knowledge, there is no report on the study of electrochemical characters for jatrorrhizine and the determination of it by electroanalytical method only. However, electrochemical techniques are simple, sensitive, rapid, inexpensive and convenient to investigate the redox mechanism of analyte. Moreover, the data obtained from electrochemical techniques are often correlated with molecular structures and pharmacological activities of drugs. Therefore, it is valuable to develop an electroanalytical method for jatrorrhizine assay.

Glassy carbon electrode (GCE) is one of the most common working electrodes used in electrochemical research [18]. And the electrode surface modifications and pretreatments have been widely used to improve the electrochemical responses of biological compounds and to construct electrochemical detectors [19–23]. Among them, electrochemical pretreatment of glassy carbon electrode (EPGCE) seems to be a simple, less time consuming and more applicable strategy in comparison to other procedures. This strategy avoids the use of some compounds that might cause environmental pollution in the modification procedure of electrode surface [24,25]. For example, EPGCE has been applied in the determination of metal ions such as copper [26] and manganese species [27], some organic molecules such as vitamin $B_2$ [28], morphine [29], uric acid and epinephrine [30]. In addition, EPGCE has also been used in the detection of biomolecules, such as DNA [31,32]. More recently, Geremedhin et al. [33] has accomplished the detection of fenitrothion in tap water and human urine

* Corresponding authors. Tel.: +86 371 677 81757; fax: +86 371 677 63654.
*E-mail address:* yebx@zzu.edu.cn (B. Ye).

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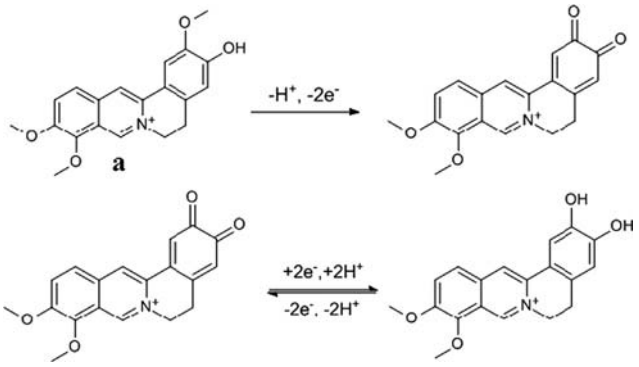


**Scheme 1.** Proposed redox mechanism of jatrorrhizine.

using a simple EPGCE. At this EPGCE, the current response of fenitrothion was linearly with its concentration in the range from $4.0 \times 10^{-7}$ to $5.0 \times 10^{-5}$ mol $L^{-1}$ and a low detection limit of $7.8 \times 10^{-8}$ mol $L^{-1}$.

In this work, a simple, inexpensive and highly sensitive electrochemical method for determination of jatrorrhizine in *T. capillipes* Gagnep was developed using the electrochemically pretreated glassy carbon electrode (EPGCE). Compared to untreated glassy carbon electrode, the EPGCE showed a significantly enhanced peak current towards jatrorrhizine oxidation. The electrochemical properties of jatrorrhizine were investigated systematically and the dynamic parameters of electrode process were obtained using various electrochemical techniques for the first time. It was found that the EPGCE exhibited improved sensitivity for the detection of jatrorrhizine.

## 2. Experimental

### 2.1. Apparatus and reagents

Electrochemical measurements were performed on a RST5000 electrochemical workstation (Zhengzhou Shiruisi Instrument Co., Ltd., Zhengzhou, China). A three-electrode system was used, consisting of a bare GCE or EPGCE (3 mm diameter) working electrode, an Ag/AgCl reference electrode and a platinum (Pt) wire counter electrode. $Fe(CN)_6^{3-/4-}$ ($5 \times 10^{-3}$ mol $L^{-1}$ containing 0.1 mol $L^{-1}$ KCl) was employed as probe and electrochemical impedance spectroscopy (EIS) was performed in frequency range of 0.1 MHz–0.01 Hz. All the experiments were carried out in a 10 mL vessel at room temperature.

Jatrorrhizine was purchased from Aladdin (http://www.aladdin-e.com/) and used as received. *T. capillipes* Gagnep was purchased from Tongrentang pharmacy (Beijing, China). Standard stock solution of Jatrorrhizine ($1 \times 10^{-3}$ mol $L^{-1}$) was prepared with methanol and kept under 4 °C. It was diluted to necessary concentration before use. 0.1 mol $L^{-1}$ phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.1 mol $L^{-1}$ $NaH_2PO_4$ and $Na_2HPO_4$. The lower pH was adjusted with 0.1 mol $L^{-1}$ $H_3PO_4$ solution. All the pH was measured with a PHS-3C (Jingke Devices Factory of Shanghai, China) pH meter with combined glass electrode. All the other chemicals were of analytical reagent grade and used as received without further purification. Double distilled water was used for all preparations.

### 2.2. Fabrication of the electrochemically pretreated glassy carbon electrode

Prior to modification, the GCE surface was polished to a mirror state using finer emery paper and 0.5 μm alumina slurry respectively. After rinsing thoroughly with water, the GCE was washed ultrasonically with absolute alcohol and double-distilled water respectively again. Electrochemically pretreated glassy carbon electrode was obtained by successive potential sweep between −1.5 and 2.5 V at 100 mV $s^{-1}$ for four cycles in pH 7.0 PBS. This was the optimal pretreated condition for fabricating the EPGCE from test. Prior to use, the EPGCE was pretreated in a 0.1 mol $L^{-1}$ PBS by cyclic scans between potentials of 0.1 and 1.0 V (20 cycles).

### 2.3. Analytical procedure

The quantitative analysis of jatrorrhizine was carried out in PBS buffer solution (pH 3.0) at room temperature unless otherwise specified. Then, the electrodes were placed into the test solution and the cyclic voltammetry (CV) or linear sweep voltammetry (LSV) were performed after being adequately stirred for 180 s at open circuit. The renewal of EPGCE was easily achieved in blank PBS (pH 7.4) by successive sweeping of two cycles between 0.1 and 1.0 V to give a regenerated electrode surface. For investigating the analytical method, LSV was employed for establishing calibration curve.

### 2.4. Preparation of real samples

For the analysis of the real sample, the *T. capillipes* Gagnep were finely powdered and homogenized in a mortar. Then, 1.0 g of the powder was transferred into 100 mL beaker containing 20 mL methanol and sonicated for 3 h. Next, the suspensions were filtrated. The above extraction steps were repeated for three times. All the methanol extract (Met-E) was mixed together and the solvent was evaporated by water bath heating to 10 mL for further analysis.

For the content determination of jatrorrhizine in *T. capillipes* Gagnep, certain volume of Met-E was mixed with 10 mL 0.1 mol $L^{-1}$ PBS (pH 3.0). Standard addition method was used and the jatrorrhizine spiked in above samples were successfully determined from the peak appeared at +0.805 V (versus Ag/AgCl) by LSV with scan rate of 0.05 V $s^{-1}$.

## 3. Results and discussion

### 3.1. Electrochemical properties of EPGCE

In general, $Fe(CN)_6^{3-}$ is used as electrochemical probe to investigate the properties of pretreated electrode surface. Here, we used it as the proof to characterize the EPGCE by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The voltammograms at bare GCE and EPGCEs fabricated with different cycles of pretreatment were obtained by using CV technique (data not shown). $Fe(CN)_6^{3-}$ ($1 \times 10^{-3}$ mol $L^{-1}$) exhibited a pair of well-defined redox peaks on the bare GCE with the peak-to-peak separation of 0.074 V, which was a nearly reversible electrode process. While using the EPGCE, the charging current increased greatly and the redox peak currents decreased gradually along with the cycles of increase, which could be attributed to the increase of oxygen-containing groups [24,34–35]. Whereas the peak-to-peak separations also increased from 0.092 V to 0.110 V, which were larger than that of bare GCE. This could be attributed to the resistance of oxygen-containing group layer. EIS was also carried out to further study the property of EPGCE (date not shown). From EIS, the values of the charge transfer resistance ($R_{ct}$) and surface capacitance ($C_d$) of different cycles of electrochemical pretreatment were obtained according to an equivalent circuit [36] and the fitting results are listed in Table 1. The values of $R_{ct}$ and $C_d$

were increased along with the increase of the cycles of electrochemical pretreatment.

Meanwhile, we optimized the electrochemical response of EPGCE towards jatrorrhizine with respect to electrochemical pretreatment cycles. We found that the EPGCE prepared by 4 cycles showed higher electrochemical response towards jatrorrhizine than pretreated by less or more than 4 cycles. Thus, EPGCE obtained by 4 cycles of electrochemical pretreatment was chosen for preparing the adopted EPGCE sensor for jatrorrhizine.

### 3.2. Electrochemical behavior of jatrorrhizine on EPGCE

Fig. 1A illustrates the cyclic voltammograms (CVs) of jatrorrhizine ($5.0\times10^{-6}$ mol $L^{-1}$) obtained at bare GCE (curve a) and EPGCE (curve b) in 0.1 mol $L^{-1}$ PBS (pH 3.0). The curve c in Fig. 1A was a blank voltammogram of EPGCE in PBS. In order to explain all phenomena thoroughly, each scan was performed by 2 cycles. Fig. 1B was the magnified curve a in Fig. 1A. On the bare GCE, jatrorrhizine exhibited very weak electrochemical reactivity in potential window between 0.1 V and 1 V. On the EPGCE, a sensitive anodic peak at $E_p$=0.845 V (P1) and a cathodic peak at $E_p$=0.552 V (P2) were presented in the 1st cycle. In the 2nd cycle, a new anodic peak at $E_{pa}$=0.616 V (P3) was appeared and the P1 was disappeared. The P2 had no change. The EPGCE had no electrochemical response in blank solution (curve c). From these experimental data, we initially estimated that the P2 and P3 were a pair of redox peaks and its active group came from the product of P1 reaction. The P1 was an irreversible anodic peak.

To further investigate the electrochemical behaviour of jatrorrhizine at the EPGCE, the scan potential window was controlled with different range to observe the electrode response of jatrorrhizine. Firstly, when the potential window was controlled between 0.1 V and 0.65 V for 4 cyclic scans, there was no any redox peak appearance (Fig. 2A). Secondly, when the potential window was set between 0.65 V and 1.0 V for 4 cyclic scans, the P1 was observed in the first cycle and then no any peak was obtained (Fig. 2B). Thirdly, when the potential window was controlled between 1.0 V and 0.1 V again and the initial potential was set up at 0.65 V going negatively for 4 cycles (Fig. 2C). Just as expected, the P2 was not observed in the first cycle and the three peaks were obtained just as the curve b in Fig. 1A. These data demonstrated the above conjecture. Finally, successive 4 cyclic scans were performed between 0.1 V and 1.0 V (Fig. 2D). The P1 disappeared after the first cycle and the peak currents and potentials of P2 and P3 almost kept unchanged in the next 3 cyclic scan.

**Table 1**
$R_{ct}$ and $C_d$ value obtained from EIS by fitting using an equivalent circuit.

| Cycles of pretreatment | 0 | 2 | 4 | 6 | 8 | 10 |
|---|---|---|---|---|---|---|
| $R_{ct}$ (Ω) | 16.1 | 18.9 | 26.3 | 45.4 | 54.4 | 61.3 |
| $C_d$ (μF/cm$^2$) | 1.4 | 30.8 | 48.1 | 70.5 | 96.2 | 106 |

### 3.3. Effect of pH and scan rate

To understand the reaction pathway and conjecture the reaction mechanism of jatrorrhizine at EPGCE, it is necessary to investigate the effect of solution pH and scan rate. Fig. 3A and B displays CVs of jatrorrhizine ($5.0\times10^{-6}$ mol $L^{-1}$) at EPGCE in 0.1 mol $L^{-1}$ PBS with different solution pH, ranged from 2.0 to 7.5. The peak potentials of P1, P2 and P3 shifted negatively with increasing pH. Plots of peak potentials versus solution pH were found to be linear over the pH range of 2.0–6.5. And the Linear regression equations were $E_{p1}$ (V)= −0.0306pH+0.9292 ($R$=0.9977), $E_{p2}$ (V)= −0.0583pH+0.785 ($R$=0.9909), and $E_{p3}$ (V)= −0.0573pH+0.187 ($R$=0.9988) (Fig. 4C). From the slope of −0.0306 V $pH^{-1}$ (P1), the process of P1 involved protons and electrons in a ratio of 1:2. And the slope of −0.0583 V $pH^{-1}$ (P2) and −0.0573 V $pH^{-1}$ (P3) were close to the Nernst slope of 0.059 V $pH^{-1}$ at 25 °C. This result indicated that there were equal number of proton and electron taking part in the redox of P2 and P3.

It was also observed that the peak current of jatrorrhizine at the EPGCE was augmented along with the increase of pH from 2.0 to 3.0 and then started to decrease for pH values higher than 3.0. Especially in pH 7.5, the electrochemical response of EPGCE to jatrorrhizine was very small. Hence, pH 3.0 was chosen as the optimum pH for further analyses.

The effect of scan rate on the redox of jatrorrhizine at the EPGCE was investigated and a superimposed voltammogram is shown in Fig. 4A. Likewise, every CV scan was cycled twice. The data of P1 was obtained from the first cycle and the P2 and P3 were from the second cycle. With the scan rate increasing, the currents of three peaks increased and peak potential of P1 and P3 shifted positively and P2 shifted negatively. For the P1, a good

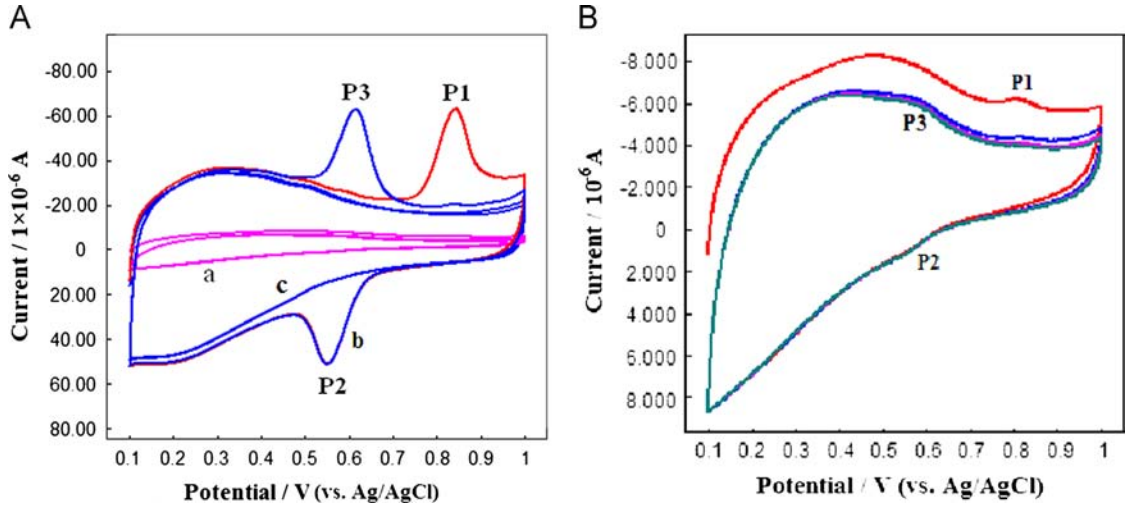


**Fig. 1.** (A) Cyclic voltammograms of jatrorrhizine ($5.0\times10^{-6}$ mol $L^{-1}$) at bare GCE (a) and EPGCE (b) and blank voltammograms of EPGCE (c); (B) The enlarged view of curve a in (A). Supporting electrolyte: 0.1 mol $L^{-1}$ PBS (pH 3.0); Scan rate: 0.1 V $s^{-1}$.

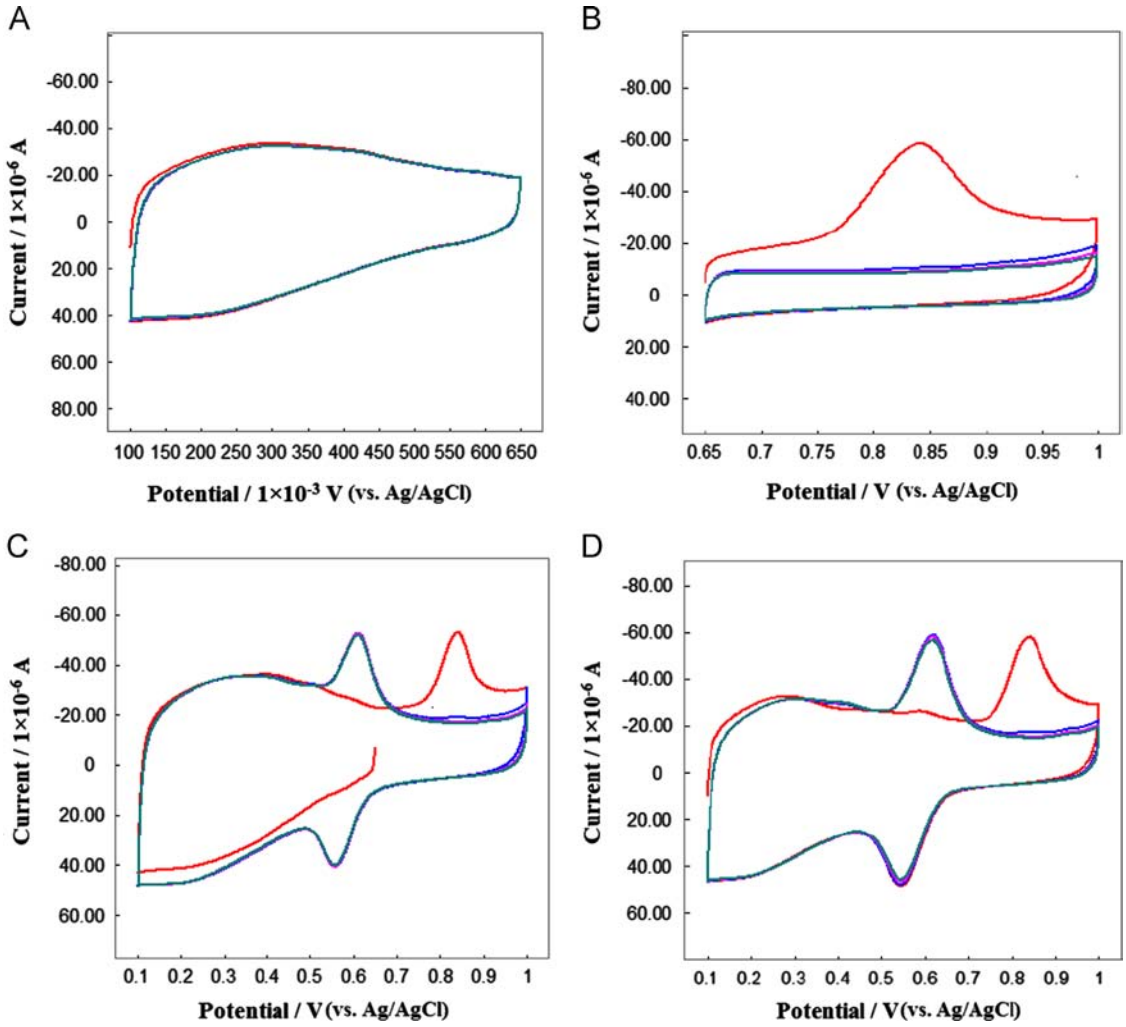


**Fig. 2.** CVs of jatrorrhizine ($5.0\times10^{-6}$ mol $L^{-1}$) within different potential window. A: between 0.1 V and 0.65 V; B: between 0.65 V and 1.0 V; C and D: between 1.0 V and 0.1 V. Other condition same as in Fig. 1.

linear relation between peak currents and scan rates could be described by following equation: $i_{p1}=-323.5\nu-6.337$ ($i_{p1}$ in μA, $\nu$ in V $s^{-1}$, $R=0.9979$), suggesting that the anodic peak P1 was controlled by adsorption. Meanwhile, a good linear relationship was exhibited between peak potential ($E_{p1}$) and ln $\nu$ (Fig. 4B, curve a). The regression equation was $E_{p1}=0.0309\ln\nu+0.9145$ ($E_{p1}$ in $V$, $\nu$ in V $s^{-1}$, $R=0.9900$). According to Laviron theory [37] for an irreversible process, following equation exists:

$$E_p(V)=E^{0'}-\frac{RT}{\alpha nF}\ln\frac{RTk_s}{\alpha nF}+\frac{RT}{\alpha nF}\ln\nu$$

where $E^{0'}$ is formal standard potential and $k_s$ is the standard heterogeneous reaction rate constant; $n$ is the transfer electron number; $\alpha$ refers to charge transfer coefficient; $\nu$, $R$, $T$ and $F$ have their usual meanings. From the slope of $E_{p1}$ vs. ln $\nu$, $n=2$ could be achieved by assuming of $\alpha=0.5$. The results indicated that two electrons were involved in the anodic P1. Then, the electron transfer coefficient ($\alpha=0.41$) and the heterogeneous electron transfer rate constant ($k_s=1.06$ $s^{-1}$) could be calculated based on the above mentioned equation. The formal standard potential ($E^{0'}=0.8087$ V) was calculated from another linear relation of $E_{p1}-\nu$ by extrapolating $\nu=0$.

For P2 and P3, the peak currents of $i_{p2}$ and $i_{p3}$ were proportional to the scan rates with linear regression equations of $i_{p2}=394.3\nu+1.663$ ($i_{p2}$ in μA, $\nu$ in V $s^{-1}$, $R=0.9978$) and $i_{p3}=-348.5\nu+2.322$ ($i_{p3}$ in μA, $\nu$ in V $s^{-1}$, $R=0.9977$), also indicating the electrode process driven by adsorption. When the scan rates increased from 0.04 V $s^{-1}$ to 0.2 V $s^{-1}$, the peak potentials of P2 and P3 ($E_{p2}$ and $E_{p3}$), shifted slightly and the peak-to-peak separation ($\Delta E_p$) was augmented from 0.046 to 0.126 V, indicating that the redox of P2 and P3 was a quasi-reversible process driven by adsorption. And the regression equation between $E_{p2}$ ($E_{p3}$) and ln $\nu$ were $E_{p2}=-0.0254\ln\nu+0.4738$ ($E_{p2}$ in V, $\nu$ in V $s^{-1}$, $R=0.9873$, Fig. 4B, curve b) and $E_{p3}=0.0263\ln\nu+0.6785$ ($E_{p3}$ in V, $\nu$ in V $s^{-1}$, $R=0.9821$, Fig. 4B, curve c). According to the above results, the electron transfer kinetics of this pair of redox reaction can be obtained using the approach developed by Laviron's equation [37]:

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

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

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

$k_s$, $\nu$, $n$, $\alpha$, $R$, $F$, and $T$ have their usual meaning. A value of 2 could be achieved for $n$ and $\alpha$ was 0.5 contained for P2 and P3 redox according to Eq. (1) and Eq. (2). Based on the Eq. (3), the value of $k_s$ was further calculated to be 0.79 $s^{-1}$.

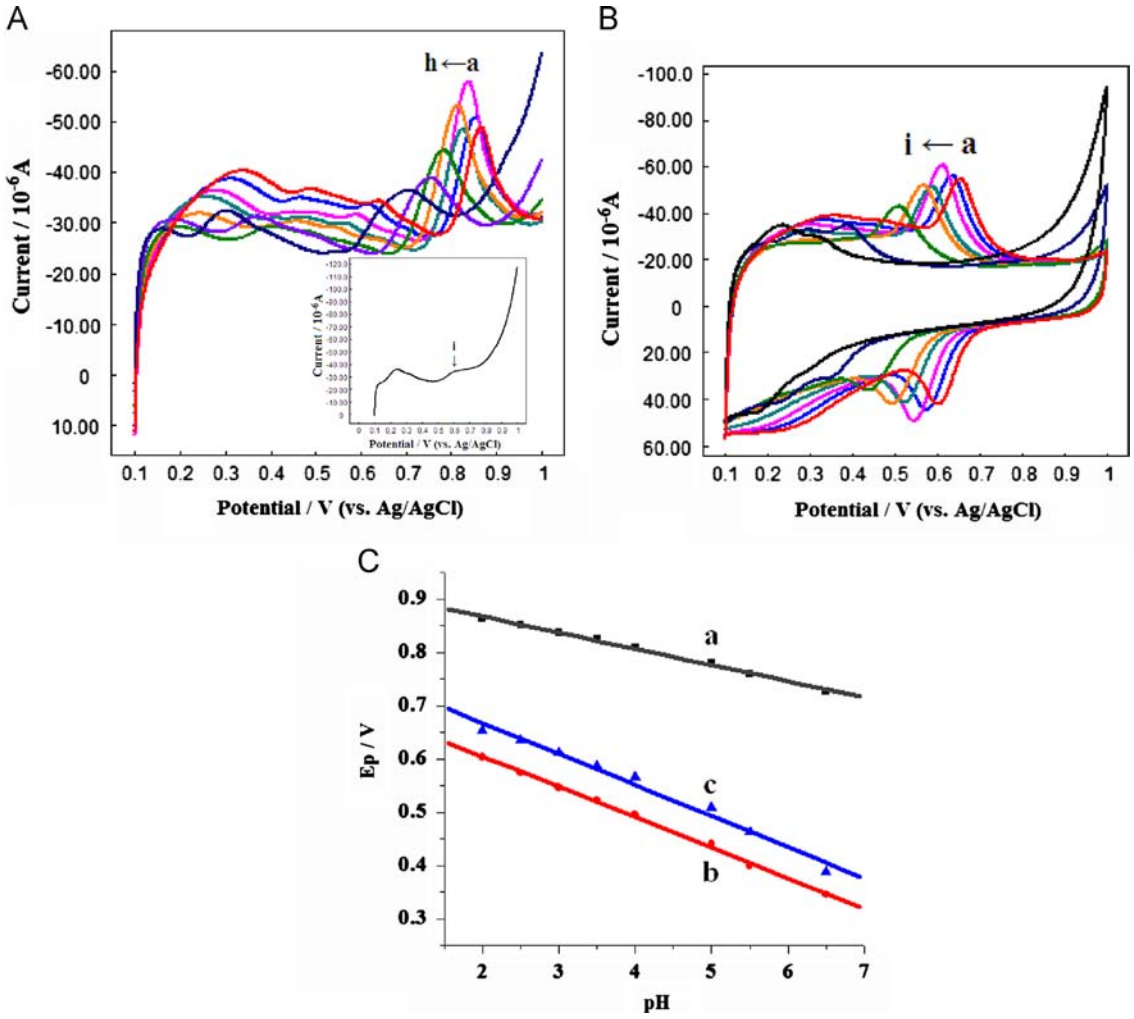


**Fig. 3.** (A) CVs of jatrorrhizine ($5.0\times10^{-6}$ mol $L^{-1}$) in different pH PBS corresponding to P1 (pH from curve a to i):2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 5.5, 6.5, 7.5 (inset); (B) corresponding to P2 and P3; (C) The relationship between the peak potentials and solution pH (except pH 7.5); Scan rate:0.1 V $s^{-1}$.

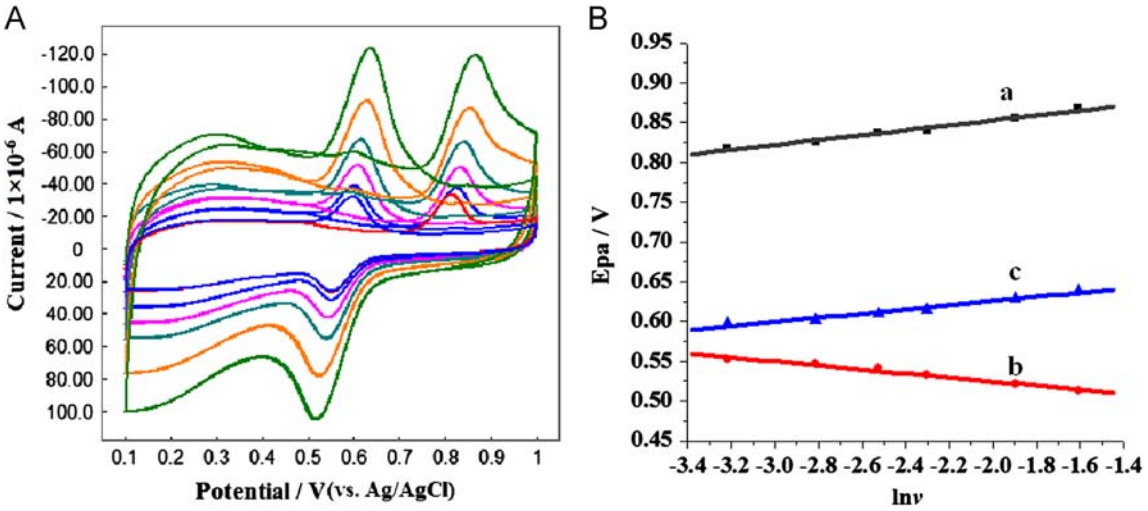


**Fig. 4.** (A) The superimposed voltammograms of jatrorrhizine ($5.0\times10^{-6}$ mol $L^{-1}$) with different scan rates (from inner to outer): 0.04, 0.06, 0.08, 0.1, 0.15, 0.2 V $s^{-1}$. (B) The relationship between $E_{p1(2,3)}$ and ln $\nu$, curve a for P1, curve b for P2, curve c for P3.

According to the data obtained above, one can conclude that the electrode reaction of jatrorrhizine on EPGCE is a two-electron and one-proton irreversible electro-oxidation process for P1 and a two-electron and two-proton quasi-reversible redox process for P2 and P3. A reasonable electrode reaction mechanism is proposed and shown in Scheme 1.

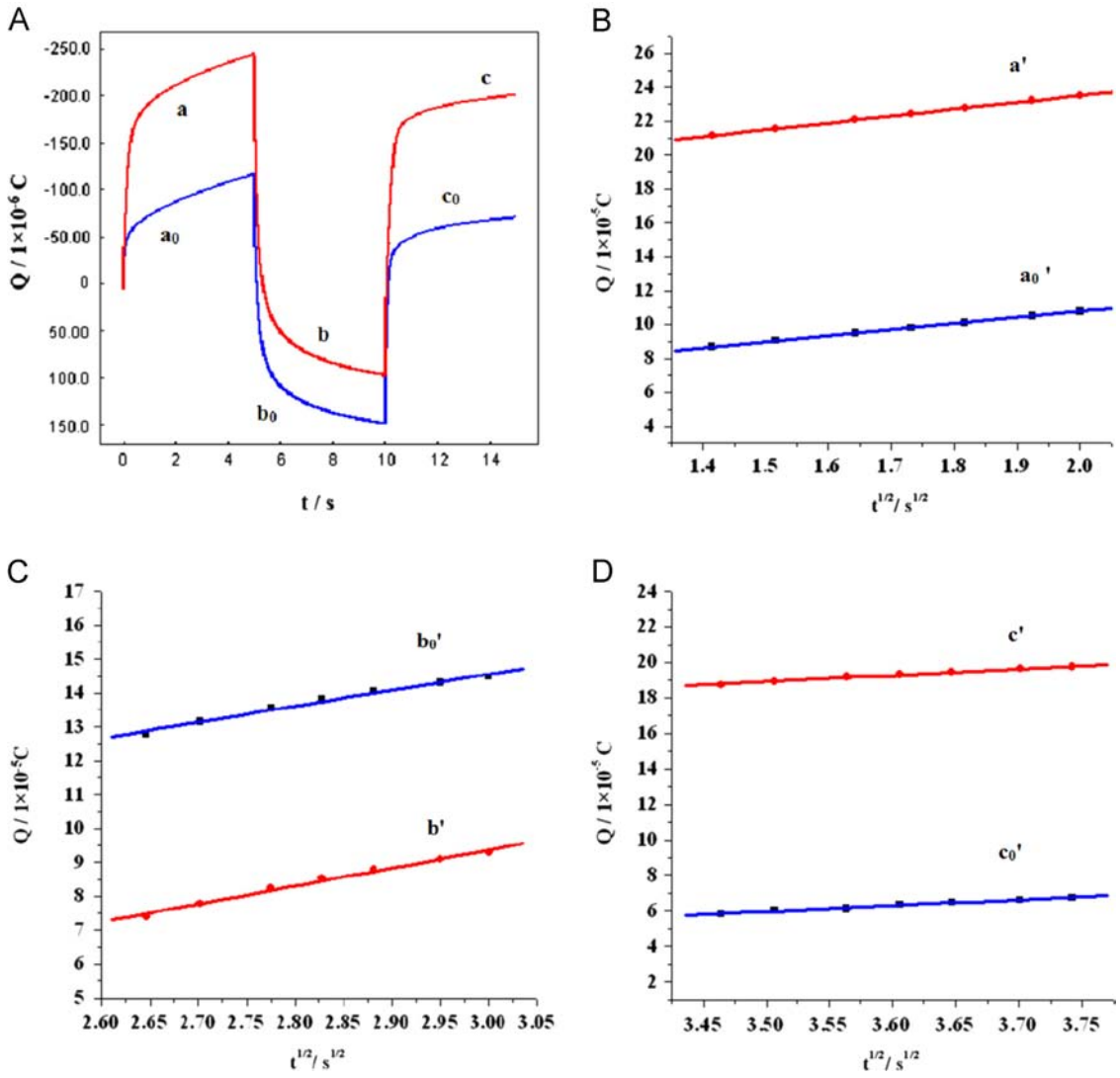


**Fig. 5.** (A) Chronocoulometric curves obtained at the EPGCE in the presence (a, b, c) and absence ($a_0$, $b_0$, $c_0$) of jatrorrhizine ($5.0 \times 10^{-6}$ mol $L^{-1}$). (B, C, D) The dependency of charge $Q$ ($10^{-5}$) vs. $t^{1/2}$, corresponding data were derived from (A).

**Table 2**
Comparison of different methods for jatrorrhizine determination (The concentration unit in the references cited had been converted to mol $L^{-1}$.)

| Method/electrode | Sample pre-treatment | Linear range ( $\times 10^{-6}$ mol $L^{-1}$) | Detection limit ( $\times 10^{-6}$ mol $L^{-1}$) | Reproducibility (R.S.D%) | Reference |
|---|---|---|---|---|---|
| HPLC–ECD[a] | Extraction and separation | 0.06–80 | 0.02 | 3.1 | [13] |
| SPME–HPLC[b] | Extraction and separation | 16.9–16,900 | 3.38 | 6.1 | [14] |
| LC–MS-MS[c] | Extraction and separation | 148–74,400 | 148 | 15 | [15] |
| qNMR[d] | Extraction | – | – | 2.3 | [16] |
| CZE[e] | Separation | 33,838–3,383,800 | 6767.6 | 6.9 | [17] |
| EPGCE | Extraction | 0.07–20 | 0.05 | 2.9 | This work |

[a] High performance liquid chromatography (HPLC)–electrochemical detection (ECD).
[b] Solid-phase microextraction–high performance liquid chromatography.
[c] Liquid chromatography–tandem mass spectrometry.
[d] Quantitative $^1$H NMR.
[e] Capillary zone electrophoresis.

### 3.4. Chronocoulometry studies

For an adsorption controlled electrode process, it is necessary to calculate the saturated adsorptive capacity ($\Gamma_{max}$) of electroactive substance at the electrode surface. For getting the $\Gamma_{max}$, the active area (A) of electrode surface must be known first. In order to get the $A$ value, 0.1 mM $K_3[Fe(CN)_6]$ was employed again as model complex and chronocoulometry technique was used based on Anson equation [38]:

$$Q_{total} = \frac{2nFAc(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads}$$

where $A$ is active area of working electrode, $c$ is concentration of substrate, $D$ is diffusion coefficient, $Q_{dl}$ is double layer charge which could be eliminated by background subtraction, and $Q_{ads}$ is Faradic charge. Other symbols have their usual meanings. Based on

the slopes of linear relationship between $Q$ and $t^{1/2}$ (data not shown), A is calculated to be 0.203 $cm^2$ and 0.308 $cm^2$ for bare GCE and EPGCE, respectively. The results indicate that the electrode active surface area is increased after electrode pretreated by electrochemical method.

Next, the saturated absorption capacity of jatrorrhizine on EPGCE was determined in 0.1 mol $L^{-1}$ PBS (pH 3.0) when in the absence and presence of $5.0\times10^{-6}$ mol $L^{-1}$ jatrorrhizine. Here, multi-potential step chronocoulometry was employed and potentials steps were performed from 0.65 V to 1.0 V (first step), 1.0 V to 0.1 V (second step) and 0.1 V–0.7 V (third step). For control, $Q$–$t$ curve was firstly recorded in blank PBS (Fig. 5A, curve $a_0$, $b_0$, $c_0$). Then the EPGCE was immerged in a jatrorrhizine solution ($5.0\times10^{-6}$ mol $L^{-1}$) for several minutes to achieve saturated absorption and then the $Q$–$t$ curve was recorded (Fig. 5A, curve a, b, c). The markers *a*, *b* and *c* were correspondence with the P1, P2 and P3 in Fig. 4. Extracting data from Fig. 5A, the corresponding $Q$–$t^{1/2}$ curves were obtained and displayed in Fig. 5B, C and D. Curves a′, b′ and c′ were obtained in jatrorrhizine solution and curves $a_0'$, $b_0'$ and $c_0'$ were in blank solution. At the first step (0.65–1.0 V), the corresponding $Q$–$t^{1/2}$ plot of curves $a_0'$ and a′ were calculated with the linear equations of $Q$ $(10^{-5}C)=3.973t^{1/2}+3.632$ ($R=0.9999$) and $Q$ $(10^{-5}C)=4.077t^{1/2}+15.372$($R=0.9999$), respectively. As shown in Fig. 5B, a bigger intercept and almost same slope were obtained from a′ comparing with $a_0'$, which further meant that the oxidation of jatrorrhizine at P1 was mainly controlled by adsorption. The $Q_{ads}$ (the difference of the two intercepts) was caused by the oxidation of adsorbed jatrorrhizine. According to the formula given by Anson [38], the value of $Q_{ads}$ was calculated to be $1.174\times10^{-4}$C. Using Laviron's theory of $Q=nFA\Gamma^*$, the $\Gamma_{max}$ value of jatrorrhizine was $1.98\times10^{-9}$ mol $cm^{-2}$ at EPGCE.

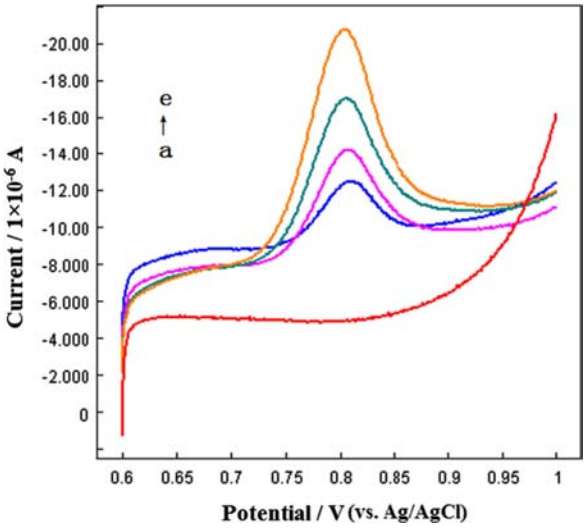


**Fig. 6.** LSV curves of EPGCE in different amount of jatrorrhizine standard solution in the presence of 50 μl Met-E (from b to e): 50 μl Met-E, b+$1.0\times10^{-6}$, b+$2.0\times10^{-6}$, b+$4.0\times10^{-6}$ mol $L^{-1}$ (Curve a was the response in blank solution; Curves c, d and e were the amount of jatrorrhizine standard solution added in the presence of 50 μl Met-E). Scan rate: 0.05 V $s^{-1}$.

For the next two steps from 1.0 to 0.1 V and from 0.1 to 0.7 V, the corresponding $Q$–$t^{1/2}$ curves are plotted in Fig. 5C and D. Obviously, the two slope values of the $Q$–$t^{1/2}$ plots were equal both in the absence and presence of jatrorrhizine, which was additional evidence for an total adsorption-driven electrode process. According to the formula mentioned above, saturated adsorption capacity values of $1.71\times10^{-9}$ mol $cm^{-2}$ and $2.02\times10^{-9}$ mol $cm^{-2}$ were calculated for the oxidative and reductive jatrorrhizine, respectively.

### 3.5. Calibration curve and detection limit of jatrorrhizine at EPGCE

Here, the current response of P1 was chosen to achieve a calibration curve. The relationship between the peak currents and the jatrorrhizine concentrations was investigated by linear sweep voltammetry (LSV). It was found that the anodic peak currents were linear with jatrorrhizine concentrations within the range from $7.0\times10^{-8}$ mol $L^{-1}$ to $2.0\times10^{-5}$ mol $L^{-1}$. The regression equation was $i_{pa}/\mu A=1.942+2.213C_{jatrorrhizine}/10^{-6}$ mol $L^{-1}$ ($R=0.9964$) with a detection limit of $5.0\times10^{-8}$ mol $L^{-1}$. Here, the detection limit was the concentration being able to give the measurable signal that can be observed by naked eye. Table 2 listed the results about the comparison of different methods for jatrorrhizine determination. It can be seen that sensitive determination of jatrorrhizine was achieved on the proposed electrode compared with other methods. Moreover, the electrochemical method is more convenient and low cost without any separation procedure.

### 3.6. Reproducibility, stability, interference

To evaluate the reproducibility and stability of the proposed sensor, LSV was employed and performed in a $1\times10^{-6}$ mol $L^{-1}$ jatrorrhizine solution. The intra-day precision of the proposed method was evaluated by five replicate measurements of the same sample in one day. The detected relative standard deviation (RSD) was of 4.8%. Meanwhile, the obtained RSD was 4.4% from three parallel pretreated EPGCEs in a same $1\times10^{-6}$ mol $L^{-1}$ jatrorrhizine solution, revealing a good reproducibility. Moreover, the sensor maintained about 90% of its initial response for a same jatrorrhizine solution after stored for one week, demonstrating that the EPGCE exhibited long-term stability.

The interference of some normal anions and cations and some organic compounds was investigated in the presence of $1\times10^{-6}$ mol $L^{-1}$ jatrorrhizine. The results suggested that 100-fold concentration of $Cu^{2+}$, $Zn^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Cl^-$, $SO_4^{2-}$, $NO_3^-$, glucose and oxalic acid had no influence on the signals of jatrorrhizine with deviations below 5%. 50-fold $Ca^{2+}$, $Mg^{2+}$, citric acid, ascorbic

**Table 3**
Determination results of jatrorrhizine in *Tinospora capillipes* Gagnep (TCG).

| Sample[a] ($\times10^{-6}$ L) | Original detected value[b] ($\times10^{-6}$ mol $L^{-1}$) | Standard added ($\times10^{-6}$ mol $L^{-1}$) | Detected total value[b] after added ($\times10^{-6}$ mol $L^{-1}$) | RSD (%) | Recovery (%) |
|---|---|---|---|---|---|
| 50.0 | 0.812 | 1.00 | 1.956 | 3.8 | 107.9 |
| | | 2.00 | 2.951 | 2.6 | 104.9 |
| | | 4.00 | 4.595 | 3.2 | 95.5 |
| 100.0 | 1.718 | 0.50 | 2.15 | 1.7 | 96.5 |
| | | 1.00 | 2.77 | 4.5 | 101.9 |
| | | 2.00 | 3.83 | 3.4 | 103.1 |

[a] 50 or 100 μl Jatrorrhizine solution (Met-E) was added to pH 3.0 PBS.
[b] Average value of three replicate measurements.

acid, 10-fold uric acid and palmatine chloride also showed no influence. In short, the interference effects of the studied compounds were negligible, which clearly proved the reasonable selectivity for the proposed method.

### 3.7. Quantitative analysis of jatrorrhizine in pharmaceutical sample

The accuracy of the method was also demonstrated by its recovery during spiked experiments by LSV. The proposed method in real sample analysis was examined in PBS containing Met-E. Fig. 6 displayed the LSV curves of EPGCE in different amount of jatrorrhizine standard solution in the presence of 50 μl Met-E. The results of recovery experiments are listed in Table 3. It can be seen that the recoveries of jatrorrhizine from the *T. capillipes* Gagnep were satisfactory with values ranged from 95.5% to 107.9%. These results demonstrated the ability of EPGCE for LSV determination of jatrorrhizine with high sensitivity and good reproducibility. Further, the content of jatrorrhizine in *T. capillipes* Gagnep was calculated to be 0.58 mg $g^{-1}$. Meanwhile, according to the chromatographic conditions in literature [39], the same sample was tested by HPLC and the content of jatrorrhizine in *T. capillipes* Gagnep was 0.61 mg $g^{-1}$. This further demonstrated the good accuracy of the proposed method.

## 4. Conclusion

In conclusion, a simple and highly sensitive electrochemical method for the determination of jatrorrhizine in *T. capillipes* Gagnep was developed using the electrochemically pretreated glassy carbon electrode (EPGCE). Using this sensor, the electrochemical properties of jatrorrhizine were investigated in detail for the first time. And the first electroanalytical method of jatrorrhizine was proposed. The EPGCE dramatically improved the electrochemical response of jatrorrhizine and enhanced the sensitivity. The detail electrochemical characters of jatrorrhizine were studied systematically and dynamic parameters of electrode process were calculated. Moreover, the EPGCE was wider linear range and lower detection limit for response of jatrorrhizine, and it also exhibited good stability and reproducibility. In practical application investigations, the EPGCE showed good recoveries and could be applied to determine jatrorrhizine in *T. capillipes* Gagnep with satisfactory results.

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