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Study On The Electrochemical Behaviour Of The Anticancer Herbal Drug Emodin

Baoxian Ye
Department of Chemistry,
Zhengzhou University,
Zhengzhou, 450052 (P.R.CHINA)
Fax : 86-371-67763220
E-mail: yebx@zzu.edu.cn

Liping Wang, Zongpei Zhang
Department of Chemistry,
Zhengzhou University,
Zhengzhou, 450052 (P.R.CHINA)

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ABSTRACT

The electrochemical behaviour of the anticancer herbal drug emodin was investigated by cyclic voltammetry (CV) at glassy carbon electrode. In 0.05 M NH_3-NH_4Cl (50% ethanol, pH 7.2) buffer solution, a pair of quasi-reversible redox peaks with peak potentials of E_{p1} = -0.688V and E_{p2} = -0.628V and an irreversible anodic peak, which was a typical anodic peak of emodin, with E_{p3} = -0.235V were appeared at scan rate of 100 mV/s. The irreversible anodic peak currents are linearly relationship with emodin concentrations in the range of 8.9×10^{-8} M to 7.8×10^{-6} M with pre-concentration 80s under -0.620V. Using established method, the emodin in herbal drug was determined with satisfactory results and separation was unnecessary. Moreover, the electrode process dynamics parameters were investigated too by electrochemical techniques.

KEYWORDS

Anti-cancer herbal drug;
Emodin;
Dynamics parameters;
Determination.

INTRODUCTION

Traditional chinese medicines (TCM) has attracted great interest in recent researchers as alternative antineoplastic therapies. Herbal medicines were one of the major resources for health-care in early eras. Currently, herbal medicines are gaining more attention from modern pharmaceutical institutes, as scientists become aware that herbal medicine is an almost infinite resource for drug development. Although the Traditional chinese medicine definition of cancer may differ from that of modern science, several chinese plants do have significant antitumor activity. Emodin is one of these nature anticancer drugs. It is the active ingredient of the Traditional chinese medicine — rhubarb. Emodin exhibits electrochemical activity by the interaction of chromophore group — the anthraquinone substitu-

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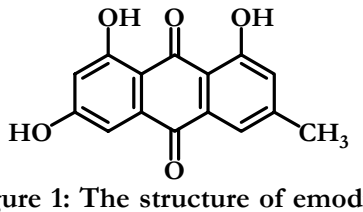

Figure 1: The structure of emodin

ents at positions 9 and 10[1]. Figure1 shows the structure of emodin[2].

Analysis of herbal medicine is an important technique, which offers many applications in biochemical, pharmaceutical and clinical research. Especially, voltammetric techniques are relatively simple, quite rapid and reasonably cheap. Furthermore, voltammetric techniques can afford the messages about the reaction mechanism and the dynamics parameters of herbal drug, which may be very helpful for understanding the pharmacological effect and the antineoplastic mechanism of emodin.

Available analytical methods about emodin included thin-layer chromatography scanning (TLC-scanning)[3], capillary zone electrophoresis with amperometric detection (CZE)[4], high-performance thin layer chromatography (HPTLC)[5], near infrared surface-enhanced Raman spectra (NIR SERS)[6], capillary electrophoresis (MEKC)[7] and so on. Chromatographic methods offer a high degree of selectivity but need relatively heavy and costly instrumentation. Furthermore, they can't offer the messages about dynamics parameters and reaction process of the herbal drug. To our knowledge no attention has paid to the reaction mechanism of emodin. The aim of our work is to present a new analytical method and the basic dynamics data about emodin. It is very useful for clinic application of emodin. For the analytical method established, it possesses high sensitivity, wide linear range (8.9×10^{-8} M ~ 7.8×10^{-6} M) and good selectivity. It is unnecessary to separate other 9, 10-anthraquinone derivative ingredients contained in rhubarb because a typical oxidation peak (-0.235V *vs.* SCE) of emodin can be used. Using this method, emodin in real traditional chinese medicine was determined with satisfactory results.

EXPERIMENTAL

Apparatus and materials

Model 650A electrochemical system (CHI Instrument Company, USA) was employed for electrochemical techniques. A standard three-electrode electrochemical cell was used for all electrochemical experiments with glassy carbon electrode (GCE) (d = 3 mm) as working electrode, a platinum (Pt) wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

Stock solutions 7.400×10^{-4} M of emodin (Checkout Institute of Biology drugs, China) were prepared with ethanol and stored at 4ºC. Other reagents used were of analytical grade. Doubly distilled water was used for all preparations. N_2 was employed to deoxygenize and all experiments were carried out at room temperature. All reported potentials are against SCE.

Procedure

Supporting electrolyte was a 0.05 M NH_3-NH_4Cl buffer solution (pH 7.2). In all case , 50% ethanol was added because of the very low solubility of emodin in aqueous solutions. When determined the concentration of emodin, a pre-concentration potential of -0.620V, a pre-concentration time 80s and NH_4Cl-HCl buffer solution (pH 5.7) were used.

RESULTS AND DISCUSSION

The cyclic voltammetry behavior of emodin at GC electrode

Cyclic voltammetry (CV) was performed in a standard electrochemical cell with a 3-electrode system. Figure 2 shows the cyclic voltammogram of emodin (7.400×10^{-5} M) in NH_3-NH_4Cl (pH 7.2) buffer solution. A pair of quasi- reversible redox peaks (P_1 and P_2) appeared with the peak potentials of E_{p1} = -0.688V and E_{p2} = -0.628V at scan rate of 100 mV/s. The pair of redox peaks were reported previously[2]. A new irreversible anodic peak (P_3) appeared at potential of -0.235V (E_{p3}). The P_3 is never reported previously and is a typical oxidation peak of emodin compared with other 9, 10-anthraquinone derivative ingredients contained in rhubarb, so we

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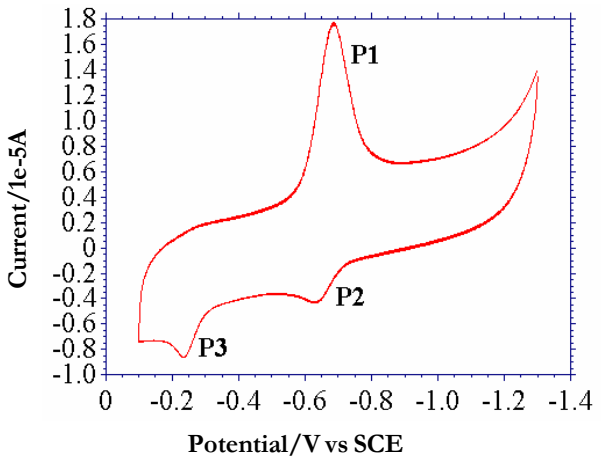

Figure 2: Cyclic voltammogram of emodin
[emodin] = 7.400×10^{-5} M
Support electrolyte: 0.05 M NH_3-NH_4Cl buffer solution (pH 7.2)
Scan rate: 100 mV/s

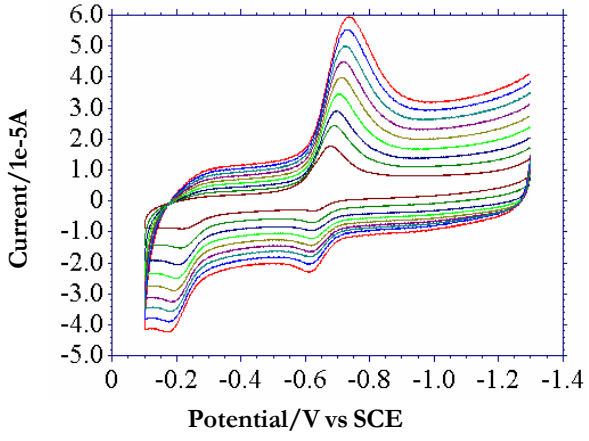

Figure 3: Cyclic voltammograms of emodin at the different scan rates
Scan rates from inner to outer: 100, 200, 300, 400, 500, 600, 700, 800, 900 mV/s
Other conditions same as Figure 2

have used this peak to research emodin in analytical park.

Effect of supporting electrolyte and pH

A series of supporting electrolytes were tested (disodium hydrogen phosphate, potassium nitrate, borax, acetate buffer, ammonium-hydrochloric buffer). Both the peak current and peak shape were taken into consideration when choose the supporting electrolytes and pH. The results showed that NH_3-NH_4Cl buffer solution (pH 7.2) gave the best response. So a 0.05 M NH_3-NH_4Cl buffer solution (pH 7.2) was selected as the supporting electrolyte.

Effect of scan rate

The effect of scan rate was studied by changing it from 100 to 900 mV/s. It was found that the peak current increased and the peak potential shifted to more positive value (P_2 and P_3) or negative value (P_1) with the increase of scan rate (Figure 3).The results show that peaks P_1 and P_2 are a pair of quasi-reversible redox peaks. Changing the initial potential between -0.1V and -1.3V, the same cyclic voltammograms were obtained. The results show that there is equilibrium between the oxidation and reduction forms in the buffer solution. P_3 is an irreversible anodic peak because no cathodic peak corresponding to it. Figure 4 shows the relationship between peak current i_p and the scan rate. The three plots of i_p vs. υ all gave a straight line. The results show that the quasi-reversible redox reaction and the irreversible oxidation reaction are all adsorption-driven process.

Relationship between the peak current i_p (P_3) and emodin concentrations

Emodin possesses 9, 10-anthraquinone structure and is one of the primary active ingredient of the traditional chinese medicine — rhubarb. There are several 9, 10-anthraquinone derivative ingredients contained in rhubarb. In order to avoiding interfere,

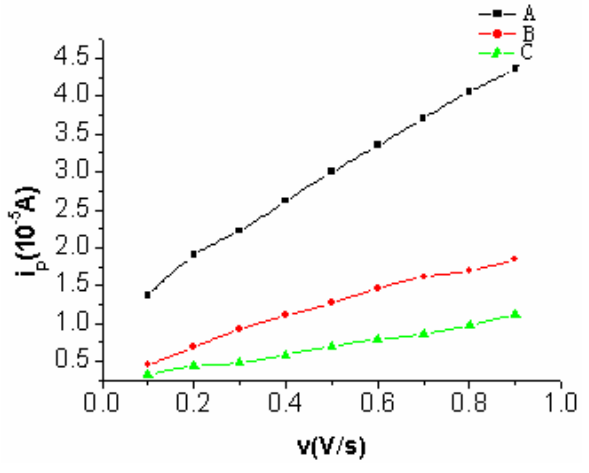

Figure 4: The relationship between peak currents and the scan rates line A — i_{p1} vs. υ; line B — i_{p3} vs. υ; line C — i_{p2} vs. υ

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the typical oxidation peak of emodin, P_3, was chosen and differential pulse voltammetry (DPV) was employed for establishing analytical method.

Because the electrochemical reaction of emodin is adsorption-driven process, pre-concentration can enhance detection sensitivity. Pre-concentration potential (E_{pre}) and time (t_{pre}) were investigated carefully. E_{pre} = -0.620V and t_{pre} = 80s were chosen. The peak P_3 shape was taken into consideration when choose the value of pH. So a 0.05 M NH_4Cl-HCl buffer solution (pH 5.7) was used. Under the select condition, the peak currents are linear relationship with emodin concentrations in the range of 8.9×10^{-8} M~7.8×10^{-6} M with the detection limit of 7.8×10^{-9} M. The linear regression equation and correlation coefficient are:

$$i_p\ (10^{-7}\text{A}) = -0.1063 + 0.2520\ C_{emdin}\ (10^{-7}\text{ M}) \quad \gamma = 0.997$$

Analytical application

Using the estiblished method, emodin in real sample-Sanhuang tablets (made from rhubarb) was determined. In order to validate the veracity, emodin standard solution was added in sample for detecting recovery. The results are listed in TABLE 1. The times of parallel determination was eight. The average content of emodin in the sample is 0.2117mg/tablet and the average recovery of emodin is 99.34.

TABLE 1: Sample determination

No	Content (mg/tablet)	Emodin added (10-2mg)	Emodin found (10-2mg)	Recovery (%)
1	0.2013	1.499	1.532	102.2
2	0.2310	1.499	1.480	98.74
3	0.2100	1.499	1.465	97.73
4	0.1998	1.499	1.547	105.0
5	0.2104	0.300	0.288	96.10
6	0.2098	0.300	0.276	92.00
7	0.2201	0.300	0.299	99.67
8	0.2109	0.300	0.310	103.3

Electrode process dynamics of emodin

1. The dynamics parameters of P_1 and P_2

For the adsorptive-driven peaks P_1 and P_2, based on the Laviron theory[8], there is the following equation:

$$i_p = \frac{nFQv}{4RT} \qquad (Q = nFA\Gamma_T)$$

This is means that the electron transfer number n can be calculated as long as the CV peak area Q is obtained under certain scan rate. From this, scan rates 50, 100, 300, 500, 700 mV/s respectively were preformed and n = 2 were obtained under all scan rates for the pair of P_1 and P_2.

Changing the solution pH from 4.12 to 8.91, the potentials of both P_1 and P_2 shift to more negative values. The slope of the E_p versus pH plot indicates that there are 2 H^+ participating in this electrode process. The linear regression equations are:

P_1: E_{red} = -0.10553-0.07345 pH
P_2: E_{ox} = -0.02094-0.07585 pH

The above results show that the number of protons and the number of electrons participating in this electrode process are 1:1, which is consistent with the previous work[2].

For a quasi-reversible interfacial reaction, we have obtained the value of α based on the following equation[9]:

$$E_p = k + \frac{RT}{\alpha nF}\ln v$$

Figure 5 shows the plot of E_p (P_1) vs. lnυ. By the slope value, we know the transfer coefficient α = 0.47 for the quasi-reversible interfacial reaction.

The apparent rate constant can be calculated by following equation[8]:

$$\lg k_s = \alpha\lg(1-\alpha) + (1-\alpha)\lg\alpha - \lg\frac{RT}{nF\upsilon} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT},$$

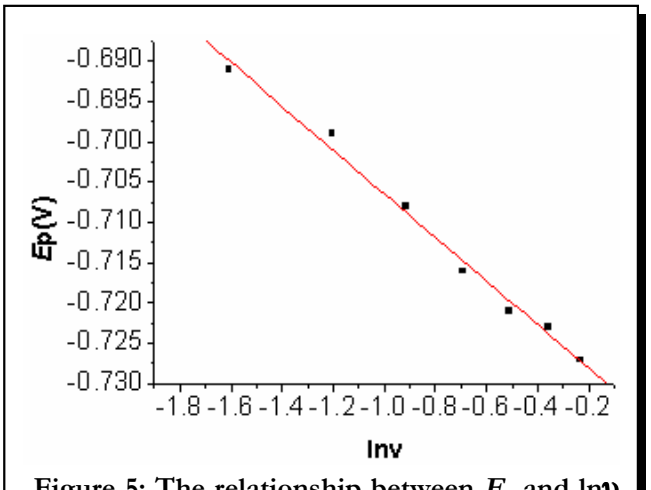

Figure 5: The relationship between E_p and lnυ

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when scan rates were 200, 300, 400, 500 mV/s, ΔE_p were 69.0 mV, 79.0 mV, 90.0 mV, 94.0 mV respectively. From this, we calculated the apparent rate constant K_s = 2.59 s^{-1}.

2. Dynamics parameters of P_3

For the adsorptive-driven P_3 and based on the Laviron theory[8], n = 2 was obtained. The linear regression equation of E_p versus pH is: E_{ox} = 0.378-0.07496 pH. From the equation and the Nernst equation: $E = E^{o} + \frac{RT}{nF}\ln\frac{[O]}{[R]} - \partial\frac{RT}{nF}\ln[H^+]$, we know the value of ∂: n = 1 (∂ was the number of protons).Then there are two electrons and two protons taking part in this reaction.

Based on the literature[8], we know α=0.5 for the irreversible reaction dominated by adsorption and the error is not more than 6%. So the value of αn is 1.0. In order to gain E^0, we made the plot of E_p vs. υ at the low scan rates. When the value of scan rate approached zero, the value of E^0 was -0.236V[10]. For an irreversible oxidation reaction controlled by adsorption, the following equation is obtained from Laviron theory[11]:

$$E_p = E^{\circ} - \frac{RT}{(1-\alpha)nF}\ln\frac{RT}{(1-\alpha)nF}\cdot\frac{K_s}{\upsilon}$$

When scan rates were 200, 300, 600, 700 mV/s, the average value of K_s = 2.11 s^{-1}.

CONCLUSIONS

The reaction of emodin at GCE has been investigated by electrochemical methods. The electrode process dynamics parameters were investigated and the same time, a sensitivity and good selectivity electroanalytical method for emodin was established. By this method erected, emodin was analyzed in traditional chinese medicines with satisfactory results and separation with other components was unnecessary.

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