A Highly-sensitive Voltammetric Sensor for Scutellarin Based On Poly-taurine Film Modified Electrode

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A highly-sensitive voltammetric sensor for scutellarin was fabricated via directly electro-polymerization of taurine at glassy carbon electrode. This sensor was simply obtained but displayed greatly improved response for scutellarin. The redox process of scutellarin was investigated systematically and some dynamic parameters of electrode reaction were calculated using electrochemical techniques in details. Under the optimum conditions, the linear range was from 8.0×10^{-8} to 4.0×10^{-6} mol L^{-1} with a low detectable limit 8.0×10^{-8} mol L^{-1}. The proposed method was used to determine scutellarin in commercial pharmaceuticals with satisfactory results..

1. Introduction

Scutellarin is the main flavonoid contained in medicinal herb scutellaria barbata D. Don, and it is also the chemical marker used by the Chinese Pharmacopoeia for evaluating the quality of Scutellaria barbata. Recently, many pharmacological studies in different animals and cells models have demonstrated that scutellarin possesses many pharmacological actions including neuroprotective effects (Machado, 2011), resisting liver fibrosis(Wang, et al., 2015), protecting against the liver injury induced by diosbulbin B (Niu, et al., 2015), inhibiting helicobacter pylori urease (Yu, et al., 2015), anti-inflammatory (Tan, et al., 2010), anti-apoptotic (Dai, et al., 2011), anti-HIV-1 efficacy (Zhang, et al., 2005). Thus it can be seen that scutellarin has important and comprehensive development values and a sensitive analytical method for scutellarin is highly needed. Some analytical method has been proposed, such as liquid chromatography with electrochemical detection(LC-ED), LC–MS/MS, HPLC-UV, capillary zone electrophoresis. Compared with these method, electroanalysis method was more rapidity, simplicity, convenience and the electrochemical techniques also help for identifying the redox characters of drug compounds and provide important information about pharmacological actions. To the best of our knowledge, although there were some articles reported about electroanalytical method in determining scutellarin, they were not very sensitive (Liu, et al., 2013; Ozalp, et al., 2009). So it is valuable to develop an sensitive electroanalytical method for scutellarin assay.

Nowadays, polymerizations of amino-acids have attracted considerable attention. Most of these are deposited by electrochemical oxidation, both potentiostatically or by cyclic voltammetry. The poly amino-acids modified electrodes were extensively applied in the determination of pharmaceutical drugs owing to their excellent electrocatalytic properties (Li，et al., 2013). Taurine (2-aminoethanesulfonic acid) is a well-known dissociated amino acid, which exhibits important physiological functions and pharmacological characteristics. It has been used to modified electrodes and applied in the electrochemical field. Generally, taurine was deposited onto bare glassy carbon electrodes (GCE) or modified GCE cycling the potential in a wide range. However, there are literatures reporting that this procedure leads to the formation of oxygen-containing functionalities and consequently to a graphite oxide film, which enhanced sensitivity towards cationic species, but not permselectivity against anionic ones. This is just advantageous to the determination under acidic conditions.

In this work, a simple and sensitive electroanalytical method for scutellarin was developed using poly-taurine modified glass carbon electrode (poly-taurine/GCE). The electrochemical properties of scutellarin were

investigated systematically at the proposed sensor. It was found that the new voltammetric sensor greatly enhanced the sensitivity for scutellarin with wide linear range. The present method was used to determine the real sample with satisfactory results.

2. Experiment

2.1 Instruments and reagents

Electrochemical experiments were carried out using a RST5000 electrochemical system (Zhengzhou Shiruisi Instrument Co.Ltd., Zhengzhou, China). A standard three-electrode system was employed, in which a GCE (3 mm diameter) or modified GCE was used as working electrode, a platinum(Pt) wire as auxiliary electrode and an Ag/AgCl as reference electrode.

All reagents were of analytical grade without further purification. Scutellarin and taurine were purchased from Aladdin Co. Ltd. (Shanghai, China). The standard stock solution of scutellarin (1×10^{-3} mol L^{-1}) was prepared with ethyl alcohol and stored under 4 °C. 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 low pH values of PBS were adjusted with 0.1 mol L^{-1} H_3PO_4. All solutions used in this work were prepared with doubly distilled water and all experiments were performed at room temperature..

2.2 Preparation of modified electrode

Prior to fabricate the poly-taurine/GCE , the bare GCE was initially polished to a mirror using 0.5 µm alumina slurry, and then washed ultrasonically with absolute alcohol and double-distilled water, respectively. Then the cleaned GCE was modified by electropolymerization of taurine (2 mM) in pH 7.0 solution, with a potential scan between -1.5 and 2.5 V at the scan rate of 0.1 V s^{-1} for 5 cycles. The modified electrode was then rinsed with double distilled water and used for further electrochemical studies.

3. Results and discussion

3.1 Electrochemical characterization of poly-taurine/GCE

To evaluate the characteristic of the modified electrode, $Fe(CN)_6^{3-/4-}$(5×10^{-3} mol L^{-1} containing 0.1 mol L^{-1} KCl) was selected as probe and cyclic voltammetry (CV) was performed. As shown in Figure1(a), compared with the bare GCE (curve a), the peak current was not changed very obviously after the modification of the GCE (curve b). While the charge current at the poly-taurine/GCE was increased obviously, which indicated that the area of the electrode was increased after the modification.

For further evaluation of the sensing characteristics, the EIS for bare GCE and poly taurine/GCE were recorded in 5 mM [$Fe(CN)_6^{3-/4-}$] as shown in Figure1(b). The semicircles obtained at lower frequency indicates the diffusion limited electron transfer process and charge transfer limited process for the higher frequency. As can be seen from Figure1(b), the semicircles diameter at poly taurine/GCE was smaller than that at bare GCE. These results demonstrated the excellent conductivity of the poly taurine film. Therefore, the polymer film could be efficiently used for the various types of electrochemical reactions.
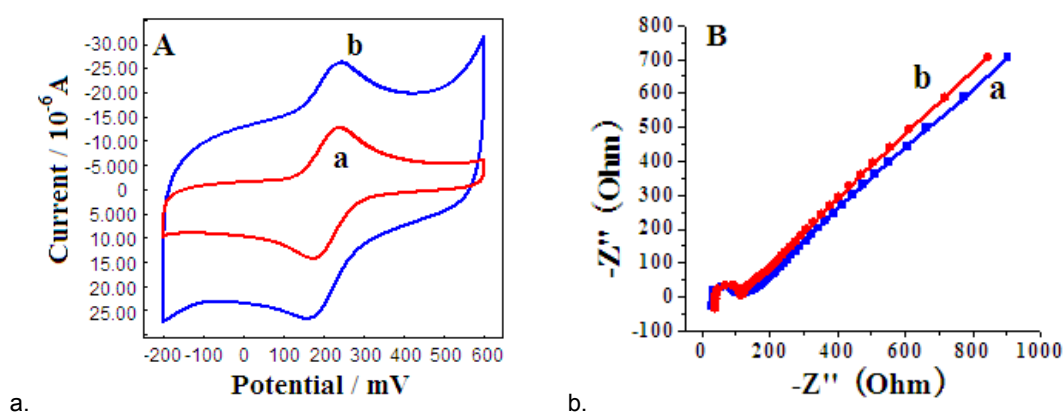

a.   b.

Figure1: a) Cyclic voltammograms of [$Fe(CN)_6$]$^{3-}$ (1.0×10^{-3}mol L^{-1}) containing 0.1 mol L^{-1} KCl and b) Nyquist plots of EIS with different electrodes at bare GCE and poly-taurine/GCE.

3.2 Electrochemical behavior of scutellarin at poly-taurine/GCE

The direct electrochemistry of scutellarin at poly-taurine/GCE was studied by CV in the potential window between -0.25 and 0.65 V. Figure 2 displayed the cyclic voltammograms of scutellarin (2×10^{-6} mol L^{-1}) at bare and poly-taurine modified GCE in PBS (pH 7.0) solution. As can be seen from Figure 2, there was a pair of very weak redox peaks at bare GCE following curve a. While at the poly-taurine/GCE following curve b, the peak currents of the redox peaks increased obviously. This improved response may be due to the introduction of N, O and S elements in the polymer film on GCE. And the above mentioned groups may provided more adsorption sites for analyte rich in hydroxyl though hydrogen bonding. Curve c was the voltammogram of poly-taurine/GCE in blank PBS, which indicated that the proposed electrode was electrochemical inert in the potential window studied.
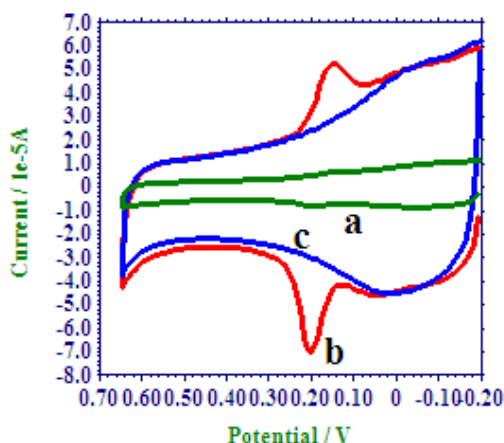

Figure 2: Cyclic voltammograms of scutellarin at bare GCE and poly-taurine/GCE and presented the blank voltammograms (Scan rate: 0.1 V s^{-1}).

3.3 Effects of solution pH and scan rate

The effect of pH value of solution on the reaction of scutellarin at poly-taurine/GCE was studied in a stationary concentration of scutellarin (2×10^{-6} mol L^{-1}). The changed from 3.0 to 9.18 pH was 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.18 and the superimposed voltammograms was shown in Figure 3(a). The redox peak potentials shifted negatively and the current also changed as pH value increased. The maximal current response was obtained at pH 7.0. Therefore, the PBS with pH 7.0 was selected for the determination of scutellarin. The relationship between the peak potential and pH value were shown in Figure 3B. Apparently, the plots of Epa and Epc versus pH value showed a good linear correlation: Epa= -0.068pH + 0.6893 (R=0.9973); Epc= -0.063pH + 0.5985 (R=0.9985). The slope of -0.068 V or 0.063 V per unit of pH was close to the theoretical value 0.059 V/pH, showing that the transferred electron number was equal to the number of protons.
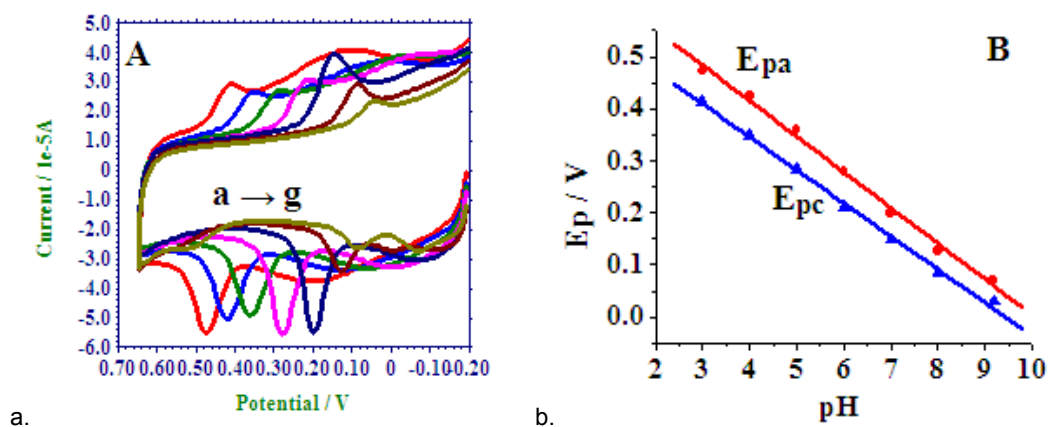

a.   b.

Figure 3: a) Cyclic voltammograms of scutellarin (2×10^{-6} mol L^{-1}) at poly-taurine/GCE with different pH of PBS and b) The relationship between the peak potential and pH (Scan rate: 0.1 V s^{-1}).

The effect of scan rate on the electrochemical properties of scutellarin was studied by varying the scan rate from 0.1 to 0.4 V s^{-1} in PBS at pH 7.0 at Figure 4.
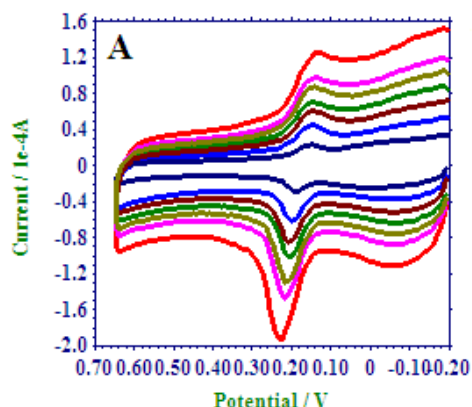

Figure 4: The voltammograms of scutellarin with different scan rates (from inner to outer: 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4 V s$^{-1)}$).

Both the anodic and cathodic currents increased linearly with the increase of the scan rate. The relationships between i_{pa} and i_{pc} and v respectively is shown in Figure 5.
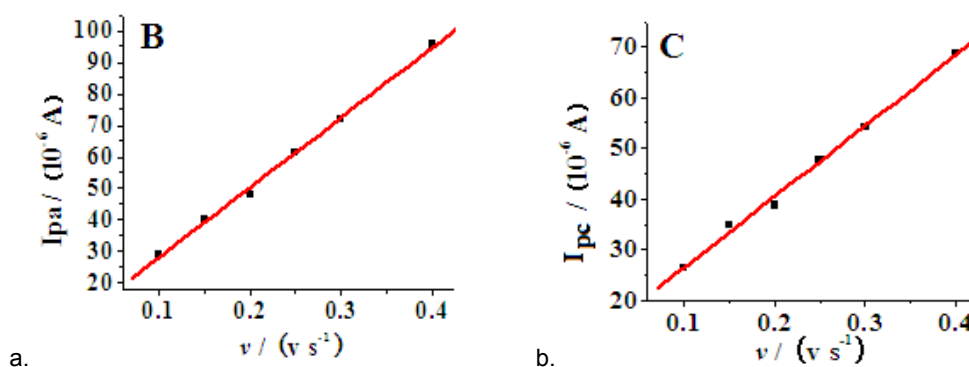

Figure 5: The relationships between a) i_{pa} and b) i_{pc} and v respectively.

The linear regression equations were expressed as i_{pa}= 221.3v +6.113(R=0.9985) and i_{pc}= 140.2v +12.42 (R=0.9979). These results suggested that the electrode reaction of scutellarin is an adsorption-controlled process. According to Laviron's theory, the electron-transfer number (n), transfer coefficient (α) and apparent rate constant (k_s) could be deduced using the following equation.

$$i_p = \frac{n^2 F^2 v A \Gamma}{4RT} = \frac{nFQv}{4RT} \qquad (1)$$

$$\lg k_s = \alpha \lg(1-\alpha) + (1-\alpha)\lg\alpha - \lg\frac{RT}{nFv} - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT} \qquad (2)$$

where Q is the peak area (known quantity) of certain scan rate; k_s is the standard heterogeneous reaction rate constant. R, T and F have their usual meanings (R=8.314 J mol^{-1} K^{-1}, T=298K, and F=96,487 C mol^{-1}); According to Eq. (1), the electron-transfer number n was calculated to be 2. We could also obtain the k_s value 1.42 s^{-1} with the assumed condition α=0.5 in Eq.(2). Therefore, a mechanism of scutellarin redox at the poly-taurine/GCE was proposed as following
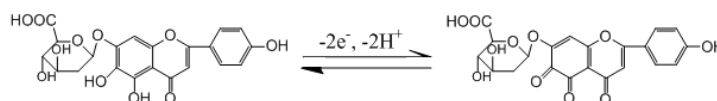
3.4 Adsorption of scutellarin at poly-taurine/GCE measured by chronocoulometry

The multi-potential steps chronocoulometry was employed to determine Q_{ads} of scutellarin at poly-taurine/GCE based on the formula given by Anson.

$$Q = 2nFAC_0 (Dt)^{1/2}\pi^{-1/2} + Q_{dl} + Q_{ads} \qquad (3)$$

Where Q_{dl} is the double-layer charge, Q_{ads} is the Faradaic charge due to the oxidation of adsorbed scutellarin. The Q-t curves were shown in Figure 5(a). Extracting data from Figure 5(a), the corresponding Q-$t^{1/2}$ curves of oxidation process were obtained as shown in Figure 5(b) and Figure 5(c). Curves a_1' and a_2' were obtained in blank solution and curves b_1' and b_2' were in scutellarin solution (2×10^{-5} mol L^{-1}). At the first step (-0.25 to 0.65 V), the corresponding Q~$t^{1/2}$ plot of curves a_1' and b_1' at Figure 5(b) were calculated with the linear equations of Q (10^{-4} C)=0.45 $t^{1/2}$+0.748 (R=1, curve a_1') and Q (10^{-4} C)= 0.59 $t^{1/2}$+1.418 (R=0.9998, curve b_1'), respectively. The linear equations of Q (10^{-5} C)=-4.9$t^{1/2}$+21.95 (R=0.9995, curve a_2') and Q (10^{-5} C)= -5.4 $t^{1/2}$+29.93(R=0.9965, curve b_2') were obtained for curves c_2' and d_2' at Figure 5(c), As shown in Figure 5(b) and Figure 5(c), the two straight lines were almost parallel in the absence and presence of scutellarin, further indicated an adsorption-controlled electrode process. According to the intercepts difference, the value of Q_{ads} was calculated to be 6.7×10^{-5} C (oxidative process) and 7.98×10^{-5} C (reductive process). The surface coverage (Γ_{max}) of scutellarin could be derived to be 4.89×10^{-9} (oxidative process) and 5.82×10^{-9} mol cm^{-2} (reductive process) at poly-taurine/GCE combining with Laviron's theory of $Q_{ads}= nFA\Gamma^*$.
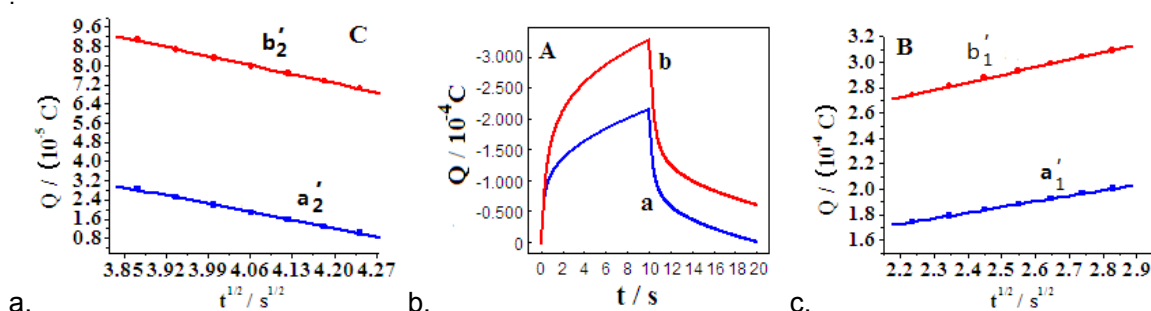

Figure 6: a) Chronocoulometric curves of poly-taurine/GCE in the absence and presence of 2.0×10^{-5} mol L^{-1} scutellarin. The corresponding Q ~ $t^{1/2}$ plots b) for oxidative process and c) for reductive process.

3.5 Calibration curve and detection limit

Firstly, to compare the sensitivity of different electrochemical methods, the volt-ampere curves of CV, linear sweep voltammetry (LSV), SWV and DPV were recorded in a scutellarin solution with a constant concentration. As a result, DPV had the best response for scutellarin and was selected to establish analytical method for scutellarin. The DPV responses of scutellarin was increased with increasing amount of analyte added. The peak currents increased linearly with the concentration of scutellarin in the range of 8.0×10^{-8} to 4.0×10^{-6} mol L^{-1} (data not shown). The linear regression equation could be expressed as I_P(10^{-6} A)=28.69C(10^{-6} mol L^{-1})+3.366 (R=0.9978) and the detection limit was 8.0×10^{-8} mol L^{-1}. This value was lower than that reported in literatures (Liu, et al., 2013; Cai, et al., 2013). Therefore, the sensitive determination of scutellarin was implemented at poly-taurine/GCE.

3.6 Interference, reproducibility and stability

Some possible interference for the detection of scutellarin was investigated in a 1×10^{-6} mol L^{-1} scutellarin solution by DPV. For controlling the peak current response error ≤5%, 100-fold concentration of Cu^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, Ca^{2+}, Cl^-, SO_4^{2-}, NO_3^-, 50-fold glucose and starch had no influence on the signals of scutellarin. In addition, the selectivity was also studied in the presence of 50-fold UA and AA Supporting electrolyte pH 7.0 PBS. The DPV curves demonstrated that the peaks of UA appear at more positive potential (0.305 V) and the current response for scutellarin decreased a little, which may be due to the competitive adsorption between scutellarin and UA. In the case of AA added, there was no new peak observed and the current was almost the same. These results indicated that the proposed electrode had credible selectivity toward the determination of scutellarin.

Next, the reproducibility of this method was estimated. The relative standard deviation (RSD) of response current of 1×10^{-6} mol L^{-1} scutellarin at five electrodes was obtained as 3.7%. The RSD of the peak currents was 4.3% for five parallel determination with one electrode. To study the stability of poly-taurine/GCE, the

electrode was kept for two weeks, the peak current of scutellarin only reduced 6.2%. These results proved that the proposed method had excellent reproducibility, repeatability and long-term stability.

3.7 Determination of scutellarin in real sample

To evaluated the practicality of the proposed method, it was employed to determine scutellarin in breviscapine tablets. Firstly, the tablets were finely powdered in a mortar; then, 0.1013 g of the powder was transferred into a 100 mL beaker containing 25 mL methanol and sonicated for 1 h. Next, the suspension was filtrated and the volume of filtrate was supplemented to 25 mL with methanol. The recoveries of detection scutellarin were satisfactory with values ranged from 98.2 to 107.5%. Further, the content of scutellarin in commerical tablet was calculated to be 19.87 mg/tablet, which was consistent with the label. These results demonstrated the ability of poly-taurine/GCE for scutellarin assay with high sensitivity and good reproducibility.

4. Conclusions

In conclusion, a simple but high-sensitive sensor, poly-taurine/GCE, was fabricated by electropolymerization of taurine at glassy carbon electrode by successive CV scanning. As a voltammetric sensor, poly-taurine/GCE could be obtained simply, and also showed improved response for scutellarin assay. Consequently, a new voltammetric method for determination of scutellarin was proposed. The advantages of present method were high sensitivity and good accuracy. In practical application investigations, the poly-taurine/GCE was utterly selective response for scutellarin without any other interference signal, and the detection results were satisfactory.

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