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Study on the Electrochemical Behaviour of Apigenin and its Interaction with DNA

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The electrochemical behavior of the antitumor herbal drug apigenin was studied in 0.1 M B-R buffer solution (50% ethanol, pH 9.0) by cyclic voltammetry (CV), normal pulse voltammetry (NPV), chronoamperometry (CA) and chronocoulometry (CC) at glassy carbon electrode. In CV, only one irreversible anodic peak with E_p = 0.580V was appeared at scan rate of 50 mV/s and a new electroanalytical method for this herbal drug was established according to this anodic peak. The peak currents are linearly relationship with apigenin concentrations in a range from 5.0×10^{-6} M to 9.0×10^{-5} M. Using the established method without pre-separation, apigenin in herbal drug was determined with satisfactory results. Moreover, the electrode process dynamics parameters were also investigated by electrochemical techniques and the possible electrode reaction mechanism was deduced. We also study the interaction of apigenin with DNA by DPV and Ultraviolet –Visible (UV) spectra. The results show apigenin doesn’t interact with DNA under the conditions.

1. Introduction

Chinese herbal drugs especially anticancer herbal drugs have attracted great interest in recent researchers (Huang et al, 2016). Herbal medicines were one of the major resources for health-care in early eras in China. Furthermore, herbal medicines are important for drug development now and scientists become aware that herbal medicine is an almost infinite resource for findiing new drugs. Many active ingredients of Traditional Chinese Medicine were found and their molecular structures were determined. Apigenin is a flavone found in vegetables, seasonings and oranges, and it possesses antioxidant activity in vitro. Potent biological effects have been described in vitro and in vivo including anti-carcinogenic, anti-inflammatory, and antimutagenic (Liu et al, 2016).

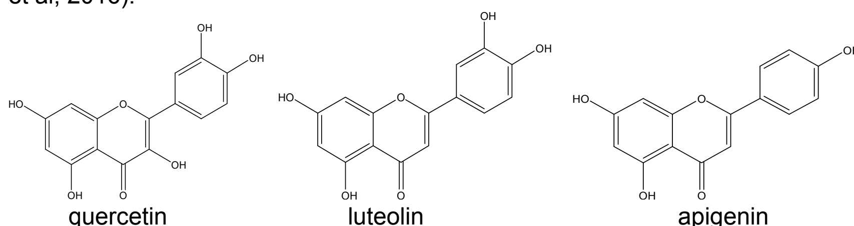


Figure 1: Molecular structures of apigenin, luteolin, and quercetin

Analysis of herbal medicine is an important technique, which offers many applications in biochemical, pharmaceutical and clinical research. Although the high-performance liquid chromatography (HPLC) has been used often for analysis of the flavonoids including apigenin (Qiao and He, 2011) use of HPLC for analysis of traditional Chinese medicines often have shortcomings such as long analysis time, low resolution, and short column lifetime, owing to easy contamination. Micellar electrokinetic capillary electrophoresis (MEKC) (Česla et al, 2012), thin-layer chromatography (TLC) (Cieśla et al, 2013) and gas chromatography (GC) (Nolvachai and Marriott, 2013) have also been used for this purpose. These methods rely on photoabsorption detection, and their sensitivities are relatively low and need relatively heavy and costly instrumentation. In this approach,

we develop a new electroanalytical method, voltammetric method, which is relatively sensitive, simple, quite rapid and reasonably cheap. It can unveil the messages about the reaction mechanism and the dynamics parameters of analytes. In the investigation of apigenin, voltammetric technique is very helpful in understanding the pharmacological effect and the antineoplastic mechanism. There are often three flavones (apigenin, luteolin, and quercetin) with similar structure (Fig. 1) coexisting in the same sample and it's hard to detect the three flavones separably at the same time. The aim of this work is to develop a new analytical method without separation and present the basic dynamics data about apigenin, which is useful for clinic study of apigenin. There is no need to separate other flavonoids contained in sample. By using this method, apigenin in real sample of Traditional Chinese Medicine was determined with satisfactory results. We also study the interaction of apigenin with DNA by DPV and UV spectra.

2. Experimental

2.1 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) (Φ = 3 mm, A = 7.07×10-2 cm2) as working electrode, a platinum (Pt) wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode respectively.

Stock solutions of 1.000×10-3 M apigenin, quercetin and luteolin (Checkout Institute of Biology Drugs, China) were prepared with nonaqueous ethanol as solvent and stored at 4°C. Flos buddlejae (Traditional Chinese Medicine) was gotten from herbal shop. Fish tests DNA (Shanghai Sangon Company, China) solutions 1.0 mg/mL was prepared with doubly distilled water. Other chemicals used in this study were analytical grade. Doubly distilled water was used for all preparations. N2 was employed to deoxygenize and all experiments were carried out at room temperature.

2.2 Procedure

Supporting electrolyte was 0.1 M B-R buffer solution (pH 9.0). In all cases, 50% ethanol was added because of the very low solubility of apigenin in aqueous solutions.

Sample preparation: 5g dried Flos buddlejae was powered in a mortar and extracted with 100mL ethanol at 70°C. The extract was concentrated under reduced pressure. After filtering extracts were quantified to 100mL with ethanol.

3. Results and discussion

3.1 The cyclic voltammetry behavior of apigenin at GC electrode

Cyclic voltammetry (CV) was performed in a standard three-electrode electrochemical cell. Fig.2 shows the cyclic voltammogram of apigenin (5.000×10-4 M) in B-R (pH 9.0) buffer solution. An anodic peak appeared at potential of 0.580V (Ep) with scan rate of 50 mV/s. Obviously, this is an irreversible electrode reaction process because no corresponding cathodic peak appears. That is, apigenin can only be oxidized and can't be reduced at electrode surface. This character may explain why apigenin possesses antioxidant activity in vitro.

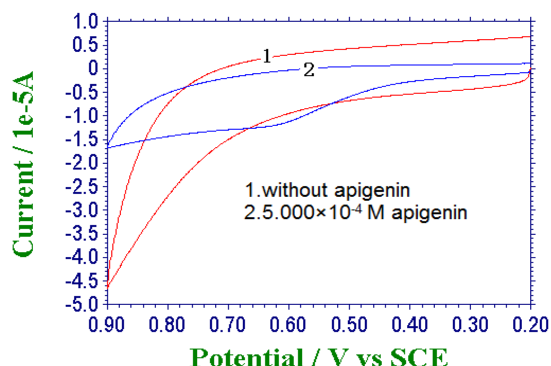


Figure 2: The cyclic voltammogram of apigenin in B-R (pH 9.0) buffer solution at the scan rate of 50 mV/s

3.2 Effect of supporting electrolyte and pH

A series of supporting electrolytes were tested (potassium nitrate, borax, acetate buffer, ammonium-hydrochloric buffer, B-R buffer). Both the peak current and peak shape were taken into consideration when

selecting the supporting electrolytes and pH. The results showed that B-R buffer solution at pH 9.0 gave the best response. In this study, we selected a 0.1 M B-R buffer solution (pH 9.0) as the supporting electrolyte.

3.3 The relationship between scan rate and peak currents

The effect of scan rate was studied in the range from 10 to 130 mV/s. It was found that the peak current increased and the peak potential shifted to more positive value with the increase of scan rate. The plot of i_p vs. $\upsilon^{1/2}$ gives a straight line. The result shows that the irreversible oxidation reaction is a diffusion-driven process.
We immersed the GCE into the apigenin solution for 10 min and shifted it to 0.1mM $K_4[Fe(CN)_6]$ or the buffer solution without apigenin to perform CV scan. The CV curve of $K_4[Fe(CN)_6]$ (Fig.3, curve 2) changed little compared to CV cuurve of no immersing electrode in apigenin solution (Fig.3, curve 1) and we can't find the oxidation peak of apigenin in the buffer solution. The result shows that there is no adsorption of apigenin on the surface of GCE. But when the GCE was performed cyclic scan in apigenin solution and then shifted it to 0.1mM $K_4[Fe(CN)_6]$. We could not find the redox peak of $K_4[Fe(CN)_6]$ by CV (Fig.3, Curve 3). This means that the production of apigenin oxidation is adsorbed strongly on the surface of GCE.

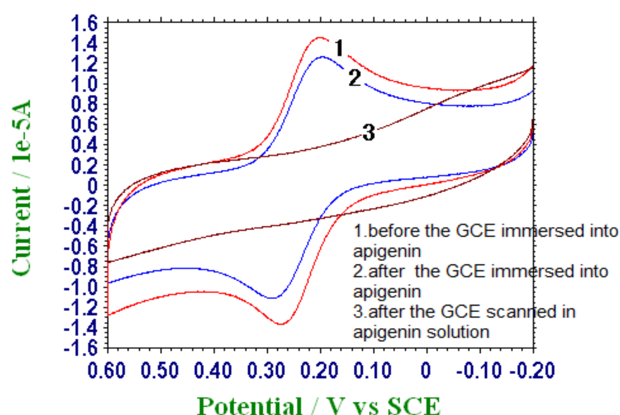


Figure 3: The cyclic voltammograms of 0.1 mM $K_4[Fe(CN)_6]$

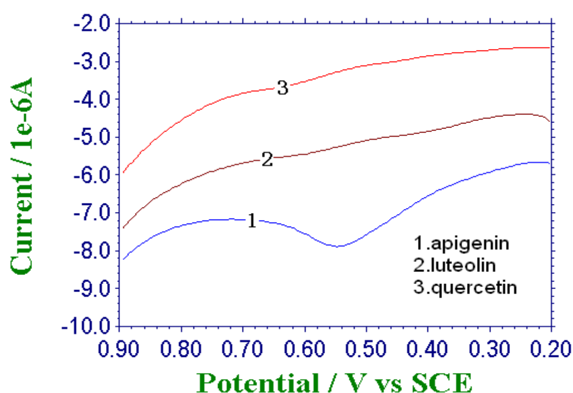


Figure 4: The DPV voltammograms

3.4 Relationship between the peak current ip and apigenin concentrations

From the Fig.4 we know luteolin and quercetin have no peak within used potential windows. So they don't interfere with the detection of apigenin. For the low electrochemical activity of apigenin at GCE, we choose differential pulse voltammetry (DPV) as detect mean to establish analytical method. Furthermore, the conditions of experiment were optimized for enhance the detection sensitivity. So the pulse width (0.01s) and the pulse period (0.5s) were selected for analyzing apigenin. Under such optimized condition, the peak currents are linearly relationship with apigenin concentrations in the range of 5.0×10^{-6} M ~ 9.0×10^{-5} M with detection limit of 1.5×10^{-6} M. The linear regression equation and correlation coefficient are:

$$i_p(10^{-8}A) = -1.4417 + 0.7123C_{apigenin}(10^{-6}M) \qquad \gamma = 0.998$$

3.5 Analytical application

Using the established method above, apigenin in real sample-Flos buddlejae was determined. The sample pretreated was mentioned in 2.2. We took 5.0mL of the pretreated sample solution as the object which we were going to determine. In order to validate the veracity, apigenin standard solution was added into the sample solution for detecting recovery. The results were listed in table 1. The times of parallel determination was six. The average content of apigenin in the sample is 0.1053mg/g and the average recovery of apigenin is 98.76%.

Table 1: The results of sample determination and recovery

No	Detection result of the sample (mg/g)	Standard solution added (mg)	Standard solution found (mg)	Recovery (%)
1	0.1002	0.2700	0.2653	98.26
2	0.1097	0.2700	0.2603	96.41
3	0.1058	0.2700	0.2739	101.4
4	0.1044	0.2700	0.2589	95.89
5	0.1100	0.2700	0.2702	100.0
6	0.1017	0.2700	0.2716	100.6

4. Electrode Process Dynamics of Apigenin

The electron transfer number (n) of electrode process was detected by NPV in 5.0×10^{-4}M apigenin solution. For the irreversible electrode reaction we can obtain the n by the following equation (1):

$$E = E_{1/2} + 2.303\frac{RT}{\alpha nF}\log\frac{i_l - i}{i} \quad (1)$$

If we suppose the α = 0.5 for the irreversible reaction and the calculation error is not larger than 6%. So, the electron transfer number n was calculated equal to 1.

The proton number (∂) of parting in electrode reaction can be calculated by Nernstian equation (2):

$$E = E^{o} + \frac{RT}{nF}\ln\frac{[o]}{[R]} - \partial\frac{RT}{nF}\ln[H^{+}] \quad (2)$$

The linear regression equation of Ep versus pH is: E_p = 0.5028-0.07441pH. Based on both above equations, we calculated the value of ∂ = 1. Hence, one electron and one proton were involved in this electrode reaction process.

The electrode reaction is driven by diffusion, so the diffusion coefficient (D) can be determined by CA and CC techniques. Adding a step potential from 0.2V to 0.9V on electrode and solution contained apigenin 5.0×10^{-4M}, the i~t and Q~t curves were recorded. D can be calculated by Controll equation (3) and (4):

$$i(t) = \frac{nFAD^{1/2}C}{(\pi t)^{1/2}} \quad (3)$$

$$Q(t) = \frac{2nFAD^{1/2}Ct^{1/2}}{\pi^{1/2}} \quad (4)$$

The straight lines of i vs. t-1/2 and Q vs. t1/2 were obtained. The slope values of two curves were then utilized to calculate D. D was calculated as 1.11×10^{-7} cm^2/s by the slope value of i vs. $t^{-1/2}$ and 4.17×10^{-7} cm^2/s by the slope value of Q vs. $t^{1/2}$. Two kinds of results are approaching. The average value of D is 2.64×10^{-7} cm^2/s.

The electrode reaction apparent rate constant (K_f) can be detected using the method described by Haoqing Wu (Wu and Li, 1998). The following equation (5) and (6) is obtained adding a step potential on a plate electrode.

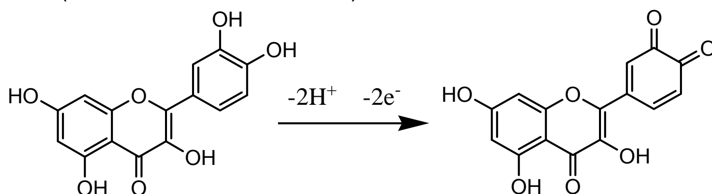
$$i(t) = nFAK_fC(1 - \frac{2H\sqrt{t}}{\sqrt{\pi}}) \quad (5)$$

$$H \equiv \frac{K_f}{D_{OX}^{1/2}} + \frac{K_b}{D_{RX}^{1/2}} \quad (6)$$

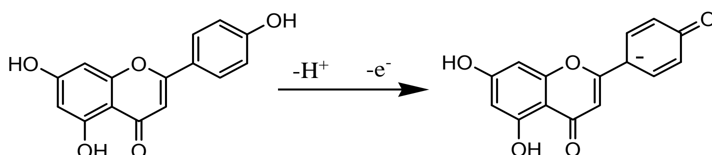
Because there was only an oxidation process of apigenin, the second part of H can be neglected. That is: $H = \frac{K_f}{D_{ox}^{1/2}}$. While t→0, the current i(t) is linear relationship with $t^{1/2}$. On plotting i(t) against $t^{1/2}$, we got the slope and intercept, and then the apparent rate constant K_f. was calculated, which was 1.3×10^{-3} s^{-1}.

5. The reaction mechanism of apigenin

Quercetin, another flavonoid compounds with similar structure of apigenin (Fig. 1), was studied by Hamid R. Zare (Hamid and Mansoor, 2005). The electrode reaction mechanism of quercetin is expressed as:



Their research was performed in 0.1M phosphate buffer (pH = 4.0). Quercetin have a cathodic peak with Epc= 0.315V and an anodic peak with Epa= 0.365V. From the structure of quercetin, we know the two neighboring hydroxyls are changed to two carbonyls by the oxidation reaction. So based on the number of electron transfer and proton involved in the electrode reaction of apigenin, we deduce the oxidation process may be expressed as:



From the deduced mechanism of apigenin, an intermediate of negative carbon free radical was formed. It may be just the free radical to polymerize and passivate the electrode surface, bring that there is no reduce peak apparent during reverse scan in CV, and there are no redox peak of K4[Fe(CN)6] (Fig.3, curve 3) after having scanned the electrode in apigenin solution.

6. The Interaction of Apigenin with DNA

PH 7.0, 0.1 M B-R buffer solution (50% ethanol) was chosen as supporting electrolyte. Fig.5 shows DPV of apigenin with and without adding DNA into apigenin solution. The peak current and the peak potential don't change after adding DNA. The result shows apigenin probably doesn't interact with DNA. We also did the UV spectra of apigenin and DNA. Fig.6 shows the UV spectra of DNA (curve 1) apigenin (curve 2), and apigenin-DNA (curve 3). DNA has an absorption peak at about 260 nm. Apigenin has three small absorption peaks at about 270nm, 325nm and 398nm respectively. One big absorption peak (curve 3) has been observed at about 270nm, which ascribes to the combination of DNA and apigenin. Curve 3 shows the absorption peaks at about 325 nm and 398nm don't change after adding DNA. So we believe that apigenin doesn't interact with DNA. The result is consistent with that from electrochemistry study. Apigenin shows the anticancer activity not by interacting with DNA but by other ways. This simultaneously exhibits the low toxic effect of apigenin to a certain extent.

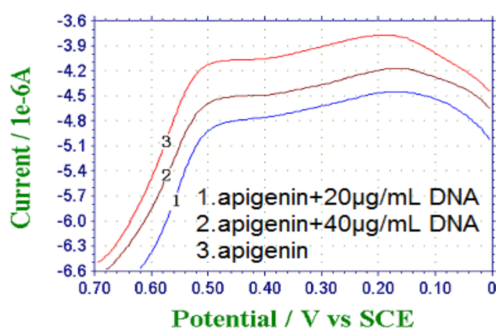


Figure 5: The DPV of apigenin with DNA

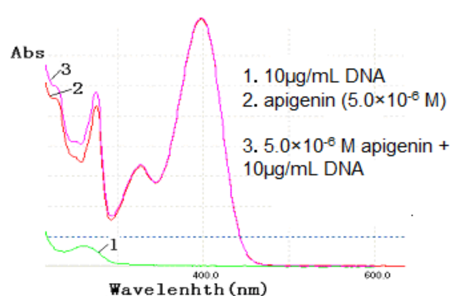


Figure 6: UV-vis spectra of apigenin and DN

7. Conclusions

The reaction of apigenin at GCE has been investigated by electrochemical methods. The electrode process dynamics parameters were investigated and the reaction mechanism was deduced. A direct electroanalytical method for determination of apigenin was established with high selectivity. Using this method, apigenin was directly analyzed in Traditional Chinese medicine samples with satisfactory results and no separation steps. We also studied the interaction of apigenin with DNA by DPV and UV spectra. The results show apigenin doesn't interact with DNA under the conditions.

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