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Study on the electrochemical behaviour of apigenin and its interaction with DNA

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ABSTRACT

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. 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.

KEYWORDS

Anti-cancer herbal drug;
Apigenin;
Electrochemical;
Dynamics parameters;
DNA.

INTRODUCTION

Chinese herbal drugs especially anticancer herbal drugs have attracted great interest in recent researchers. 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[1], and it possesses antioxidant activity in vitro[2,3]. Potent biological effects have been described in vitro and in vivo including anti-carcinogenic, anti-inflammatory, and antimutagenic[4–7].

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[8,9], 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)[10], thin-layer chromatography (TLC)[11] and gas chromatography (GC)[12,13] 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 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 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.

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} cm^2) 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. 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. N_2 was employed to deoxygenize and all experiments were carried out at room temperature.

THE CYCLIC VOLTAMMETRY BEHAVIOR OF APIGENIN AT GC ELECTRODE

Cyclic voltammetry (CV) was performed in a standard three-electrode electrochemical cell. Figure 1 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 (E_p) 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[2,3].

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[14]:

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

Based on the literature[15], we know that if we suppose the $\alpha = 0.5$ 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:

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

The linear regression equation of E_p versus pH is: E_p = 0.5028-0.07441 pH. Based on both above equations, we calculated the value of $\partial = 1$. 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^{-4}M, the *i*~t and Q~t curves were recorded. D can be calculated by Controll equation[14].

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

The straight lines of *i* vs. $t^{-1/2}$ and Q vs. $t^{1/2}$ 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[16]. The following equation is obtained adding a step potential on a plate electrode.

$$i(t) = nFAKfC\left(1 - \frac{2H\sqrt{t}}{\sqrt{\pi}}\right) \qquad H \equiv \frac{K_f}{D_{OX}^{1/2}} + \frac{K_b}{D_{RX}^{1/2}}$$

Because there was only an oxidation process of apige-

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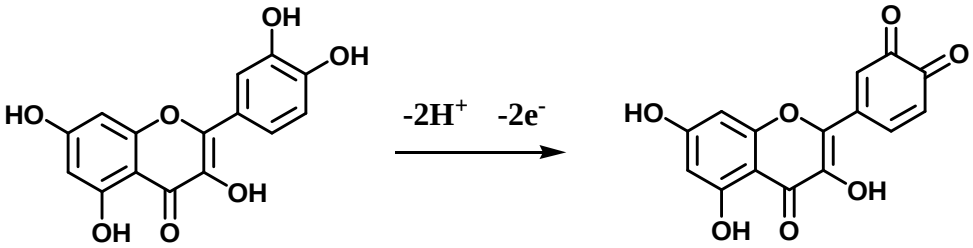
nin, the second part of H can be neglected. That is:

$H = \frac{K_f}{D_{OX}^{1/2}}$. 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 $1.3\times10^{-3}s^{-1}$.



Their research was performed in 0.1M phosphate buffer (pH = 4.0). Quercetin have a cathodic peak with E_{pc} = 0.315V and an anodic peak with E_{pa} = 0.365V. From the structure of quercetin, we know the two neighboring

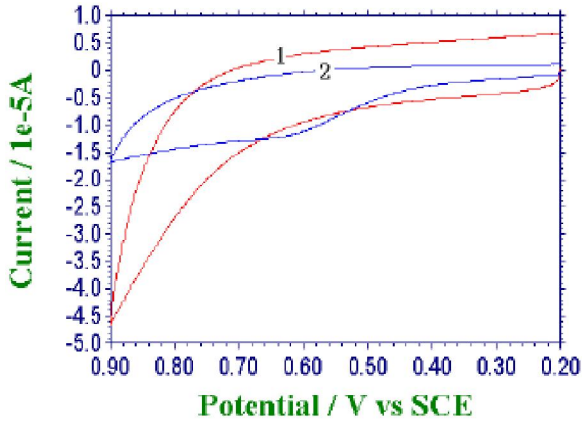


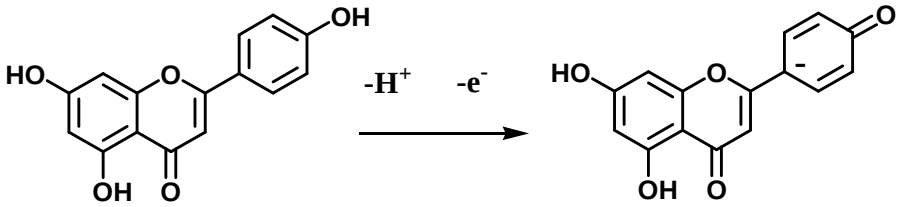
Figure 1 : The cyclic voltammogram of apigenin in B-R (pH 9.0) buffer solution at the scan rate of 50 mV/s Curve 1): the B-R buffer solution without apigenin; Curve 2): 1) + 5.000×10^{-4} M apigenin

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 $K_4[Fe(CN)_6]$ after having scanned the electrode in apigenin solution.

THE REACTION MECHANISM OF APIGENIN

Quercetin, another flavonoid compounds with similar structure of apigenin (Figure 1), was studied by Hamid R. Zare[17]. The electrode reaction mechanism of quercetin is expressed as:

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:



THE INTERACTION OF APIGENIN WITH DNA

pH 7.0, 0.1 M B-R buffer solution (50jpethanol) was chosen as supporting electrolyte. Figure 2 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. Figure 3 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.

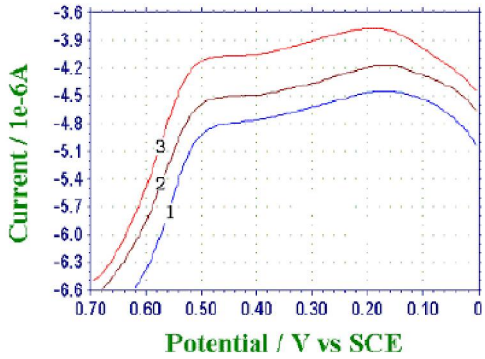


Figure 2 : The DPV of apigenin with different concentration of DNA in B-R (pH 7.0) buffer solution (50% ethanol); Curve 1): apigenin+20μg/mL DNA; Curve 2): apigenin+40μg/mL DNA; Curve 3): apigenin (5.0×10^{-6} M)

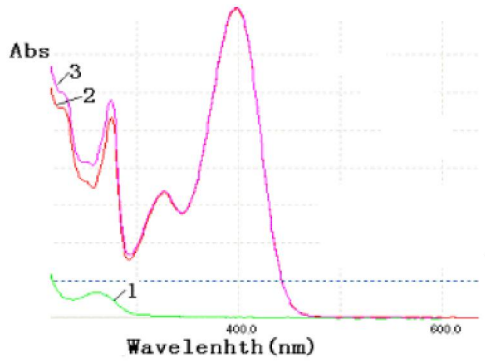


Figure 3 : UV-vis spectra of apigenin and DNA in B-R (pH 7.0) buffer solution; Curve1) 10μg/mL DNA; Curve2) apigenin (5.0×10^{-6} M); Curve3)5.0×10^{-6} M apigenin +10μg/mL DNA

REFERENCES

[1] B.Fernandez de Simon, J.Perez Ilzarbe, T.Hernandez, C.Gomez Cordoves, I.Estrella; Importance of phenolic compounds for the characterization of fruit juices. J.Agric.Food.Chem., **40**, 1531 **(1992)**.

[2] C.G.Fraga, V.S.Martino, G.E.Ferraro, J.D.Coussio, A.Boveris; Flavonoids as antioxidants evaluated by in vitro and in. situ liver chemiluminescence. Biochem.Pharmacol., **36**, 717 **(1987)**.

[3] S.A.B.E.Van Acker, D.J.Van den Berg, M.N.J.L.Tromp, D.H.Griffioen, W.P.Van Bennekom, W.J.F.Van der Vijgh, A.Bast; Structural aspects of antioxidant activity of flavonoids. Free.Radical.Bio.Med., **20**, 331-342 **(1996)**.

[4] D.F.Birt, D.Mitchell, B.Gold, P.Pour, H.C.Pinch; Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. Anticancer.Res., **17**, 85-91 **(1997)**.

[5] H.Wei, L.Tye, E.Bresnick, D.F.Birt; Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. Cancer.Res., **50**, 499-502 **(1990)**.

[6] S.J.Lee, K.H.Son, H.W.Chang, J.C.Do, K.Y.Jung, S.S.Kang, H.P.Kim; Antiinflammatory activity of.flavonoids: Mouse ear edema inhibition. Arch.Pharm.Res., **16**, 18-24 **(1993)**.

[7] M.L.Kuo, K.C.Lee, J.K.Lin; Genotoxicities of nitropyrenes and their modulation by apigenin tannic acid, ellagic acid and indole-3-carbinol in the Salmonella and CHO systems. Mutat.Res., **270**, 87-95 **(1992)**.

[8] E.N.Salka, O.D.Lars; Column-switching high-performance liquid chromatographic assay for determination of apigenin and acacetin in human urine with ultraviolet absorbance detection. J.Chromatogr.B., **713**, 379-386 **(1998)**.

[9] L.P.Li, H.D.Jiang, H.H.Wu, S.Zeng; Simultaneous determination of luteolin and apigenin in dog plasma by RP-HPLC. J.Pharma.Biomed.Anal., **37**, 615-620 **(2005)**.

[10] T.F.Jiang, X.Du, Y.P.Shi; Determination of flavonoids from paulownia tomentosa (Thunb) steud by micellar electrokinetic capillary electrophoresis. Chromatogr., **59**, 255-258 **(2004)**.

[11] M.Zelijam, M.S.Marica; Determination of phenolic glucosides in gentiana piasezkii by capillary zone electrophoresis. J.Pharma.Biomed.Anal., **24**, 353-359 **(2001)**.

[12] D.G.Watson, A.R.Pitt; Analysis of flavonoids in tablets and urine by gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry. Rapid.Commun.Mass.Spectro., **12**, 153-156 **(1998)**.

[13] R.Christov, V.Bankova; Gas chromatographic analysis of underivatized phenolic constituents from propolis using an electron-capture detector. J.Chromatogr, **623(1)**, 182 **(1992)**.

[14] Z.X.Zhang, E.K.Wang; Principle and methods of electrochemistry. China Science Press, Beijing., **(2000)**.

[15] E.Laviron; General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. J.Electroanal.Chem., **101**, 19-28 **(1979)**.

[16] H.Q.Wu, R.H.Li; Electrochemical kinetics. China Higher Education Press, Beijing and Springer-Verlag Berlin Heidelberg, **(1998)**.

[17] R.Z.Hamid, N.Mansoor, N.Navid; Electrochemical behavior of quercetin: Experimental and theoretical studies. Journal of Electroanalytical Chemistry, **584**, 77-83 **(2005)**.