Electrochemical Behaviours of Daidzein at Multi-Wall Carbon Nanotubes Modified Glassy Carbon Electrode

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A simple and sensitive analytical method is presented for voltammetric determination of daidzein based on its electrochemical oxidation at a multi-wall carbon nanotube modified glassy carbon electrode ((MWCNT/GCE). Compared with bare glassy carbon electrode, the MWCNT/GCE exhibited excellent catalytic and enhancement effect on the electrochemical oxidation of daidzein. A series of parameters including supporting electrolyte, pH of supporting electrolyte, scan potential and scan rate were optimized. Under the optimal conditions, the linear range of daidzein concentration was 6.0×10^{-6}-1.0×10^{-4} mol/L (R = 0.9991) with a detection limit of 7.2×10^{-7} mol/L (S/N = 3), and the RSD for 2.8×10^{-5} mol/L daidzein solution was 1.8 % (n = 11). This method was successfully used in the determination of daidzein in tablet. The mechanism investigation showed that the reaction of daidzein at the modified electrode was a process with one proton and one eletron transfer.

Key Words: Carbon nanotube, Chemically modified electrode, Daidzein, Electrochemical behaviour.

INTRODUCTION

Isoflavonoids are plant polyphenolic antioxidants[1] which are primarily found in plants of the leguminosae, especially in soybean[2]. Recent research reveals that isoflavonoids also exist abundantly in Radix Puerariae (RP)[3] which was used in traditional medicine in China. One of the major isoflavonoids occurring in Radix Puerariae is daidzein (7,4'-dihydroxy-isoflavone)[4]. Investigations showed that daidzein has significant pharmacological and therapeutic effects in anoxia[5,6], cerebra lischemia[7,8] and cardio-cerebrovascular disease[9] and has anti-inflammatory[10,11], antitumor[12,13] and cardioprotective[14,15] effects. Consequently, daidzein has been broadly employed in medical and clinical applications. Several analytical methods such as high-performance liquid chromatography (HPLC)[16,17], ultraviolet spectrophotometry (UV)[18] and gas chromatography-mass spectrography (GC-MS)[19,20] have been utilized for quantitative determination of daidzein. However, both HPLC and GC-MS methods involve tedious processes and time-consuming procedures, while the UV method suffers from a lack of high sensitivity.

The molecular structure of daidzein contains electro-chemical active groups such as phenolic hydroxyl (Fig. 1), which make it possible to determine daidzein using an electro-chemical technique[21]. Carbon nanotubes have been extensively used in electrode modification for electrochemical studies,
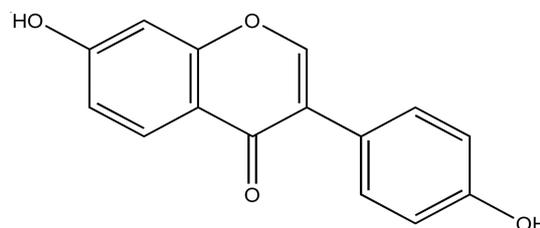
Fig. 1. Structure of daidzein

due to their high specific surface area, excellent structural, electronic and mechanical properties[22]. It could therefore be an effective material used in preparation of a modified electrode for daidzein measurement. However, to our best of knowledge, there is no report on the determination of daidzein using a multi-wall carbon nanotubes modified glassy carbon electrode (MWCNT/GCE).

The aim of this work is to develop a simple and sensitive electrochemical method to detect daidzein in pharmaceutic preparations using a modified electrode prepared by dropping the multi-walled carbon nanotube suspension, which was pretreated under a ultrasonic process, to the surface of a glassy carbon electrode (GCE). The electrochemical behaviour of daidzein at the modified electrode was investigated by cyclic voltammetry (CV) compared with that at the bare glassy carbon electrode. Experimental parameters of the analytical procedure were optimized and the method was applied to the determination

of daidzein in tablets. The possible electrode reaction mechanism was also discussed.

EXPERIMENTAL

All the chemicals used were of analytical grade and deionized water was used throughout the experiments. Daidzein was purchased from Chinese Chemicals and Biological Material Institute (Beijing, China). A stock solution of 1.0×10^{-3} mol/L daidzein was prepared by dissolving 0.0254 g daidzein in 2 mL ethanol and diluting to 100 mL, which was further diluted to a suitable concentration just before usage. The sulphuric acid stock solution (0.1 mol/L) was prepared by diluting 2.72 mL 98 % concentrated sulphuric acid (Shanghai Shiyi Chemicals Reagent Co. Ltd.) to 500 mL with deionized water. Multi-wall carbon nanotubes was obtained from Chengdu Institute of Organic Chemistry Co. Ltd., Daidzein tablets was purchased from Liaoning Yicheng Pharmaceutical Co. Ltd.

Cyclic voltammetric measurements were carried out with a CHI 600C electrochemical workstation (Shanghai CH Instruments, China). A three-electrode system was used, with glassy carbon electrode or MWCNT/GCE as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. Any ultrasonic oscillations of reagents and samples were performed using an ultrasonic cleaner (KQ-2200E, Kunshan Ultrasonic Instrument Co. Ltd., China). All the chemicals and samples were weighed using an electronic analytical balance (Mettler-Toledo Instruments Shanghai Co. Ltd).

Preparation of modified electrode

Pretreatment of glassy carbon electrode: Glassy carbon electrode (3 mm in diameter) was carefully polished with emery paper firstly and then was treated with alumina slurry on polishing cloth until a mirror was obtained; after rinsed with deionized water, the electrode was ultrasonically cleaned for 5 min in 1:1 (v:v) nitric acid, ethanol and deionized water, respectively and then dried in air.

Pretreatment of MWCNT and preparation of modified electrode: Before usage, MWCNT was pretreated for purification and functionalization purposes. 200 mg of MWCNT was ultrasonicated for 4.5 h in 50 mL of 6 mol/L HCl to remove impurities like amorphous carbon and metals. It was filtered and washed thoroughly with deionized water until the pH of the washing was 7. The purified MWCNT was further treated by ultrasonication in a mixture of nitric acid and sulfuric acid in 1:3 ratio for 6.5 h. It was then washed several times with deionized water until the washing was neutral and dried at 100 ºC in a drying oven for 12 h. Afterwards, 10 mg of the pretreated MWCNT was dispersed into 10 mL of N,N-dimethylformamide by ultrasonication for 0.5 h. 12 µL of the uniform suspension solution of MWCNT was dropped to the surface of pretreated glassy carbon electrode using a micro-injector pipette. The electrode was dried under a infrared lamp until a steady MWCNT film was obtained.

Electrochemical measurements: Cyclic voltammetry was performed using 10 mL aliquots of solution containing between 6.0×10^{-6} and 1.0×10^{-4} mol/L daidzein with 0.5 mol/L NaCl and 1.0×10^{-4} mol/L H_2SO_4 added as a supporting electrolyte. The initial and final potentials were both set at

+0.3 V and the switching potential was set at +1.0 V. An equilibration period of 100 s, at 0 V, was used prior to each scan; the scan rate was 250 mV/s. To recover its electrochemical activity after each measurement, the electrode was scanned in a blank buffer solution until the resultant cyclic voltammetry curve became stable.

RESULTS AND DISCUSSION

Electrochemical behaviour of daidzein on MWCNT/GCE: Fig. 2 shows the cyclic voltammetric behaviour of 8.0×10^{-5} mol/L daidzein at various electrodes in a solution containing 0.5 mol/L NaCl and 1.0×10^{-4} mol/L H_2SO_4. It is seen that daidzein has a weak oxidation peak at glassy carbon electrode around +0.773 V which is not good enough for analytical purpose (curve 1); while daidzein exhibits a sensitive and well-defined oxidation peak at MWCNT/GCE around +0.814 V(curve 3), whose peak current is much greater than that obtained at GCE, indicating that electrode modified with multi-walled carbon nanotubes possesses very good catalytic activity towards the electrochemical oxidation of daidzein. During the reverse scan, daidzein shows a very weak reduction peak at MWCNT/GCE around +0.740 V, suggesting that the the oxidation products generated during the forward scan reduce at the electrode; as this reduction peak is quite weak, it could be considered that the electrode reaction of daidzein at MWCNT/GCE is a irreversible process.
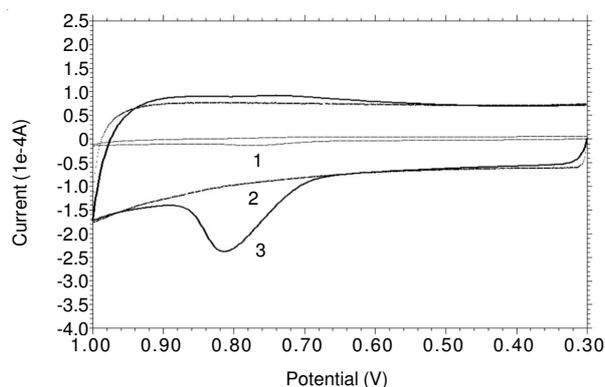
Fig. 2. Cyclic voltammograms of daidzein (8.0×10^{-5} mol/L) in 0.05 mol/L NaCl-1.0×10^{-4} mol/L H_2SO_4 solution (blank) at bare GCE (1), MWCNT/GCE (3) and blank at MWCNT/GCE (2). Scan rate: 250 mV s^{-1}

Optimization of experimental conditions

Selection of supporting electrolyte: The votammetric behaviours of 8.0×10^{-5} mol/L daidzein in 0.2 mol/L Na_2HPO_4-NaH_2PO_4, 0.2 mol/L NaOH, 0.2 mol/L AcOH-NaOAc, 0.2 mol/L NaCl, 0.2 mol/L H_2SO_4 and 0.2 mol/L HCl solutions at MWCNT/GCE were investigated, respectively. The results demonstrated that, daidzein showed a stable peak with good shape and high current in NaCl solution. The effect of NaCl concentration on peak current was further investigated in the range 0-0.5 mol/L, the results showed that the peak current increased with the increasing NaCl concentration, but when NaCl reached 0.5 mol/L, the peak current maintained constant, thus we used 0.5 mol/L NaCl as the supporting electrolyte in this study.

Effects of modified amount of MWCNT: The thickness of the MWCNT film on the GCE surface is determined by the amount of MWCNT-DMF suspension dropped on the GCE surface. The peak current increased significantly when the MWCNT suspension added onto GCE surface increased from 3.0-12.5 μL, while decreased after exceeded 12.5 μL. This is probably due to the compromise effect of the good electro-chemical performance of MWCNT film and the enhancement of resistance resulted from the increase in the thickness of the film. As a result, an appropriate amount for the fabrication of a MWCNT modified GCE is 12.5 μL of 1 mg/mL MWCNT suspension.
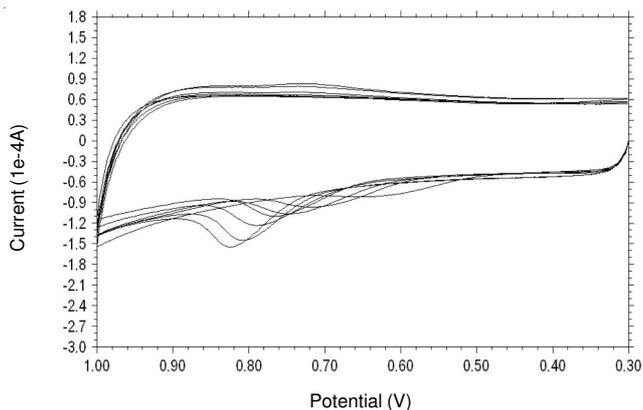
Fig. 3. Effect of pH (3.7, 4, 4.3, 4.9, 5.4, 6, and 7.4) on peak current and peak potential of 8.0×10^{-5} mol/L daidzein in 0.5 mol/L NaCl

Influence of pH: The effects of pH on the peak current and peak potential of 8.0×10^{-5} mol/L daidzein in 0.5 mol/L NaCl were investigated in the pH range of 2-10, the acidity of the solutions were adjusted using H_2SO_4 or NaOH. It was observed that daidzein peak current remained constant when pH value ranged from 2.0-3.7, while decreased from 3.7-7.4; it was also observed that some impurity peaks occurred when pH was less than 3.7 and some flocculations formed in the solution when pH was greater than 7.4. Taken together, a pH value of 3.7 was chosen as the optimum pH. As in that case the H_2SO_4 concentration in the solution was 1.0×10^{-4} mol/L, thus the supporting electrolyte solution was consisted of 0. 5 mol/L NaCl and 1.0×10^{-4} mol/L H_2SO_4.

It was also found that the oxidation peak potential of daidzein shifted negatively with the increase of pH values and the potentials (E_p) *versus* pH have a good linear relation in the pH range of 3.7-7.4 (Fig. 4). The linear regression equation is E_p (V) = 0.9967-0.0467 pH (r = 0.9985), suggesting that proton participated in daidzein oxidation reaction.

Effect of scan potential: It was showed that the variation of initial potential of the cyclic voltammetric scan did not make much difference in the peak current in the range of -0.2 to +0.3 V, therefore, the initial potential of +0.3 V was used in this experiment. However, the current decreased gradually and peak shape became worse when the switching potential was higher than +1.0 V. Thus, +1.0 V was employed in this research as the switching potential.

Effect of scan rate: The effect of potential scan rate on the cyclic voltammetric peak of daidzein was investigated in the range from 40-250 mV/s (Fig. 4). The peak current
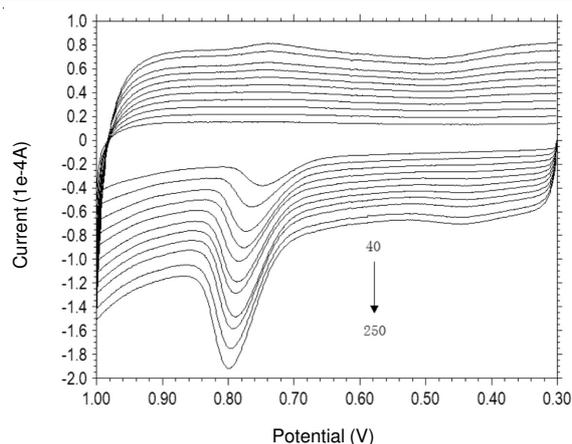
Fig. 4. Voltammograms of 8.0×10^{-5} mol/L daidzein at MWCNT/GCE with different scan rates. Scan rates: 40, 60, 80, 100, 120, 140, 160, 180, 200, 230 and 250 mV/s

increased with the scan rate, as shown in Fig. 5, and the square root of peak current has a good linearly relationship with the scan rate, the linear equation is I_p (μA) = 9.7468 $v^{1/2}$ (mV/s) -39.822 (r = 0. 9960), this indicated that daidzein reaction process is of the characteristics of diffusion controlled. In this study, a scan rate of 250 mV/s was employed.

It was also observed from Fig. 5 that the peak potential shifts slightly to more positive values, *i.e.*, from 0.76V to 0.80 V, with the increasing scan rate; and there is a linear correlation between the peak potential and the natural logarithm of scan rate (Fig. 6), the regression equation is E_p = 0.0238 ln v + 0.6675, r = 0.9980.
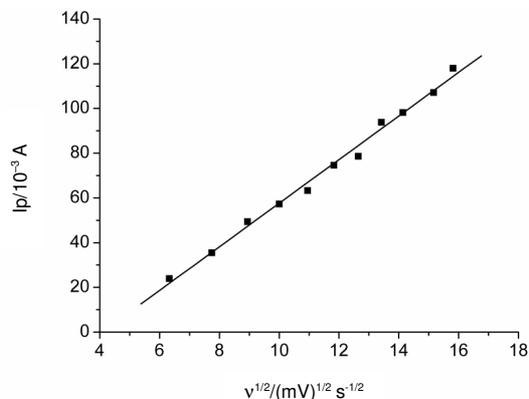
Fig. 5. Effect of scan rate on peak current
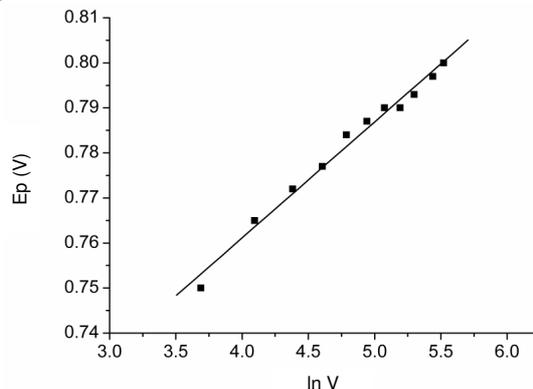
Fig. 6. Effect of scan rate on peak potential

Linear range and detection limit: The calibration curve of peak current *versus* daidzein concentration obtained under the optimal conditions was linear in the range 6.0×10^{-6}-1.0×10^{-4} mol/L (Fig. 7). The regression equation was I_p (µA) = 1.4792C (µmol/L) + 11.819, with a correlation coefficient of 0.9991. The detection limit was 7.2×10^{-7} mol/L and the relative standard deviation (RSD) for 2.8×10^{-5} mol/L daidzein solution was 1.8 % in 11 duplicate measurements.
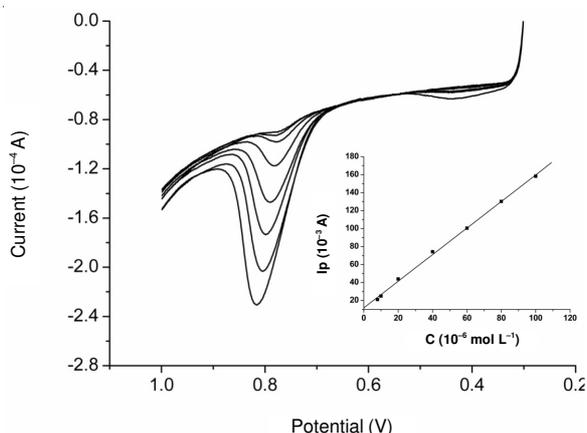
Fig. 7.   Linear scan voltammograms of daidzein with various concentration at MWCNTs/GCE. Daidzein: 0.006, 0.008, 0.01, 0.02, 0.04, 0.06, 0.08, 0.10 mmol/L; scan rates: 250 mV/s. Insert is the calibration curve of daidzein

Interference studies: To evaluate the selectivity of the present proposed method, effects of common inorganic ions, excipients and additives commonly used in pharmaceutical preparation were investigated by adding these potential interfering species into the 4.0×10^{-5} mol/L standard solution of daidzein. A substance was considered not to interfere when it caused a relative error in daidzein peak current no more than ± 5 %. The results showed that 6000-fold K^+, NO_3^-, 2000-fold Zn^{2+}, NH_4^+, Ca^{2+}, starch, 500-fold citric acid, dextrin, glucose, 250-fold lactose, sucrose, 30-fold Fe^{3+}, maltose, talc powder, 5-fold magnesium stearate, did not interfere with the determination of daidzein.

Analytical applications: Ten daidzein tablets, which were manufactured by Liao Ning Yicheng Pharmaceutical Co. Ltd. and purchased locally, were weighted and ground into a uniform fine powder. A portion of the powder, equivalent to about 50 mg of daidzein, was accurately weighed and dissolved in 100 mL of anhydrous ethanol in a brown flask, the solution was diluted appropriately with anhydrous ethanol to the linear range of determination. Under the optimal conditions, sample determination and recovery experiments were performed simultaneously. The results were shown in Table-1.

Mechanism of electrode reaction: For the irreversible electrode process, the relationship between the oxidation peak potential and scan rate is described by the equation[23]:

$$E_p = E_0 + 0.780\left(\frac{RT}{\alpha nF}\right) + \left(\frac{RT}{\alpha nF}\right)\ln\left(\frac{D^{1/2}}{k}\right) + \left(\frac{RT}{2\alpha nF}\right)\ln\left(\frac{\alpha nF}{RT}\right) + \left(\frac{RT}{2\alpha nF}\right)\ln v$$

where α is the transfer coefficient, D is the diffusion coefficient, k the rate constant for the electrode reaction, F is the Faraday constant, R is gas constant, n is the number of electron transfer.

As seen in previous section, the regression equation of daidzein peak potential and the natural logarithm of scan rate was $E_p = 0.0238 \ln v + 0.6675$. Therefore, the value of αn was calculated to be 0.5389 from the slope (0.0238) of the above equations. For most systems, the value of α is from 0.3-0.7. If assuming $\alpha = 0.5$, then, n was estimated to be 1 for the oxidation of daidzein at MWCNT/GSE.

In order to confirm the number of electron transfer in this electrode reaction, controlled potential electrolysis of 1.0 mL of 6×10^{-6} mol/L daidzein solution with MWNTs/GCE was performed at + 0.9 V for 860 s. The result shows that the net charge of the electrolysis is 7.3727×10^{-4} C. The number of electron transfer (n) was calculated by Faraday equation: n = Q/F (CV) = 1; here, Q = charge in coulombs, n = electron of transfer, F = Faraday constant, C = daidzein concentration, V = solution volume. The result was well agreed with that obtained from E_p equations.

On the other hand, due to the Nernst equation is $E_p = E_0 - 0.059$ (m/n) pH and the relationship of the daidzein potential and pH value obeys equation E_p (V) = 0.9967-0.0467 pH, thus, the number of proton (m) in the daidzein oxidation is easily calculated: 0.059 m/n = 0.0467, then m ≈ 1. This indicated that the reaction of daidzein at the MWCNTs/GCE electrode at +0.814 V was the process with one electron and one proton transfer.

The structure of daidzein shows that there are two phenolic hydroxyls connecting to A ring and B ring, respectively. The previous investigation showed that the reaction mechanism of flavonoid compounds mainly involved the participation of the phenolic hydroxyl in B ring[24], therefore, it was presumed that phenolic hydroxyl in B ring is more easily oxidized at a low potential than that in A ring, in addition, the semiquinone radical intermediate forming in B ring was more stable than that forming in A ring. In the investigation of flavonoid antioxidants, Zhang[25] found that the phenolic hydroxyl in B ring was more active than that in A ring and more easily oxidized. Based on the above results, the mechanistic pathway for the oxidation of daidzein was postulated as shown in Fig. 8.

Conclusion

A new electrochemical method for the determination of daidzein using a MWCNT/GCE based on the good electrochemical behaviour of daidzein at MWCNT/GCE was

TABLE-1								
DETERMINATION OF DAIDZEIN IN THE SAMPLE AND ITS RECOVERY TEST (n = 5)								
Sample lot number	Labeled amount (mg)	Detectable amount (mg)	RSD (%)	Added (mg)	Found (mg)	Recovery (%)	Average recovery (%)	RSD (%)
20100101	50.0	51.3	1.4	19.9, 59.6, 99.3	71.9, 114.2, 151.3	103.5, 105.5, 100.7	103.2	1.9
20100102	50.0	48.1	1.9	31.8, 63.6, 95.3	80.5, 108.8, 142.8	101.9, 95.4, 99.4	98.9	2.7
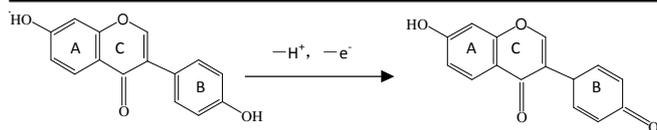
Fig. 8. Oxidation reaction mechanism of daidzein at MWCNT/GCE

demonstrated. The stoichiometric relationship between peak current and daidzein concentration make possible to sensitively determine daidzein even with a simple set-up. It was believed that the electrode reaction of daidzein was the process accompanied by one electron and one proton transfer. The prepared modified electrode is of good stability and durability. The proposed method is not only simple and convenient, but also sensitive and user-friendly. It had been applied to the determination of daidzein in tablets with satisfactory results.

ACKNOWLEDGEMENTS

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