Article

Electrochemical Behavior and Determination of Chlorogenic Acid Based on Carbon Nanotubes Modified Screen-Printed Electrode

Xiao-Yan Ma [1,2], Hong-Qiao Yang [1,2], Hua-Bin Xiong [1,2], Xiao-Fen Li [1,2], Jin-Ting Gao [1,2] and Yun-Tao Gao [1,2,*]

[1]   The Engineering Laboratory of Polylactic Acid-Based Functional Materials of Yunnan,
     School of Chemistry and Biotechnology, Yunnan Minzu University, Kunming 650500, China;
     18788481659@163.com (X.M.); yanghqiao2010227@163.com (H.Y.); xionghuabin@ynni.edu.cn (H.X.);
     wjplxf24@163.com (X.L.) 15198774152@163.com (J.G.)
[2]   Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry
     of Education, Yunnan Minzu University, Kunming 650500, China
*   Correspondence: ymz409@163.com or gaoyuntao@ynni.edu.cn

Abstract: In this paper, carbon nanotubes modified screen-printed electrode (CNTs/SPE) was prepared and the CNTs/SPE was employed for the electrochemical determination of antioxidant substance Chlorogenic acids (CGAs). A pair of well-defined redox peak of CGA was observed at the CNTs/SPE in 0.10 mol·L-1 acetic acid-sodium acetate buffer (pH 6.2) and electrode process is adsorption-controlled. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) method for the determination of CGA were proposed based on the CNTs/SPE. Under the optimal conditions, the proposed method exhibited linear ranges from 4.73×10-7 to 4.45×10-5 mol·L-1, the linear regression equation was Ipa(μA) = 4.1993 C (mol·L-1)+1.1039 (r = 0.9976) and the detection limit for CGA could reach 3.25×10-6 mol·L-1. The recovery of matrine was 94.74~106.65% (RSD = 2.92%) in coffe beans. The proposed method is quick, sensitive, reliable, and can be used for the determination of CGA.

Keywords: Chlorogenic acid; screen-printed electrode; carbon nanotubes

1. Introduction

Chlorogenic acids (CGAs) (Figure 1) are a group of polyphenolic compounds widely distributed in different plant materials including many foods and beverages [1], especially in coffee that has one of the highest concentrations of CGA of all plant constituents [2]. Many reports have indicated that diet rich in CGA compounds plays a great role in preventing various diseases associated with oxidative stress such as cancer, cardiovascular, aging and neurodegenerative disease [3,4].
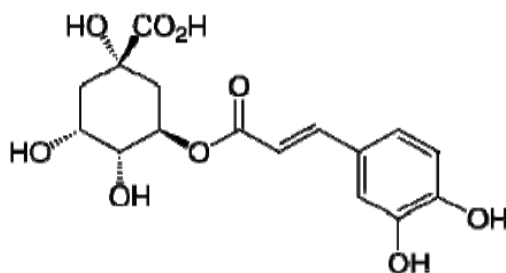

Figure 1. The structure of CGA

Several methods have been developed for determination of chlorogenic acid and its derivatives

in coffee beans and other plants. The most widely used methods are HPLC [5-7], capillary electrophoretic [8,9], and Miceller electrokinetic chromatography [10,11].   Although these developed methods have been efficient for the quantification of CGA and its derivatives, they have been criticized as being tedious, time consuming; and most of the instruments with these methods are very expensive. In addition, UV-Vis spectrophotometer method is simple, fast and inexpensive for the determination of CGA in coffee beans; however, a direct spectral determination in coffee beans is relatively difficult, because of the spectral overlap with caffeine.

During recent years, electrochemical methods have been widely investigated in the determination of phenolic compounds due to their simplicity, low cost, high sensitivity and rapid response [12,13]. Furthermore, caffeic acid as another important component of coffe can be proadly studied by cyclic voltammetry methods [14,15]. Nevertheless to the best of our knowledge, the electrochemical determination of CGA has been reported barely.

Screen-printed electrodes (SPEs) are especially recommended as a large-scale production of electrodes with the properties of ease of use and portability [16,17]. Also, miniaturized screen-printed electrodes are disposable and suitable for working with sample microvolumes [18,19]. Screen-printed electrodes modified with carbon nanotubes (CNTs/SPE) improve electronic transfer properties, resulting in high sensitivity and low detection limits, decreased overpotentials, ease of mass production, practicality [24], and they are described as useful electroanalytical tools for the development of analytical applications [20-23]

In this paper, we applied a simple and fast way to detect CGA with a high sensitive voltammetric analysis method by using carbon nanotubes material modified screen-printed electrode. Here, a carbon nanotubes material modified screen-printed electrode was prepared. The electrochemical behavior of CGA at CNTs/SPE was investigated, a sensitivity electrochemical analysis method of differential pulse voltammetry (DPV) was developed for the determination of CGA. Furthermore, the proposed method can be used to be the quantitative determination of CGA in coffe beans.

2. Materials and Methods

2.1. Instruments, materials and reagents

All electrochemical experiments were conducted with a ZAHNER Zennium IM6 Electrochemical Workstation (ZAHNER-elektrik GmbH & Co. KG, Kronach, Germany) with an integrated screen-printed three electrode device: a carbon working electrode, a carbon counter electrode, and an Ag/AgCl reference electrode.

The carbon nanotubes(CNTs, purity>95%) were purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences; The screen-printed electrodes(910 pastat mini, 80mm×54m×23mm) were purchased from Methrom, Co., Ltd. In China; The coffe beans(Pu`er University in Yunnan province, China); 0.10 mol·L^{-1} sodium hydrogen phosphate-potassium dihydrogen phosphate buffer, 0.10 mol·L^{-1} phosphate buffer solution (PBS), 0.10 mol·L^{-1} citric acid buffer, 0.10 mol·L^{-1} acetic acid-sodium acetate buffer, a certain concentration of Chlorogenic acid (purity>98%)was purchased from Sigma, USA, and saved at 4℃.

Other reagents used were of analytical-reagent grade. Twice-distilled water was used throughout all experiments.

2.2. Experimental Methods

2.2.1. Preparation of carbon nanotubes modified screen-printed electrode

Before modifying the working electrode at the integrated SPEs, the SPEs was first washed with distilled water and dried by N_2 stream. Then the SPEs was pre-anodized in a 0.1 M (pH = 7.4) PBS containing 0.1 M KCl by applying an anodic potential of +1.9 V (vs. Ag/AgCl) for 120 s. The CNTs/SPEs was prepared by coating 5 μL 0.3 mg·mL^{-1} of the CNTs homogeneous suspension onto the SPEs and then dried at room temperature overnight. All modified electrodes were cleaned by cyclic voltammetric technique between –0.5 and +0.5 V at a scan rate of 50 mV·s^{-1} in PBS (pH 7.4) until a stable cyclic voltammetric response was obtained, and then rinsed with water and dried under a nitrogen stream[25].

2.2.2. Electrochemical analysis

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed in the three-electrode cell in 0.10 mol·L^{-1} acetic acid-sodium acetate buffer solution (pH=6.2) between the potential range of -0.5 V and +0.6 V at a scan rate of 0.15 V·s^{-1}. The DPV conditions were: pulse width of 50 ms, pulse amplitude of 180 mV and pulse interval of 50 ms.

3. Results

3.1. The cyclic voltammetry and differential pulse voltammetry behaviors of CGA at CNTs/SPE.

Figure 2 displays the CV curve of CGA in the 0.10 mol·L^{-1} acetic acid-sodium acetate buffer solution (pH 6.2) at CNTs/SPE, and the scan rate is 0.15 V/s with the potential range from -0.5V to 0.6 V. A pair of redox peak was obtained at CNTs/SPE. The oxidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) of CGA were 0.08V and -0.19 V(vs. Ag/AgCl), respectively, $\Delta E = E_{pa}-E_{pc} = 0.27$ V(vs. Ag/AgCl). The ratio of oxidation peak current and reduction peak ($I_{pa}:I_{pc}$) was 0.42, implying that the electrode process of CGA at CNTs/SPE is quasi-reversible.
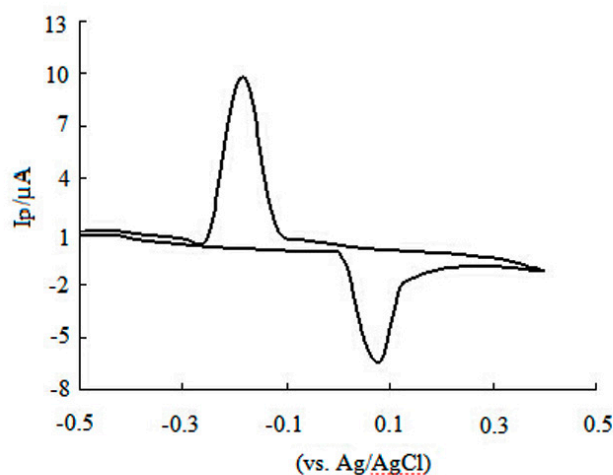

Figure 2. The cyclic voltammetry curves of CGA at CNTs/SPE

3.1. Influence of supporting electrolyte and pH

Several supporting electrolytes such as 0.10 mol·L^{-1} sodium hydrogen phosphate-potassium

dihydrogen phosphate buffer(a), 0.10 mol·L^{-1} phosphate buffer solutions (PBS)(b), 0.10 mol·L^{-1} citric acid buffer(c), 0.10 mol·L^{-1} acetic acid-sodium acetate buffer(d) were tested at CNTs/SPE (shown in Figure 3). A pair of CV redox peak is observed in the four supporting electrolyte, while, Well-defined CV response with high redox peak of CGA was obtained in 0.10 mol·L^{-1} acetic acid-sodium acetate buffer.
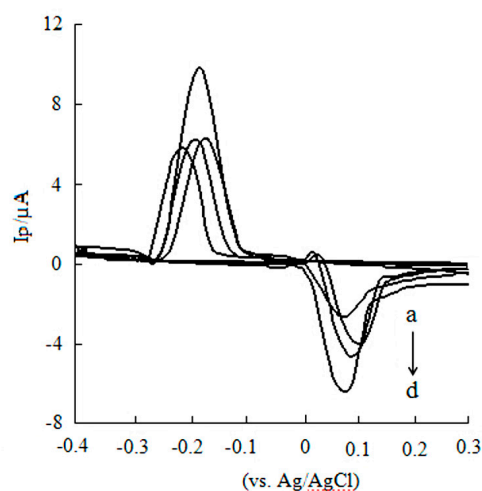

Figure 3. The influence of different supporting electrolytes on peak current: (a) 0.10 mol·L^{-1} sodium hydrogen phosphate-potassium dihydrogen phosphate buffer; (b) 0.10 mol·L^{-1} phosphate buffer solutions; (c) 0.10 mol·L^{-1} citric acid buffer; (d) 0.10 mol·L^{-1} acetic acid-sodium acetate buffer.
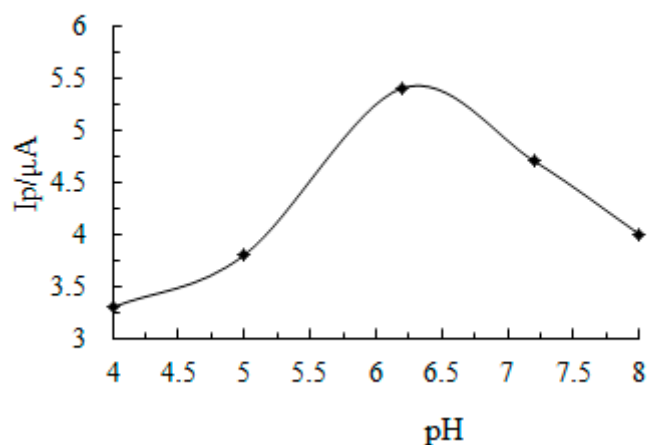

Figure 4. The influence of buffer solution pH to peak current

The influence of pH was investigated in 0.10 mol·L^{-1} acetic acid-sodium acetate buffer, as shown in Figure 4, the oxidation peak current of CGA increases with the increasing of pH from 4.0 to 6.2, and then reaches its maximum at pH 6.2, while, the oxidation peak current decreases as pH increasing above 6.2. Therefore, 0.10 mol·L^{-1} acetic acid-sodium acetate buffer (pH 6.2) was chosen as the optimal supporting electrolyte for subsequent experiments.
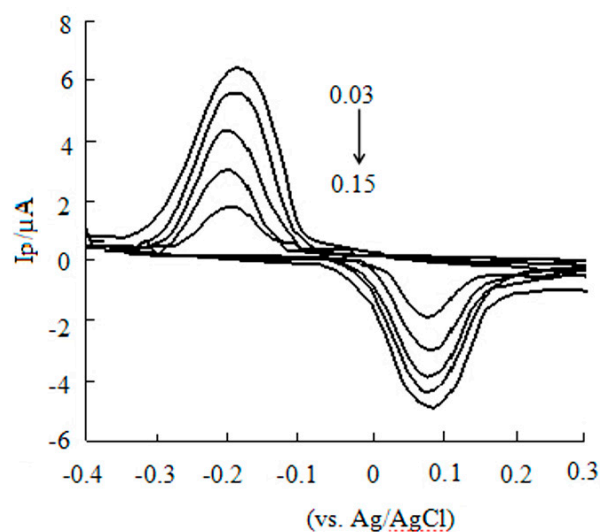
3.2. Influence of the scan rate

Figure 5. The cyclic voltammetry curves of CGA at different scan rates

Figure 5 shows the effect of scan rate on the CV response of CGA at CNTs/SPE in the 0.10 mol·L^{-1} acetic acid-sodium acetate buffer solution (pH 6.2). It is found that both the oxidation peak current (I_{pa}) and reduction peak current (I_{pc}) are linear to the scan rate (υ) in the range of 0.03 to 0.15 V·s^{-1}, the linear regression equations of I_{pa} and I_{pc} are $I_{pa}(\mu A)= 15.887v+2.8809$ （r=0.9969） and $I_{pc}(\mu A)= 16.798v+4.1028$(r=0.9955), respectively. And this indicates that the electrochemical process of CGA at CNTs/SPE is adsorption-controlled. The maximum peak signal-to-noise ratio for CGA was achieved at the scan rate of 0.15 V·s^{-1}. The scan rate of 0.15 V·s^{-1} was therefore selected in this work.

The relationship between the peak current (I) and electron transfer number (n) to comply with equation (1) in the electrode reaction according to the Laviron theory [26].

$$I = \frac{n^2 F^2 A \Gamma_T v}{4RT} = \frac{nFQv}{4RT} \quad (1)$$

While, F (96485 C·mol^{-1}), A (cm^2), Γ_T (mol·cm^{-2}) and v (mV·s^{-1}) in the formula are the Faraday constant, the electrode surface area, the adsorption quantity and the scan rate, respectively. Q=nFAΓ_T, Q is the peak area of a single process of cyclic voltammetry (with quantity of electricity). The oxidation peak electron transfer number（n） was calculated to be 2.01 (v=0.15 V·s^{-1}) in this electrode reaction, the txidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) of CGA were 0.08V and -0.19 V (vs. Ag/AgCl), respectively. $\Delta E= E_{pa}-E_{pc} =0.27$ V (vs. Ag/AgCl), the ratio of oxidation peak current and reduction peak ($I_{pa}:I_{pc}$) was 0.42, implying that the electrode process of CGA at CNTs/SPE is quasi-reversible.

3.4. The linear range and detection limit.

Well-defined oxidation peak DPV response with high peak current of CGA was observed at CNTs/SPE. CNTs/SPE was the working electrode, the different concentrations of CGA was added into acetic acid-sodium acetate buffer solution (pH 6.2), then the differential pulse voltammetry analysis was used in pulse width of 50 ms, pulse amplitude of 180 mV, pulse interval of 50 ms in the potential range of -0.5~0.6 V. We found that the oxidation peak current value was linearly related to the concentration of CGA in the range of 4.73×10^{-7}~4.45×10^{-5} mol·L^{-1} and the detection limit was 3.25×10^{-6}

mol·L^{-1}. The regression equation was: I$_{pa}$(μA) = 4.1993c (mol·L^{-1})+1.1039 (r=0.9976).

3.5. Determination of CGA in green coffe beans.

The coffe beans(From Pu`er University in Yunnan province, China) were ground to powder with a mortar. And the ground coffe sample was defatted with hexane(1:6; w/v) for 8 h in a Soxhlet extraction system. Then, CGA was extracted from the defatted coffe powder with water using the microwave-assisted extraction(MAE) lab station(Shanghai new apparatus of microwave chemical technology co., LTD, China) for 5 min under the conditions of 800 w, 50 °C and Liquid-solid ratio of 5:1[27]. According to the proposed method, the CNTs/SPE was appilied to the determination of CGA in coffe beans. And the result is shown in Table 1. The standard deviation(RSD. %) is found to be 1.33-4.77% and the recovery is 94.74-106.65%, the determination result of CGA is in good agreement with that specified by HPLC.

Table 1. Measurement results of CGA in coffe beans.(n=5)

CGA Sample	By this method (mg/g)	RSD (%)	Added (mg/g)	Found (mg/g)	Recovery (%)	By HPLC (mg/g)
1	13.23	4.77	10.00	24.11	106.65	14.71
2	21.12	3.14	10.00	30.01	94.74	19.78
3	25.21	2.47	10.00	36.03	103.25	23.92
4	18.74	1.33	10.00	28.92	100.96	19.03

4. Conclusions

The carbon nanotubes material modified screen-printed electrode (CNTs/SPE) was prepared and it was applied to the electrochemical behavior research and determination of CGA. The differential pulse voltammetry (DPV) method for the determination of CGA was proposed based on the CNTs/SPE with the several advantages, quick, sensitive and reliable. Therefore, the proposed method can be used for the determination of CGA in the coffe beans

Author Contributions:

Conflicts of Interest:

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