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# Determination of Rutin and Puerarin in Teas and Pharmaceutical Preparations Using Poly (Evans Blue) Film-Modified Electrodes

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## ABSTRACT

Single-walled carbon nanotubes (SWCNTs)/poly (Evans blue)(poly-EB) film-modified glassy carbon (GC) electrodes were fabricated for the voltammetric determination of rutin and puerarin in teas and pharmaceutical preparations. The SWCNTs/poly-EB film-modified electrode showed excellent electrocatalytic activity towards the oxidation of rutin and puerarin. Two well-defined oxidation peaks of rutin and puerarin appeared at 510 and 810 mV, respectively. The peak current depended linearly on the concentration of rutin and puerarin in the range from  $1.6 \times 10^{-7}$  to  $2.0 \times 10^{-5}$  mol/L and  $3.0 \times 10^{-7}$  to  $4.6 \times 10^{-5}$  mol/L, respectively, with the detection limit of  $8.2 \times 10^{-8}$  mol/L for rutin and  $1.2 \times 10^{-7}$  mol/L for puerarin. The modified electrode was successfully applied to the determination of rutin and puerarin in teas and pharmaceutical preparations.

Key words: poly (Evans blue), carbon nanotubes, modified electrode, rutin, puerarin

## INTRODUCTION

Rutin (3',4',5,7-tetrahydroxyflurone-3 $\beta$ -D-rutinoside) and puerarin (4',7-dihydroxy-8 $\beta$ -D-glucosylisoflavone) are widely distributed in plants. The chemical structures of rutin and puerarin are shown in Figure 1. Rutin exhibits broad biochemical and pharmacological activities, including anti-bacterial, anti-inflammation, anti-oxidant, anti-viral and anti-tumor actions<sup>(1-5)</sup>. Puerarin exerts comprehensive biological actions, exhibiting protective effects on hypercholesterolemia<sup>(6)</sup>, diabetic retinopathy<sup>(7)</sup>, liver fibrosis<sup>(8)</sup>, inflammation<sup>(9)</sup> and oxidation<sup>(10)</sup>. Therefore, it is important to develop simple and reliable protocols for the determination of these compounds in foods and pharmaceutical preparations.

A number of methods have been developed for the determination of rutin, including spectrophotometry<sup>(11)</sup>, chemiluminescence<sup>(12)</sup>, capillary electrophoresis and high performance liquid chromatography (HPLC)<sup>(13,14)</sup> as well as electrochemical methods<sup>(15-17)</sup>. For the determination of puerarin, HPLC<sup>(18-20)</sup>, capillary electrophoresis<sup>(21-23)</sup>, spectrophotometry<sup>(24)</sup>, chemiluminescence<sup>(25)</sup> and near infrared spectroscopy<sup>(26)</sup> have been reported. However, electrochemical protocols for the determination of these

compounds are still limited. The direct redox reactions of these compounds at bare electrodes are irreversible and high overpotentials are required, which results in rather poor selectivity and reproducibility in the electrochemical determination. A possible solution for this problem may be the use of modified electrodes. For example, Tysczuk *et al.* determined rutin in tablets by adsorptive stripping voltammetry using in-situ plated lead film-modified electrodes<sup>(15)</sup>.

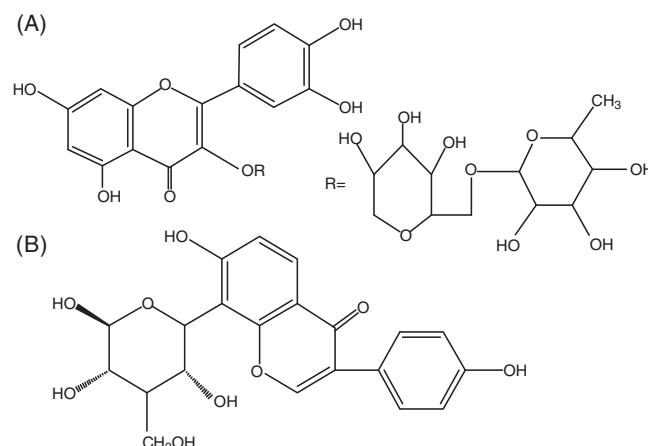
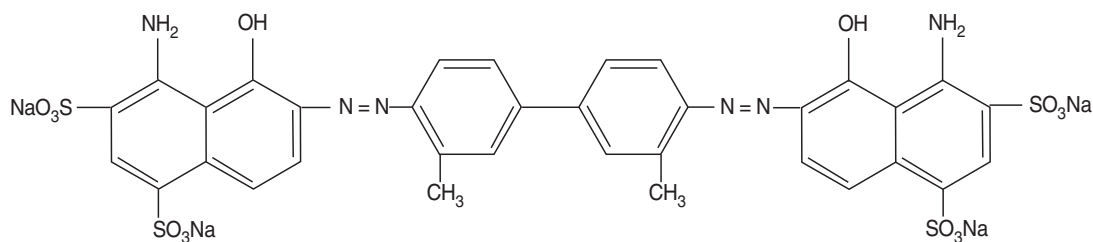


Figure 1. The chemical structures of rutin (A) and puerarin (B).

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**Figure 2.** The chemical structure of Evans blue.

Recently, conducting polymer-modified electrodes have been extensively studied for the development of high-performance electrochemical sensors<sup>(16,27-30)</sup>. Lin *et al.* used conducting polymers prepared by an electrochemical polymerization of Evans blue (EB, Figure 2) and found that the poly-EB film-coated electrodes can be used for the simultaneous determination of dopamine, ascorbic acid and uric acid<sup>(31)</sup>. However, there has been no report on the electrochemical determination of rutin and puerarin on poly-EB film-modified electrodes. Therefore, we have prepared poly-EB and single-walled carbon nanotubes (SWCNTs) composite film-coated electrodes for the determination of rutin and puerarin. In the present work, we report that SWCNT/poly-EB composite film-modified electrodes can successfully be used for the determination of rutin and puerarin in teas and pharmaceutical preparations.

## MATERIALS AND METHODS

### I. Reagents

Evans blue (EB) was purchased from Sigma Co. (USA). SWCNT was purchased from Chengdu Institute of Organic Chemistry (China). Rutin and puerarin were purchased from Tianjin Yifang Technology Co. Ltd. (China). All other reagents are of analytical grade. All solutions were prepared with deionized water (18.2 MΩ) and solutions were deoxygenated by purging with pre-purified nitrogen gas.

### II. Apparatus

Voltammetric measurements were carried out with a CHI-660A electrochemical workstation (Shanghai CH Instruments Co., China). A conventional three-electrode configuration was used with a poly-EB film-modified GC electrode (3 mm in diameter) as working electrode, an Ag/AgCl electrode as reference electrode and a platinum wire as auxiliary electrode. Chromatographic determination of rutin and puerarin was performed with a Waters-2690 chromatographic system (Waters Co., USA) equipped with a photodiode detector. A PHSJ-3F pH meter (Shanghai Leici Co., China) was used to adjust the pH of buffer solutions. All electrochemical measurements were carried out in a one-compartment voltammetric cell (25 mL) at room temperature (ca. 20°C).

**Table 1.** The determination of rutin and puerarin in teas and pharmaceutical preparations (n = 3)

Sample	Compound	SWCNT/poly-EB/GC electrode (μg/mL)	HPLC (μg/mL)	Deviation (%)
A	Rutin	7.16 (3.6) <sup>a</sup>	6.96 (2.7) <sup>a</sup>	2.9
B	Rutin	8.18 (2.6) <sup>a</sup>	8.60 (2.9) <sup>a</sup>	-4.9
C	Rutin	3.98 (3.9) <sup>a</sup>	4.12 (3.2) <sup>a</sup>	-3.4
D	Rutin	2.96 (4.0) <sup>a</sup>	2.86 (3.3) <sup>a</sup>	3.5
E	Rutin	2.86 (3.3) <sup>a</sup>	3.00 (2.6) <sup>a</sup>	-4.7
F	Puerarin	3.53 (3.0) <sup>a</sup>	3.43 (2.2) <sup>a</sup>	2.9
G	Puerarin	3.75 (2.8) <sup>a</sup>	3.90 (2.8) <sup>a</sup>	-3.8
H	Puerarin	3.44 (3.1) <sup>a</sup>	3.29 (3.1) <sup>a</sup>	4.6

A: Maiping tablet, B: Compound rutin tablet, C: Kuding tea, D: Languiren tea, E: Dianhong tea, F: Jiangzhining tablet, G: Daidzein tablet, H: Tongmai pill.

<sup>a</sup>Relative standard deviation (%) of 3 measurements.

Deviation refers to the relative deviation of the determination results between HPLC and electrochemical methods.

### III. Samples

Three kinds of tea and five pharmaceutical preparations were selected (Table 1) for electrochemical and HPLC analysis for rutin and puerarin content. These samples are commercially available. The pharmaceutical preparations were selected because they contain puerarin or rutin that is commonly contained in tea.

### IV. Preparation of Modified Electrodes

The GC electrode was polished with 0.3 and 0.05 μm alumina slurry, and then rinsed with ethanol, H<sub>2</sub>SO<sub>4</sub> (0.5 mol/L) and water successively. Poly-EB film was formed on the surface of GC electrode by scanning electrode potential between -0.2 and 1.8 V repeatedly in 0.5 mol/L of H<sub>2</sub>SO<sub>4</sub> containing 1 mmol/L of EB. A typical procedure for solubilizing SWCNT was as follows: SWCNT (5 mg) was dispersed in 5 mL of N,N-dimethylformamide (DMF) and sonicated for 40 min. SWCNT/Poly-EB/ composite film-modified GC electrode was prepared by dropping 6 μL of the SWCNT solution onto the surface of the poly-EB film-modified GC electrode and the solvent was evaporated.

## V. Measurements

Cyclic voltammetry was carried out in a quiescent solution with a scan rate of 100 mV/s. Chromatographic analysis was performed on C<sub>18</sub> column (25 cm × 4.6 mm i.d.) using water: methanol mixture (4 : 6 v/v) as the mobile phase at a flow rate of 1 mL/min. The analytes were monitored at 225 nm with a photodiode detector. The procedure for preparing rutin and puerarin samples was as follows: teas or pharmaceutical preparations were carefully grounded and 1.0 g of the resulting powder was put in 50 mL of 60% methanol to extract analytes under sonication for 60 min at 60°C. The extracted solutions were filtered prior to cyclic voltammetry and HPLC measurements.

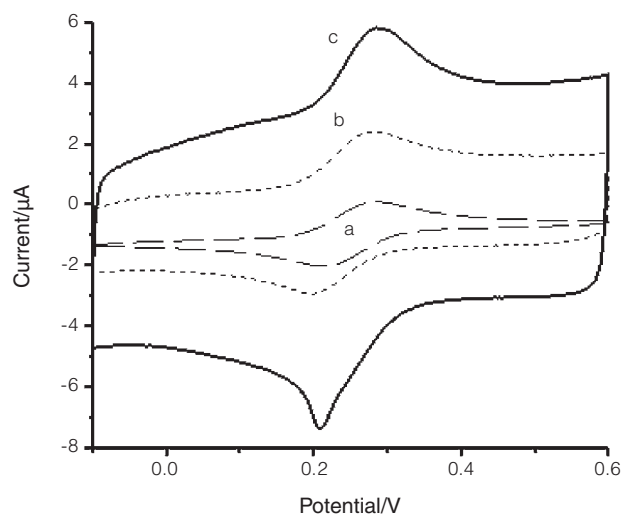
## RESULTS AND DISCUSSIN

### I. Redox Reaction of $[\text{Fe}(\text{CN})_6]^{3-}$ -Ion on SWCNT/poly-EB Film-Modified Electrode

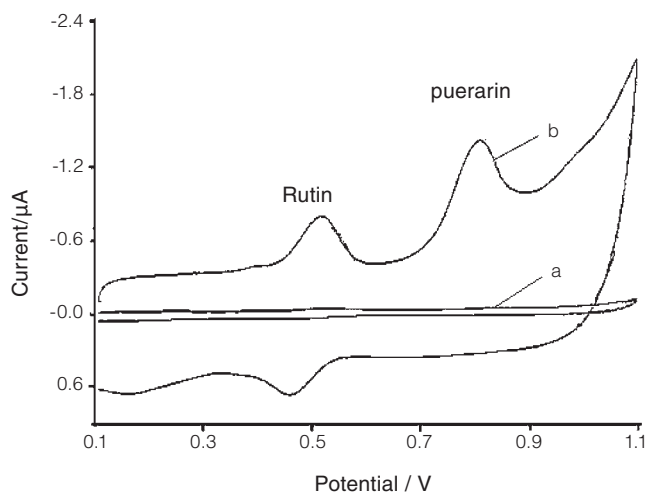
Redox reactions of  $[\text{Fe}(\text{CN})_6]^{3-}$  ion were studied on a bare GC and SWCNT/poly-EB film-modified GC electrodes. The electron transfer characteristics of the  $[\text{Fe}(\text{CN})_6]^{3-}$  ion should be influenced when the electrode surface was modified. Figure 3 shows the CVs of 5.0 mmol/L of  $[\text{Fe}(\text{CN})_6]^{3-}$  solution on bare GC and SWCNT/poly-EB/GC electrodes as well as that on the electrode modified with SWCNT. A couple of well-defined redox peaks of  $[\text{Fe}(\text{CN})_6]^{3-}$  ion were observed on bare GC electrode (curve a), while the peak currents on the SWCNT/poly-EB/GC electrode (curve c) markedly increased in comparison with those on the bare electrode. This suggests that the SWCNT/poly-EB/GC electrode exhibited higher activity in electron transfer between the  $[\text{Fe}(\text{CN})_6]^{3-}$  ion and electrode surface. The higher response of the SWCNT/poly-EB/GC electrode may be ascribed to the joint effects of poly-EB and SWCNT, because the redox current of SWCNT/poly-EB/GC film-modified electrode was significantly increased than that of SWCNT/GC electrode (curve b). These results suggest a possible use of the SWCNT/poly-EB/GC electrode for trace analysis of ions and molecules.

### II. CV of Rutin and Puerarin on SWCNT/poly-EB/GC Electrode

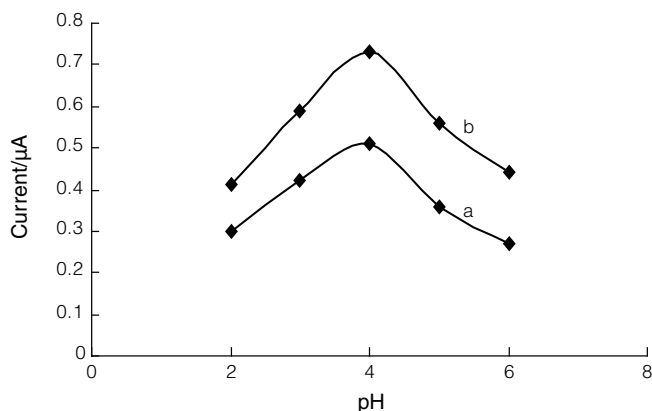
Figure 4 compared the CV responses in the mixed solution of  $0.8 \times 10^{-6}$  mol/L of rutin and  $1.9 \times 10^{-6}$  mol/L of puerarin on bare GC and SWCNT/poly-EB/GC electrodes. The electrochemical response of rutin and puerarin on unmodified GC electrode was rather poor (curve a), probably due to a low concentration of the samples. In contrast, the CV on the SWCNT/poly-EB/GC electrode (curve b) gave rise to well-defined oxidation peaks at 510 and 810 mV for rutin and puerarin, respectively. The oxidation peak currents at the SWCNT/poly-EB/GC electrode were significantly higher than those on unmodified GC electrode. It should be noted here that the oxidation peaks for rutin and puerarin are



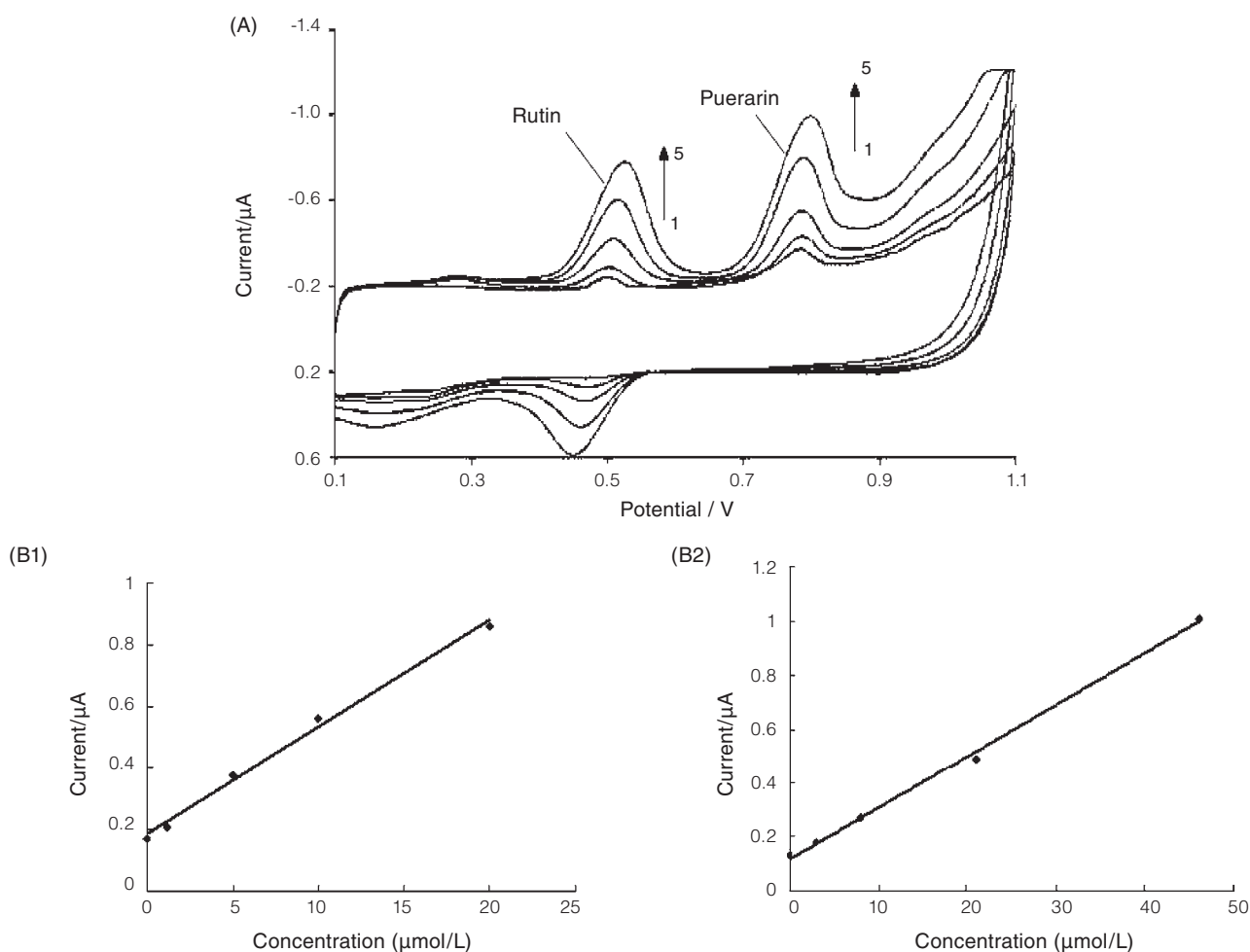
**Figure 3.** Cyclic voltammograms of SWCNT/poly-EB/GC (c), SWCNT/GC (b) and bare GC electrode (a) in 5.0 mmol/L of  $[\text{Fe}(\text{CN})_6]^{4-/3-}$ ; scan rate: 100 mV/s.



**Figure 4.** CVs of  $0.8 \times 10^{-6}$  mol/L of rutin and  $1.9 \times 10^{-6}$  mol/L of puerarin in the phosphate buffer solution (pH 4.0) at a scan rate of 100 mV/s. a) bare GC electrode; b) SWCNT/poly-EB/GC electrode.



**Figure 5.** The effect of pH on oxidation peak currents of analytes (a) 0.8  $\mu\text{mol/L}$  of rutin; (b) 1.9  $\mu\text{mol/L}$  of puerarin; scan rate: 100 mV/s.



**Figure 6.** (A) CVs for increasing concentrations of rutin and puerarin on the SWCNT/poly-EB/ GC electrode, the concentration of rutin (from 1 to 5):  $1.6 \times 10^{-7}$ ,  $1.6 \times 10^{-6}$ ,  $5.0 \times 10^{-6}$ ,  $1.0 \times 10^{-5}$  and  $2.0 \times 10^{-5}$  mol/L; the concentration of puerarin (from 1 to 5):  $3.0 \times 10^{-7}$ ,  $3.0 \times 10^{-6}$ ,  $1.0 \times 10^{-5}$ ,  $2.0 \times 10^{-5}$  and  $4.6 \times 10^{-5}$  mol/L, (B1) a calibration plot over the range for rutin and (B2) a calibration plot over the range for puerarin.

well separated from each other, suggesting a possible use of the modified electrode for simultaneous determination of rutin and puerarin.

### III. Effects of pH

The effect of pH on the electrochemical response of rutin and puerarin on the SWCNT/poly-BE/ GC electrode was evaluated in the pH range from 2.0 to 6.0. Figure 5 plots the oxidation peak current for  $0.8 \times 10^{-6}$  mol/L of rutin and  $1.9 \times 10^{-6}$  mol/L of puerarin. For both compounds, a higher response was observed at pH 4.0. Therefore, pH 4.0 phosphate buffer solution (disodium hydrogen phosphate-potassium dihydrogen phosphate) was used as the supporting electrolyte in the following measurements.

### IV. Calibration, Lower Detection Limit, Reproducibility and Storage Stability

Figure 6A plots the peak currents for rutin and puerarin as a function of the concentration. It can be seen

that the peak currents increased with the concentrations of rutin and puerarin. Under the above optimum conditions, the modified electrode exhibited a linear relationship between the peak current and the concentrations of rutin and puerarin in the range of  $1.6 \times 10^{-7}$ -  $2.0 \times 10^{-5}$  mol/L and  $3.0 \times 10^{-7}$ -  $4.6 \times 10^{-5}$  mol/L, respectively, as shown in Figure 6B1 and B2. For evaluating the reproducibility of the response, the electrochemical determinations were performed 10 times repeatedly with an identical electrode in the solution containing  $0.8 \times 10^{-6}$  mol/L of rutin and  $1.9 \times 10^{-6}$  mol/L of puerarin at pH 4.0. The relative standard deviations were 4.57 and 4.26% for the 10-time measurements of rutin and puerarin, respectively, showing that the reproducibility of the present protocol is satisfactory. The storage stability of the modified electrode was also evaluated by measuring response after storing the modified electrode for 8 days. The peak currents for rutin and puerarin decreased very slightly (ca. 1.5% of the original response for  $5.0 \times 10^{-6}$  mol/L of rutin and puerarin was reduced after 8 days), showing an acceptable stability of the modified electrode.



## V. Interference Studies

In order to assess the possible use of the electrodes for the analysis of real samples, the interference of contaminants on the response signals was studied. The effect of possible interfering compounds on the CV response was verified using  $5.0 \times 10^{-6}$  mol/L of rutin and puerarin solutions containing excess amounts of interfering compounds. It was found that interference from metal ions and organic compounds was rather low. For example, interfering current originating from  $1.0 \times 10^{-4}$  mol/L of metal ions (or 200-times higher concentration than rutin and puerarin) (i.e.,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Hg}^{2+}$  ions) was less than 5% of the oxidation current for  $5.0 \times 10^{-6}$  mol/L of rutin and puerarin. The interfering signals from the organic compounds including ascorbic acid, oxalic acid, glucose, tartaric acid, tannin and starch were slightly higher than those from the metal ions, though more than 100-times higher concentrations were required for inducing 5% deviation. Thus, SWCNT/poly-EB film-modified GC electrode was found to be rather insensitive to foreign substances, suggesting the usefulness of the electrode in the analysis of real samples.

## VI. Determination of Rutin and Puerarin in Teas and Pharmaceutical Preparations

The SWCNT/poly-EB film-modified GC electrode was used for the determination of rutin and puerarin in teas and pharmaceutical preparations and the results were compared with the data obtained by HPLC (Table 1). The results obtained by the two methods were in good agreement with small deviation (less than 5%), demonstrating the usefulness of SWCNT/poly-EB/GC electrode as an electrochemical sensor for rutin and puerarin determination.

## CONCLUSIONS

The present study demonstrated that SWCNT/poly-EB composite film-coated GC electrode exhibited electrocatalytic activity in the oxidation of rutin and puerarin. The composite film-modified electrode showed a good performance for the determination of rutin and puerarin in teas and pharmaceutical preparations. The composite film-modified electrode exhibited a selective response to rutin and puerarin, even in the presence of metal ions and organic small molecules. Therefore, simultaneous or independent measurements of the two compounds are possible without interference. The present protocol may be useful for sensitive, simple and inexpensive determination of rutin and puerarin in foods.

## ACKNOWLEDGMENTS

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