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Conductive Carbon Black-Graphene Composite for Sensitive Sensing of Rutin

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A conductive carbon black-graphene (CCB-GR) modified glassy carbon electrode (CCB-GR/GCE) has been fabricated and used for determination of rutin. Transmission electron microscopy (TEM) results indicated that CCB-GR was successfully prepared. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) results showed that CCB-GR/GCE exhibited an excellent electrochemical performance. The sensing platform showed wide linear responses for rutin from 1×10^{-9} to 1×10^{-4} mol L^{-1} with a detection limit of 3.3×10^{-10} mol L^{-1} (S/N = 3). The CCB-GR/GCE showed excellent sensitive with good stability toward the determination of rutin content in tablet.

Keywords: Graphene, Conductive carbon black, Rutin, Electrochemical sensor

1. INTRODUCTION

Graphene (GR), an atomic-layer thick two-dimensional (2D) carbon nanostructure, has attracted great attention in many research field since it was reported in 2004 [1-2]. It possesses unique properties such as fast electron transportation, high surface area, high thermal conductivity, excellent mechanical strength and high mobility of charge carriers [3-5]. Due to its specific electrochemical characteristics, such as high electrocatalytic activity, outstanding conductivity, high mobility of charge carriers and rapid heterogeneous electron transfer, graphene has been used as the electrode material [6-10].

Conductive carbon black (CCB) is another kind of carbon material, its nano-particles are arranged with chain-like structures, and the surface of particles is composed of graphite-like crystal. It possessed many fascinating properties such as low cost, large surface area, excellent electric conductivity and strong adsorptive ability. Due to its unique properties, CCB is used in preparation of carbon paste electrode [11-12]. The incorporation of conductive carbon black into graphene layers could not only inhibit the agglomeration of graphene, but also increased the electrical conductivity of the electrode. Based on these, the conductive carbon black-graphene (CCB-GR) modified electrode were prepared.

Rutin, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-({[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}methyl)oxan-2-yl]oxy}-4H-chromen-4-one, was a kind of natural flavonoid compound, which is mainly active component of many traditional Chinese medicines. Owned a number of physiological effects, such as anti-inflammatory [13], anti-tumor [14], anti-bacteria [15], haemostat and antioxidant [16], etc, rutin was widely used in the clinic as the therapeutically medicine. So it was necessary to establish simple and sensitive practicable methods to determine rutin in therapeutically medicine or plants. Various analytical methods had been used to determine rutin, such as high-performance liquid chromatography (HPLC) [17], capillary electrophoresis (CE) [18], resonance light scattering [19], UV-vis spectrophotometry [20], which usually needed complex operation and large amounts of toxic organic. Compare with these methods, electrochemical methods had advantages such as sensitive, selective, rapid and facile techniques in analytical chemistry in many fields.

In this study, we prepared a simple and general method to prepare CCB-GR composite. The CCB-GR composite was demonstration to be an effective material for fabricating a novel electrochemical sensing platform. The electrochemical behavior of rutin on conductive carbon black-graphene modified glassy carbon electrode (CCB-GR/GCE) was investigated by cyclic voltammetry (CV), from which enhanced peak currents were obtained. Moreover, the fabricated CCB-GR/GCE was successfully applied to detection rutin by differential pluse voltammetry (DPV) with highly sensitivity.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Rutin ($C_{27}H_{30}O_{10}\cdot 3H_2O$, Mr = 664.56), was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). CCB (HG-1P, density: 1.7-1.9 g/cm^3; particle size: 35-50 nm; BET surface area: 10-130 m^2/g; ash: 1.75%) was purchased from Guanghua Chemical Plant (Zibo, China). Graphite was provided by Qingdao Fujin graphite Co., Ltd (Qingdao, China). Graphene (GR) was synthesized by the modified Hummers method [21]. All the other chemicals were of analytical grade and used without further purification. The 0.2 mol L^{-1} phosphate-buffered solution (PBS) with various pH was prepared by using the stock solutions of 0.2 mol L^{-1} NaH_2PO_4 and 0.2 mol L^{-1} Na_2HPO_4.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI-660C electrochemical workstation (CHI Inc. Ltd., USA) coupled with a conventional three- electrode cell. A glassy carbon electrode (GCE, 3 mm in diameter) and the modified electrodes were used as the working electrodes. The auxiliary electrode was a platinum wire. All the transmission electron microscope (TEM) images were obtained by JEOL JEM-2100 at 90kV.

2.2. Synthesis of CCB-GR composite

A 1.0 mg the synthesized GR and 1.0 mg CCB powder were mixed completely in 1.0 mL double-distilled water with ultrasonic agitation for 8 h to form CCB-GR composite.

2.3. Preparation of the modified electrodes

Before the surface modification, the GCE was carefully polished with 0.3 μm and 0.05μm alumina slurries to obtain a mirror-like surface. After sonication in water and ethanol successively for 20 s, the electrode was rinsed with water, and then dried under an infrared lamp. The CCB-GR/GCE was prepared by casting 4 μL of as-prepared CCB-GR suspension onto the surface of cleaned GCE. The solvent was then evaporated under an infrared heat lamp. As controls, CCB/GCE and GR/GCE were also fabricated with the similar procedures for the preparation of CCB-GR/GCE by only replacing CCB-GR hybrid materials with GR or CCB, respectively.

2.4. Electrochemical measurements

All electrochemical experiments were carried out at room temperature. The CV experiments were carried out by scanning a 0.2 mol L^{-1} PBS (pH 7.0) containing a single or mixed analytes at an applied potential range from 0.1 to 0.8 V with different scan rates between 20 and 300 mV s^{-1}. The DPV was performed at potentials from 0.2 to 0.7 V. The impedance measurements were performed in a 5.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ redox probe solution with 0.5 mol L^{-1} KCl.

3. RESULTS AND DISCUSSION

3.1. Characterization of composite

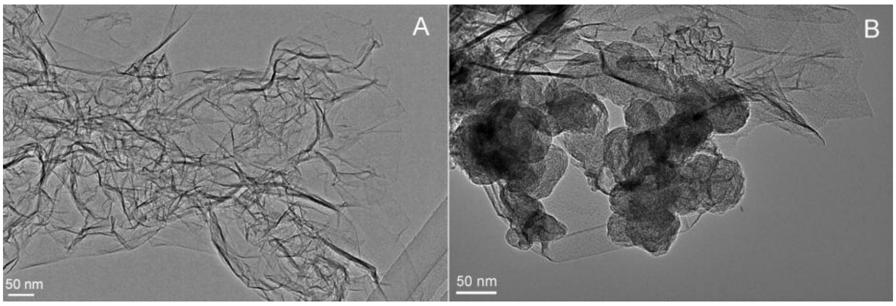


Figure 1. TEM images of (A) GR and (B) CCB/GR composite

The TEM was used to characterize the surface of different modified electrodes and the results were shown in Figure 1. Figure 1A showed the typical TEM image of graphene. The translucent films suggested the sample showed the crumpled and wrinkled flake-like structure. Figure 1B showed the TEM image of CCB-GR composite film, it was apparent that conductive carbon black was combined graphene by mechanical mixing.

The incorporation of conductive carbon black into graphene layers could not only inhibit the agglomeration of graphene, but also increased the electrical conductivity of the electrode. Conductive carbon black could not only increase the distance between the graphene, but also provide rapid diffusion path for more edge plane participation. The conductive carbon black spacers ensure the high electrochemical utilization of graphene layers as well as the open nano-channels provided by three-dimensional CCB-GR material [22].

3.2. Electrochemical behaviors of $[Fe(CN)_6]^{3-/4-}$ on the modified electrodes

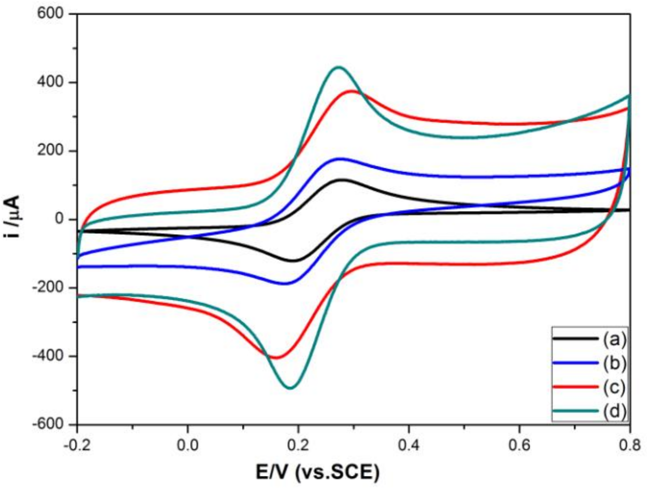


Figure 2. Cyclic voltammograms of the bare GCE (a), CCB/GCE (b), GR/GCE (c) ,CCB-GR/GCE (d), in 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.5 mol L^{-1} KCl solution at the scan rate of 100 mV s^{-1}.

Cyclic voltammograms of different modified electrodes were recorded in a 5.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution with the results shown in Figure 2. A pair of redox peak of $[Fe(CN)_6]^{3-/4-}$ was observed on CCB (Figure 2b) with the peak-to-peak separation (ΔE_p) as 136 mV at the scan rate of 100 mV/s. While on GR (Figure 2c), both redox peak currents increased obviously with the ΔE_p value decreased to 100 mV. While on CCB/GR/GCE (Figure 3d), the redox peak currents were further increased with the ΔE_p value decreased to 88 mV. The smallest ΔE_p of redox probe $[Fe(CN)_6]^{3-/4-}$ on CCB-GR/GCE suggested that the presence of CCB-GR composite film on the electrode surface further accelerate the electron transfer rate.

3.3. Electrochemical behaviors of rutin on the modified electrodes

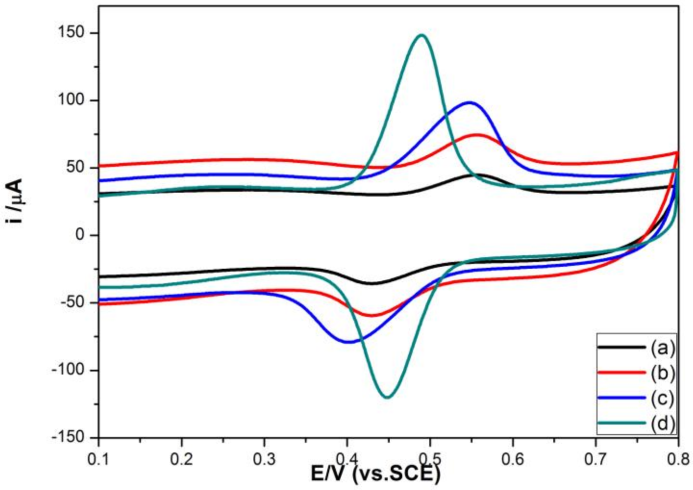
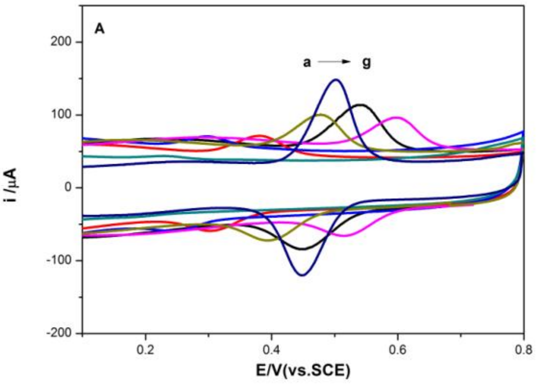


Figure 3. Cyclic voltammograms of 1×10^{-5}mol L^{-1} rutin at the bare GCE (a), CCB/GCE (b), GR/GCE (c), CCB-GR/GCE (d), in 0.2 mol L^{-1} PBS (pH 3.5) at scan rate of 100 mV s^{-1}.

A pair of well-defined redox peaks appeared on the different electrodes for rutin solution, indicating that the redox reaction of rutin had taken place on the different electrodes. As shown in Figure 3, on the CCB (Figure 3b), there were poor redox current peaks. On the GR (Figure 3c), the electrochemical signals were obviously amplified owing to the excellent conductivity of GR. The signals on CCB-GR/GCE (Figure 3d) were higher than the CCB and GR. The CCB-GR/GCE which combined the excellent properties of conductive carbon black and graphene, promoted the electrochemical reaction of rutin, achieved the sensitive rutin determination.

3.4. Optimization of parameters

3.4.1. Effect of pH



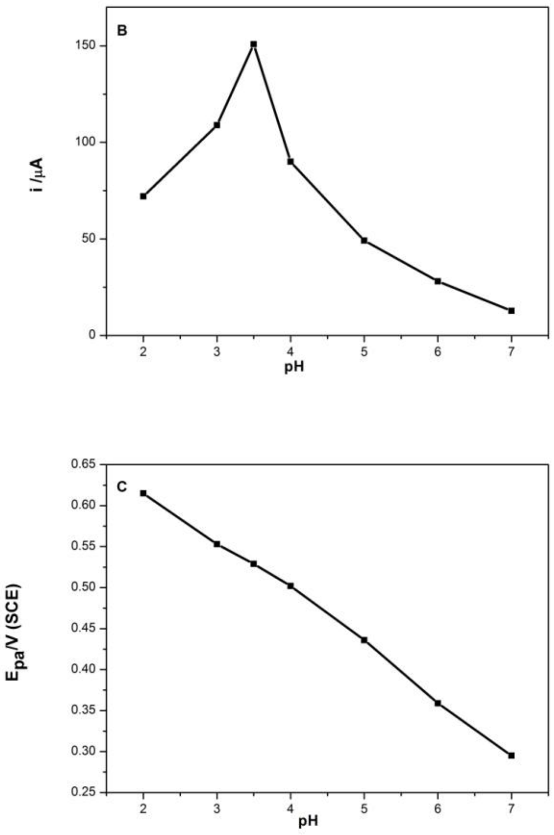


Figure 4. (A) Cyclic voltammograms of 1.0×10^{-5} mol L^{-1} rutin at CCB-GR/GCE with different pH (a-g: 7.0, 6.0, 5.0, 4.0, 3.5, 3.0, 2.0) at scan rate of 100 mV s^{-1}. (B) Effect of pH value on anodic peak currents. (C) Effect of pH value on anodic peak potentials.

As shown in Figure 4A, the effect of buffer pH on the electrochemical responses of rutin on the CCB-GR/GCE was investigated from pH 2.0 to 7.0. Figure 4B illustrated the influence of buffer pH on anodic the peak current. With solution pH rising, the peak current increased, and it achieved a maximum at about pH 3.5, then it decreased. This was related to the proton taking part in the electrochemical reaction. In basic solution, the electrochemical reaction became more difficult due to the shortage of proton [23]. In addition, at a high pH, rutin turned to anions, giving rise to the electrostatic repulsion between rutin and the CCB-GR, which also makes the peak current decrease. Therefore PBS in pH 3.5 buffer solution was chosen as the optimal supporting electrolyte for rutin determination in the following experiments.

Figure 4C illustrated the influence of buffer pH on anodic peak potential. When the pH changed from 2.0 to 7.0, there was a linear relationship between the anodic peak and the pH value as follows $E_{pa} = -0.064\ \mathrm{pH} + 0.751$ ($R = 0.9980$). The slope was a negative value, indicating the electrochemical process of rutin at the CCB-GR/GCE was an oxidative process. The absolute value of the slope 64 mV was close to the theoretical value of 59 mV, indicating that the electron transfer was accompanied by an equal number of protons in electrode reaction.

3.4.2. Effect of scan rate

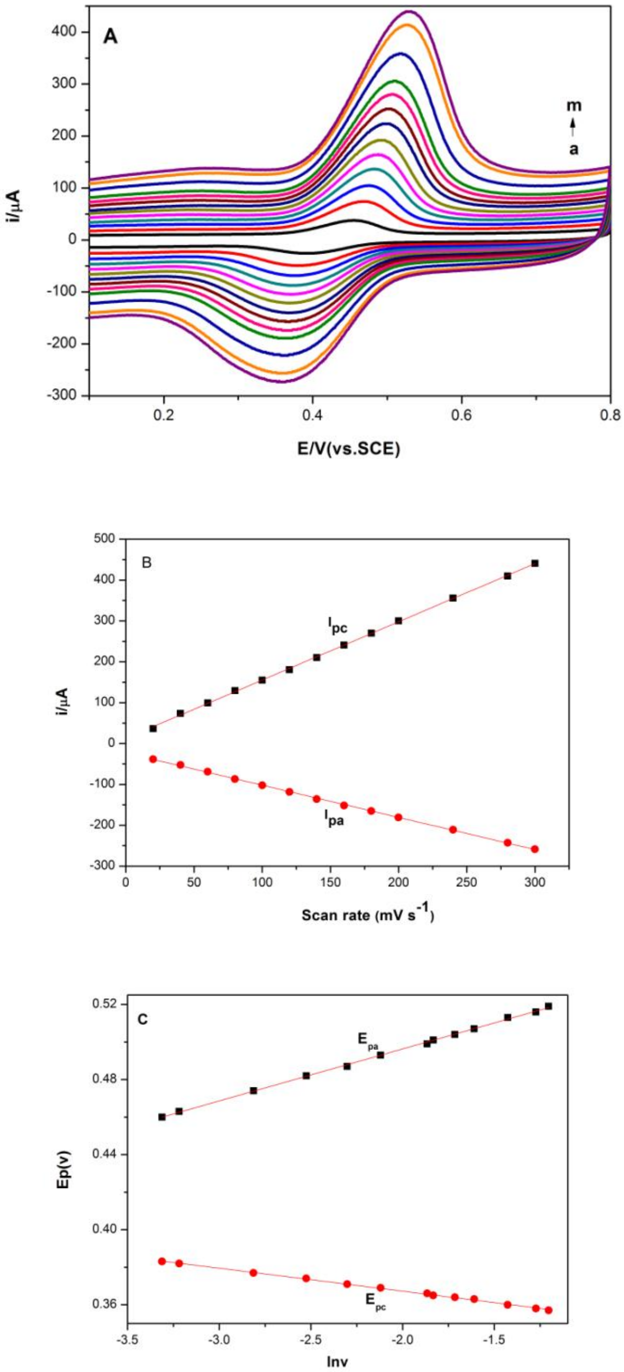


Figure 5. (A) Effect of different scan rate (a-k: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240, 280, 300) on the redox behavior of 1.0 × 10^{-5} mol L^{-1} rutin in 0.2 mol L^{-1} PBS (pH 3.5) at CCB-GR/GCE. (B) Effect of scan rate on peak current. (C) Effect of scan rate on peak current peak voltage.

The effect of scan rate on the redox peak currents of 10^{-5} mol L^{-1} rutin on the CCB-GR/GCE at pH 3.5 PBS buffer was studied. Figure 5A showed the CV curves of rutin obtained in the range of from 20 to 300 mV s^{-1} in order to investigate whether or not the redox behavior of rutin was due to rutin diffusion or adsorption on the CCB-GR/GCE. The anodic peak currents of rutin showed a linear relationship with the increase of scan rates from 20 to 300 mV s^{-1} (Figure 5B), and the regression equation was i_{pc} (μA) = 12.2682+0.6459ν (mV s^{-1}) R = 0.9993, i_{pa} (μA) = -11.5720-0.3937ν (mV s^{-1}) R = 0.9995, indicating that the electrode process was adsorption controlled. The adsorption of rutin at the surface of the CCB-GR/GCE could be ascribed to the π-π interaction between the benzene ring of the rutin and the benzene ring of the graphene [24]. The oxidative and reductive potentials were linearly dependent on the lnν (Figure 5C) with the regression equation of E_{pa} = 0.5516+0.0277lnν (V s^{-1}) R = 0.9992, E_{pc} = 0.3428-0.122lnν (V s^{-1}) R=0.9983, respectively.

3.4.3 Accumulation time

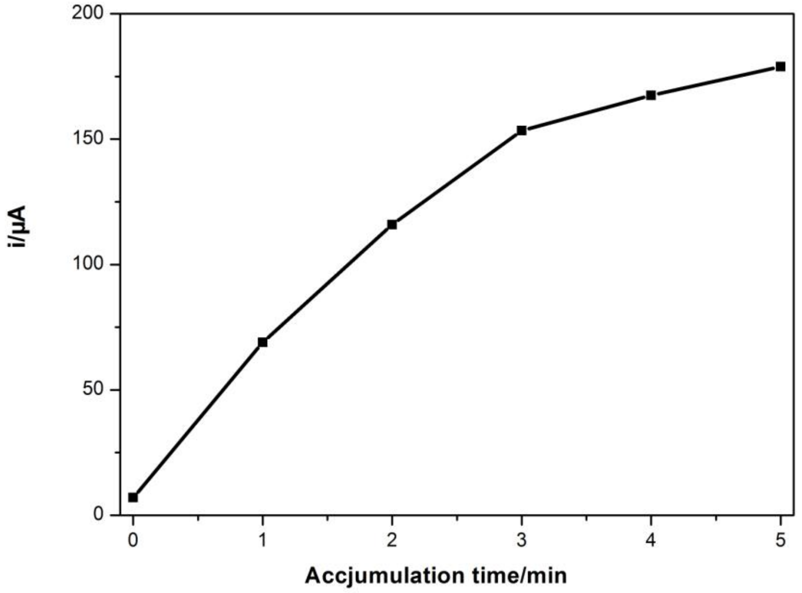


Figure 6. The effect of accumulation time on the peak current of 1×10^{-5} mol L^{-1} rutin.

Accumulation can effectively increase the adsorption of rutin on the electrode surface, and then enhance the redox response and improve the detection sensitivity. Figure 6 illustrated the influence of accumulation time on the peak current of rutin. When extending the accumulation time from 0 to 3 min, the peak current of rutin linearly increased, suggesting that accumulation obviously increased the determining sensitivity. However, the oxidation peak current of rutin increased slightly with further increasing the accumulation time from 3 to 5 min, indicating that the amount of rutin tended to a limiting value at the CCB-GR/GCE. Considering sensitivity and working efficiency, 3 min accumulation was employed.

3.5. Determination of rutin

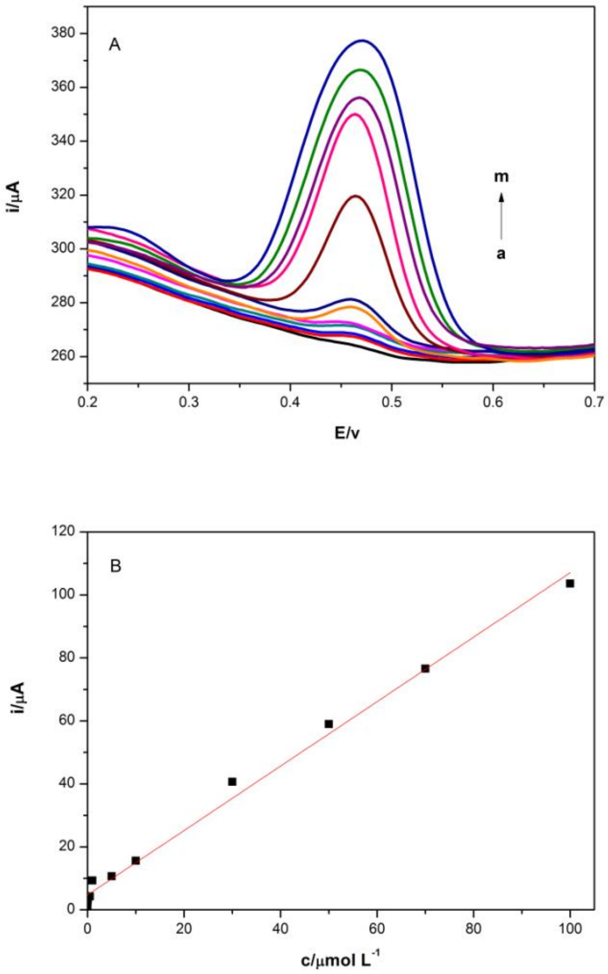


Figure 7. (A) Differential pulse voltammograms of CCB-GR/GCE in 0.2 mol L^{-1} PBS (pH 3.5) containing different concentration (a - l: 1×10^{-9}, 1×10^{-8} , 5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 3×10^{-5}, 5×10^{-5}, 7×10^{-5}, 1×10^{-4} mol L^{-1}) of rutin at scan rate of 100 mV s^{-1}. (B) Concentration calibration curve of the DPV current response for rutin.

The linear range and limit of detection were studied using DPV under the optimized conditions (Figure 7). The oxidation peak current of rutin (i_{pa}, μA) was proportional to its concentration (μmol L^{-1}) over the range of 1×10^{-9}-1×10^{-4} mol L^{-1}, with the following equation of i_{pa} (μA) = 4.7373+1.023c, (R = 0.9926) and the detection limit was 3.3×10^{-10} mol L^{-1} (S/N = 3). In comparison with previously reported electrochemical methods using different modified electrodes (Table 1) for rutin detection, the as-made CCB-GR/GCE showed both a broader range and a lower detection limit, which may be attributed to the combined advantages of the strong accumulation efficiency of CCB-GR/GCE for rutin, the large specific surface area and upstanding electric conductivity of GR.

The modified electrode owed good reproducibility and the relative standard deviation (RSD) 10 continuous detections were 2.6% for 1×10^{-5} mol L^{-1} rutin. After 3 weeks storage at room temperature in the dark, the modified electrode remained 96.8% of its initial current value, which indicated that CCB-GR/GCE had good stability.

Table 1. Comparable of different modified electrodes for rutin determination

Modified electrode	Linear range (μmol L^{-1})	Detection limit (μmol L^{-1})	Reference
β-CDEP/GCE	0.13-2	0.079	[25]
PAO-GR/CILE	0.03-800	0.0083	[26]
Nafion/GO/CILE	8.0×10^{-2}-100	0.016	[27]
GR-MnO2/CILE	0.01-500	0.0027	[28]
β-CD@CRG/Nafion/GCE	6.0×10^{-3}-10	0.002	[29]
GR/CILE/GCE	0.07-10^{3}	0.024	[30]
CCB/ G/GCE	1×10^{-3}-1×10^{3}	0.00033	This paper

3.6. Interference

The potential interference for the determination of rutin was studied. Under the optimized conditions, the oxidation peak current of rutin was individually measured in the presence of different concentrations of interferents and then the change of peak current was checked. It was found that 1000-fold concentrations of vitamin C, glucose, glycine, Cu^{2+}, Fe^{3+}, Al^{3+} and Mn^{2+}; 300-fold concentrations of hesperidin, tangeretin, naringin, morin and citromitin; 100-fold concentrations of quercetin and galangin; almost had no influence on the determination of 0.1 mg L^{-1} rutin when the peak current change was below 10%.

3.7. Analysis of real samples

To further evaluate the practicability of these electrochemical sensors, the rutin content in compound rutin table (20 mg/tablet) was analyzed by the proposed method. Table 2 presented the results with three parallel measurements. The recovery was performed with the standard addition method to evaluate the accuracy of the sensor. Measured results were satisfactory with the recovery of 97.5-101.3%, suggesting that this method could be applicable for the determination of rutin in pharmaceutical samples.

Table 2. Determination of rutin in compound rutin tables and the recovery data (n=3).

Sample	Specified (μmol L^{-1})	Detected (μmol L^{-1})	Added (μmol L^{-1})	Total (μmol L^{-1})	Recovery (%)	RSD (%)
1	60.2	59.8	50.0	107.4	97.5	2.53
2	60.2	59.6	50.0	111.2	101.3	2.35
3	60.2	59.2	50.0	108.0	98.0	2.78

4. CONCLUSION

A novel CCB-GR composite for electrochemical electrode was prepared by ultrasonic agitation and applied for the electrochemical detection of rutin. CCB offered high surface area and superior conductivity. GR also served with high surface area, excellent conductivity and provide excellent interfacial contact between CCB and GN for fast electron transport. As combining the excellent properties of CCB and GN, the CCB-GR composite modified GCE enhanced the electrochemical performance of rutin. Under the optimized conditions, the analytical performance was displayed for rutin detection from 1.0×10^{-9} to 1.0×10^{-4} mol L^{-1} with a low limit of detection of 3.3×10^{-10} mol L^{-1} (S/N = 3). A sensitive, rapid and simple electrochemical method was developed for the detection of rutin.

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