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Electrochemical Sensor for Simultaneous Determination of Theophylline and Caffeine Based on a Novel poly(folic acid)/graphene Composite Film Modified Electrode

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In this work, a novel poly(folic acid)/ graphene composite film modified electrode was developed to construct an electrochemical sensor for simultaneous determination of theophylline (TP) and caffeine (CAF). The fabrication and characterization of nanocomposite film were investigated in details by electrochemistry and scanning electron microscopy. This new electrochemical sensor displayed superior electrocatalytic property to the oxidation of TP and CAF, which obviously improved the sensitive detection of the two compounds in the terms of high sensitivity and wide linear range. Under the optimized experimental conditions, the detection limits were obtained to be 0.03 μ mol L^{-1} for TP and 0.08 μmol L^{-1} for CAF, respectively. The novel sensor was successfully used for the simultaneous detection of TP and CAF in food samples.

Keywords: Folic acid; Graphene; Chemically modified electrode; Theophylline; Caffeine

1. INTRODUCTION

Theophylline (1, 3-dimethylxanthine, TP) and caffeine (1, 3, 7-trimethylxanthine, CAF) are naturally occurring alkaloid that are widely found in plant products and beverages. It has been reported that both of TP and CAF can cause various physiological effects, such as relaxation of bronchial muscle, gastric acid secretion and stimulation of the central nervous system [1,2]. Furthermore, TP is widely employed as a bronchodilator drug in the management of various asthmatic conditions, whereas high amounts of TP can cause insomnia, anorexia and heart-burn tachycardia [3]. CAF is usually used as an additive in energy drinks and soft drinks. However, the side effects such as trembling, cardiovascular disease would occur when excessive CAF was ingested in human body [4]. Therefore, it is important to simultaneously determine TP and CAF in food analysis and clinical diagnosis.

In recent years, several analytical methods including spectrophotometry [5,6], high performance liquid chromatography [7,8], gas chromatography [9] and capillary electrophoresis [10], have been developed in the literature for the simultaneous determination of TP and CAF. However, these techniques require expensive instruments, skilled operators and complicated procedures. Electrochemical detection is an alternative method which has attracted attention due to its fast response, low-cost instrument, simple and high sensitivity. In recent literature, a few of electrochemical sensor have been reported for the individual determination of the two analytes [11-24]. However, simultaneous determination of TP and CAF is still faced with challenges. Firstly, TP and CAF have more positive oxidation potentials, which may lead to large background currents. Secondly, the oxidation products of the two compounds can adsorb strongly on the electrode surface, which result in low sensitivity and poor reproductivity [17]. In order to improve the detection sensitivity and reproductivity, development of new electrochemical sensors to accomplish simultaneous electrochemical determination of TP and CAF has still been an attractive research subject. In this work, we prepared a new poly(folic acid)/ electrodeposited graphene composite film modified electrode to develop a simple and sensitive voltammetric method for simultaneously determining TP and CAF.

Since graphene (GR) was discovered in 2004, it has become widely used carbon nanomaterial in electrochemical sensors due to its unique electrical and catalytic properties [25]. Because GR has better conductivity and the ability to promote electron-transfer reaction, it usually used with other functional materials to fabricate electrochemical sensors[26,27]. Recently, electropolymer/GR modified electrodes have been extensively studied because incorporation of GR into conducting polymers can lead to new composite materials possessing the properties of each component composites with a synergistic effect, which have showed excellent analysis characteristics [28-32]. However, few research on poly (vitamin) / GR modified electrode have been described in the literature.

Folic acid (FA), also called vitamin B_9, plays an important role in DNA synthesis and the metabolism of homocysteine[33]. As an electroactive B-group vitamin , FA has been detected by electrochemical method and applied to electrochemical sensor construction. Zheng et al. immobilized FA on the surface of carbon paste electrode by electropolymerization to develop its new application to create a dopamine sensor [34]. Rasa et al. proposed a possible electropolymerisation mechanism of FA and optimised the polymerization conditions [35]. In this work, we reported for the first time on a poly (FA) / GR composite film to modify glassy carbon electrode (GCE). So far, GR modified electrodes usually was prepared by the chemical or electrochemical reduction of drop-casted graphene oxide (GO) film on the electrode surface [36-38]. Such preparation methods have intrinsic limitations including incomplete reduction of GO and lack of control of film thickness. Herein, we prepared GR modified film directly from a dispersion containing graphene oxide by one-step electrodeposition technique, then poly(FA) film was deposited on the top of GR film by electropolymerization. The prepared new composite film possessed excellent electrocatalytic activity for oxidation reactions of TP and CAF, which showed excellent analysis characteristics such as high sensitivity, wide linearity, good selectivity and reproducibility for the simultaneous determination of TP and CAF. Therefore, the prepared poly (folic acid) / electrodeposited graphene composite film modified glassy carbon electrode (PFA/GR/GCE) was satisfactorily used for the simultaneous determination of TP and CAF in tea and soft drinks by voltammetric method.

2. EXPERIMENTAL

2.1. Reagents

TP, CAF and FA were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Graphene oxide (GO, 0.5~5μm diameter, 0.8~1.2 nm thickness, >99% single layer ratio and >99% purity) were obtained from Nanjing Xian Feng Nano Material Technology Co., Ltd. All chemicals were of analytical grade. The stock solutions of 0.067 mol L^{-1} KH_2PO_4 and Na_2HPO_4 was mixed to prepare phosphate buffer solutions (PBS) with various pH values. The purified water used in the experiments was doubly distilled water.

2.2. Instrumentation

Electrochemical measurements were carried out with a LK3200A Microcomputer-based electrochemical system (LANLIKE, Tianjin, China). A conventional three-electrode cell with 3.0 mm glassy carbon disk electrode as working electrode, a saturated calomel electrode as reference electrode, and a platinum sheet electrode as the counter electrode was used. All pH measurements were made with a pHs-3B digital pH meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass electrode. Scanning electron microscopy (SEM) was performed by using a JSM-6700F Scanning electron microscope (Japan Electron Company, Japan).

2.3. Fabrication of the PFA/GR/GCE

The GO suspension was prepared by dispersing 10.0 mg GO in 10 ml PBS (pH=9.2) under sonication for 2 h. Graphene film was electrochemically deposited on a clean GCE by cyclic voltammetry with potential scanning between 0.5 and -1.8 V at a scan rate of 50 mV s^{-1} for 25 cycles in the fresh GO suspension. The modified electrode was washed with doubly distilled water, and dried at room temperature. The obtained electrode was taken as GR/GCE. Then the PFA/GR/GCE was obtained by cyclic sweeping from -2.0 to +2.0 V at 50 mV s^{-1} for 15 cycles in pH 6.8 phosphate buffer solution containing 1.0×10^{-3} mol L^{-1} FA.

For comparisons, PFA/GCE was prepared by changing the components under the same procedures.

2.4. Measurement procedures

Cyclic voltammetric and differential pulse voltammetric measurements were employed with three electrodes in 10 ml PBS without deaeration. The cyclic voltammograms and differential pulse voltammograms were recorded in the range from 0.6 to 1.6V. All experiments were performed at room temperature. The standard addition method was used for analyzing the food samples.

2.5. Sample Preparation

For tea samples, 2.0 g of tea leave was exactly weighed and added into 70 mL of boiling double distilled deionised water for 30 min. After filtration, the filtrate was collected into a 100 mL volumetric flask and diluted to marker with doubly distilled water for further measurement.

For drink samples, carbonated drink should be degassed for 3 min in an ultrasonic bath before further measurement.

3. RESULTS AND DISCUSSION

3.1 Fabrication and characterization of PFA/GR nanocomposite film

Cyclic voltammetry was used to form graphene film on bare GCE and the electropolymerization film of FA on GR/GCE. Fig. 1 showed the cyclic voltammograms of the synthesis of GR modified film by one-step electrodeposition technique directly from a dispersion containing graphene oxide, where one cathodic peak (peak A) and one anodic peak (peak B) are observed. The cathodic peak around -0.75V is attributed to the irreversible electrochemical reduction of GO [38]. The cathodic peak current was significantly reduced to disappeared after the first cycle, suggesting that oxygen-containing groups in GO were completely reduced at low negative potentials and the reduced graphene was fabricated at the electrode surface [39]. The anodic peak around 0.25 V is ascribed to the electrochemical oxidation of some redox groups on the graphene plane [40]. The persistent increase of the anodic peak current with successive potential scans indicated that the deposition of graphene onto the electrode directly from GO dispersion was achieved. The CVs of formation of PFA/GR/GCE were shown in Fig. 2. In the first cycle, two anodic peaks appeared with potential at 0.0 V (peak A) and 0.33 V (peak B), and one cathodic peak was observed at about -0.54 V (peak C). From the subsequent cycles, both the oxidation and reduction peak currents increased with successive scanning and tended to be stable after 12 scans. The above facts reflected the electropolymerization processes of FA and the continuous growth of the PFA film [35]. After modification by fifteen electropolymerizing scans, a uniform stable blue polymer film was formed on the GR/GCE surface, which indicated that PFA was electrodeposited on the surface of GR/GCE.

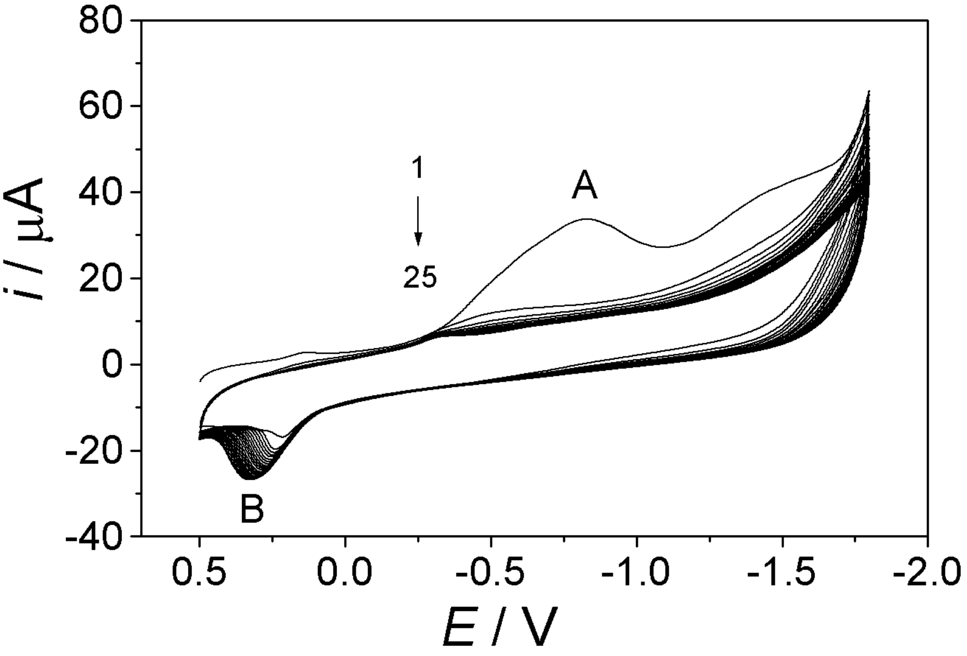

Figure 1. Cyclic voltammograms of GO at bare glassy carbon electrode in electrodeposition process from 1 to 25 cycles.

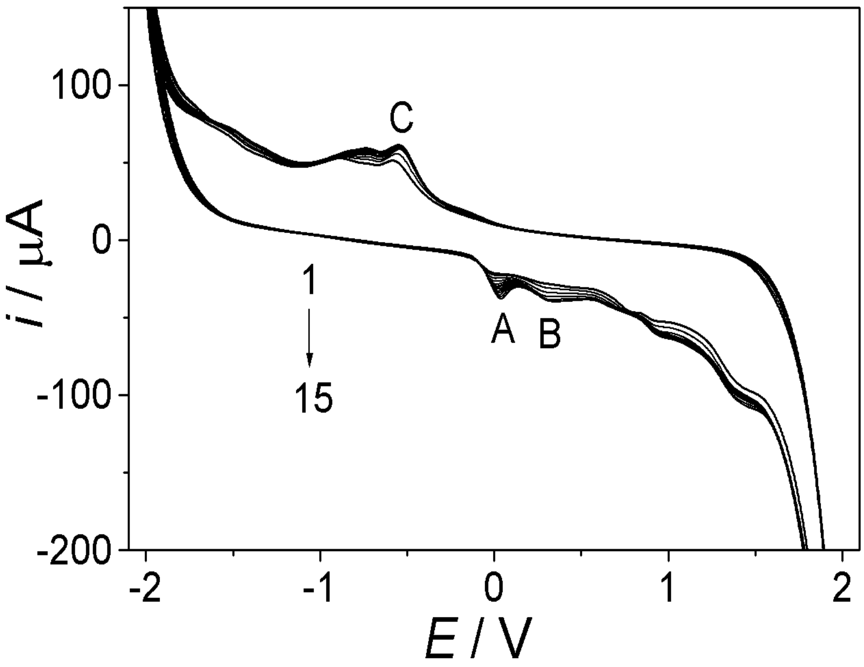

Figure 2. Cyclic voltammograms of GR modified electrode in 1 mM FA. Supporting electrolyte: pH 6.8 phosphate buffer; Number of scans: 15; Scan rate:50 mV s^{-1}

Fig. 3 showed the typical SEM images obtained for GR film and PFA/GR film on the GCE surface. It can be observed that the electrochemically synthesized graphene (Fig. 3A) displayed their well exfoliated, typical wrinkled surface morphology. In Fig. 3B, the SEM image of PFA/GR nanocomposite manifests the morphology of the wrinkled graphene covered by PFA. FA can enter inside the foldings and tiny wrinkles of GR, and grow over the GR film, then a homogeneous nanostructured composite was formed by electropolymerization process.

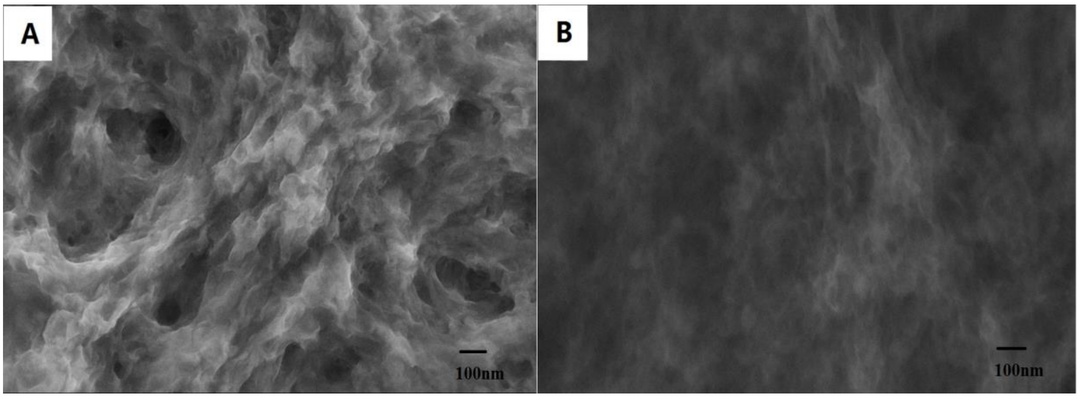

Figure 3. SEM images of (A) electrodeposited graphene film, (B) poly (folic acid)/ electrodeposited graphene composite film.

3.2 Electrochemical behavior of TP and CAF in a mixture at the modified electrode

Fig. 4 displayed the CVs of TP and CAF in pH 4.5 PBS at bare GCE and different modified electrodes. At a bare GC electrode, both compounds exhibited poor current responds (curve a), which revealed that bare GC electrode is not suitable for the stable determination of TP and CAF. As shown in curve b, the peak currents of TP and CAF at GR/GCE are 1.5 and 2.4 times larger than those at the bare GCE, respectively. This fact revealed that the GR film can enhance current responses of two compounds because GR has good conductivity and large surface area [26]. In contrast, TP and CAF showed bigger anodic peaks at the PFA/GCE (curve c), which confirmed that polymer film has the ability to enhance current responses of TP and CAF. In great contrast to bare GC electrode, the PFA/ GR/GCE oxidized TP and CAF at 1.18V and 1.44V, respectively with significantly enhanced peak current response in a mixture (curve d). The oxidation currents obtained for TP and CAF were 3.3 and 3.7 fold higher than those at bare glassy carbon electrode. Furthermore, the PFA/GR modified GC electrode gave birth to a more reversible electron transfer process to TP and CAF based on the lowered overpotential and more negative shifts of oxidation peak potentials [42]. The above results demonstrated that this new electrochemical sensor exhibited excellent electrocatalytic activity towards the oxidation of TP and CAF. The PFA film possesses larger real surface area, better conductivity and superior electrochemical catalytic property towards detection of various analytes such as dopamine [34,35]. The PFA film was deposited on the graphene modified electrode, which constructed a novel conductive composite film. As an inorganic-organic hybrid materials, the PFA/GR nanocomposite film further increased the electroactive surface area and promoted the electron transfer rate of analytes, which can result in higher electrocatalytic activity towards the oxidation of TP and CAF [31,32] .

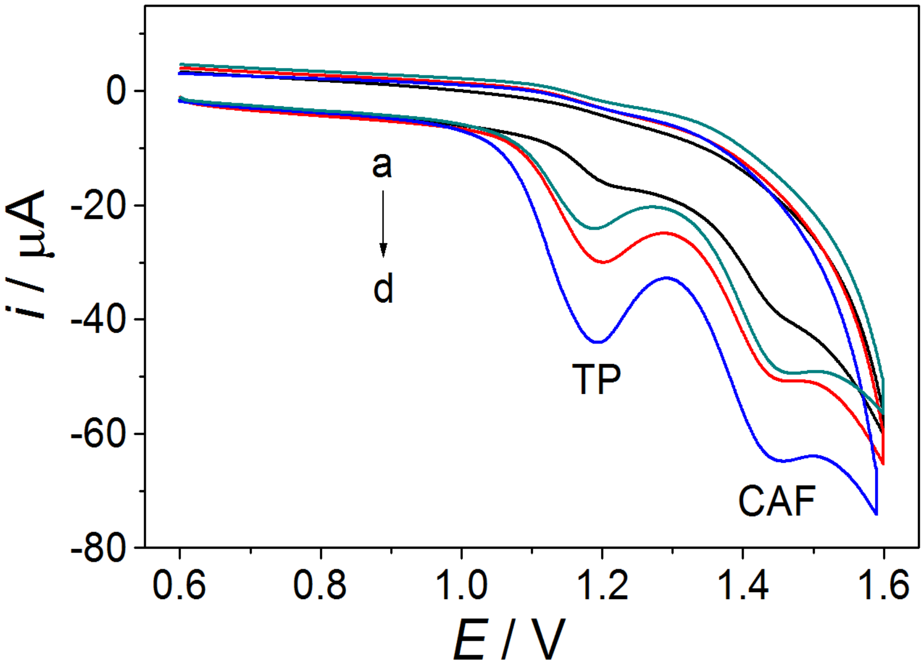

Figure 4. CVs of different electrodes in a mixture containing TP and CAF in phosphate buffer (pH 4.5). Electrode : (a) bare GCE, (b) GR/GCE, (c) PFA/GCE, (d) PFA/GR/GCE; TP: 200 μmol L^{-1}, CAF: 300 μmol L^{-1}; Scan rate: 100 mV s^{-1}

3.3 Influence of scan rate on the oxidation of TP and CAF at the modified electrode

The effects of scan rate on the anodic peak currents of TP and CAF were studied by cyclic voltammetry (Fig. 5). With the scan rate increasing in the range of 20 mV s^{-1} to 240 mV s^{-1}, the oxidation peak currents (i_{pa}) increased continuously. A good linearity between the scan rate (v) and i_{pa} of TP was obtained, suggesting a adsorption -controlled process on the modified electrode surface [41]. The regression equations was i_{pa} (μA) =5.466+0.06702ν (mV s^{-1}) with a correlation coefficient (r) of 0.9992. The i_{pa} of CAF linearly increased with the square root of the scan rate ($\nu^{1/2}$), indicating the redox process of CAF was controlled by diffusion process [42]. The linear regression equation was found to be: i_{pa} (μA) = -3.168+3.230$\nu^{1/2}$ (mV s^{-1}) for CAF with a correlation coefficient of 0.9992.

In addition, the influence of scan rate on the oxidation peak potential (E_{pa}) was investigated. With increasing scan rate, E_{pa} of the two species shifted to more positive potentials , and the plot of E_{pa} versus the logarithm of scan rate presented a linear relation, which demonstrated that the oxidation reactions of TP and CAF at PFA/GR/GCE are irreversible [42]. The linear relation between peak potential and natural logarithm of scan rate were E_{pa} (V) = 1.082+0.01939 ln ν (mV s^{-1}) (r = 0.9970) for TP, E_{pa} (V) = 1.260+0.03114 ln ν (mV s^{-1}) (r = 0.9943) for CAF, respectively. As for an irreversible electrode process, based on the theory of Laviron [41] and the slope of E_{pa} versus ln v [42], the electrontransfer coefficient α was calculated as being 0.662 for TP and 0.412 for CAF, respectively (25˚C).

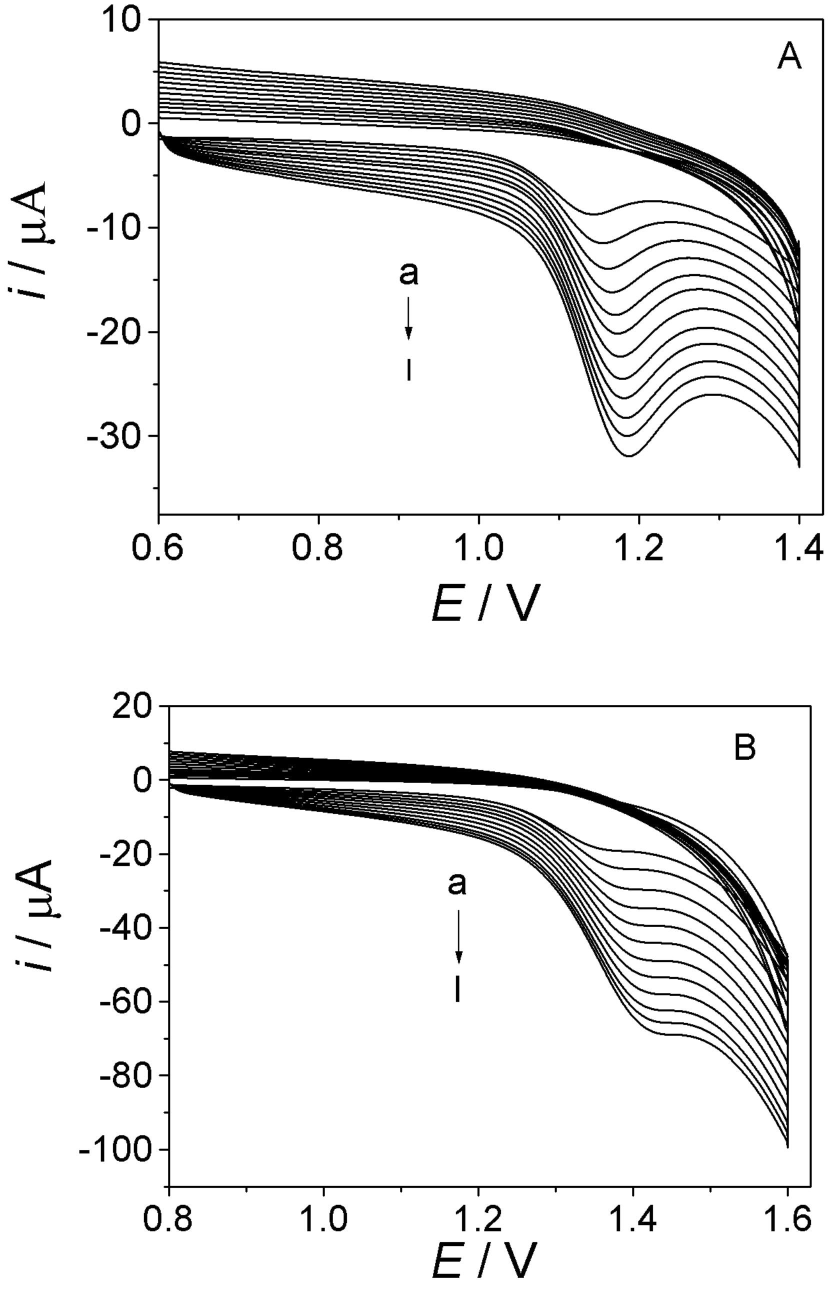

Figure 5. Cyclic voltammograms of 80 μmol L^{-1} TP (A), 300 μmol L^{-1} CAF (B) at PFA/GR/GCE at different scan rates: (a) 20, (b) 40, (c) 60, (d) 80, (e)100, (f) 120, (g)140, (h)160, (i) 180, (j) 200,(k) 220, (l) 240 mVs^{-1}.

3.4 Effect of pH on the oxidation of TP and CAF at PFA /GR/ GCE

The effect of pH on the current response of TP and CAF in the pH range 3.0-8.0 was investigated by CV. It can be seen from Fig. 6A that the peak current of TP increased with increasing pH from 3.0 to 4.5, then it decreased when pH increased further. Although the peak current of CAF increased with increasing pH value, oxidation peak of CAF was out of shape. Therefore, pH of 4.5 was selected as optimum pH value for simultaneous detection of TP and CAF. Compared to other buffers at pH 4.5 such as Britton-Robinson buffer, acetate buffer solution, the best response in terms of peak current and peak shape was obtained in phosphate buffer solution. Hence, pH 4.5 phosphate buffer was chosen as the optimum detection medium in this work.

Fig. 6B showed the plot of the anodic peak potential (E_{pa}) as a function of pH. The peak potential for oxidation of TP shifted negatively with increasing pH. Plot of E_{pa} versus pH was found to be linear over the pH range 3.0-8.0 with correlation coefficient of 0.9998. The linear behaviour between the applied potential and pH with slope of 0.050 V/pH is close to the anticipated Nernstian value (0.059 V/pH), indicating that the proportion of the electrons and protons involved in the redox of TP was 1:1[42]. With pH increased the peak potentials for CAF was almost unchanged, indicating that no proton involved in redox processes [42].

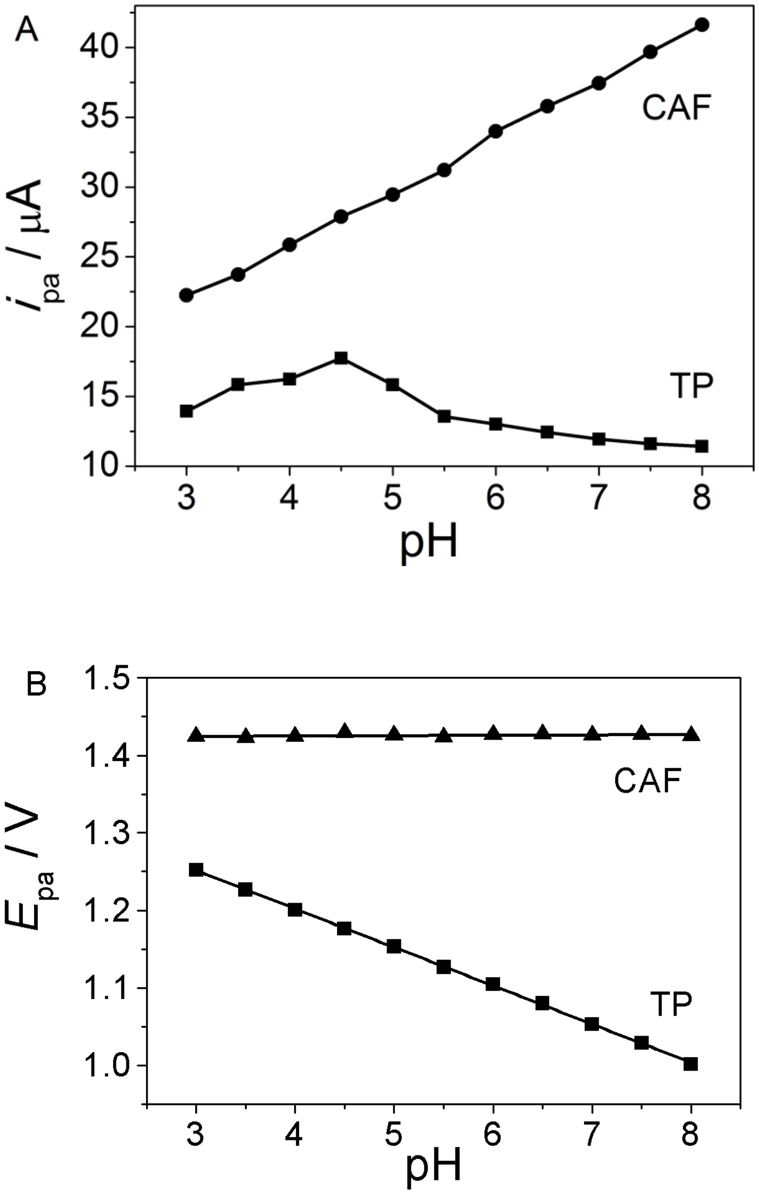

Figure 6. Effect of pH on peak current (A) and peak potential (B) of 80 μmol L^{-1} TP and 400 μmol L^{-1} CAF in phosphate buffer at a scan rate of 100 mV s^{-1}.

3.5 Simultaneous determination of TP and CAF

DPV was used for the determinationof TP and CAF at PFA/GR/GCE in order to get higher sensitivity and better resolution than CV [42]. The optimum DPV parameters for quantitative detection was chosen as follows: The pulse amplitude is 50 mV and pulse width is 100 ms. Fig. 7 showed the DPVs obtained from the different concentration of TP and CAF at the modified electrode, which demonstrated that the oxidation peak currents of TP and CAF were linearly increased with simultaneous concentration increment. The calibration parameters for the simultaneous determination of TP and CAF are listed in Table 1. Considering the above results, it can be concluded that the PFA/GR nanocomposite film can obviously improve the sensitive detection of the two compounds in the terms of high sensitivity and wide linear range. These results were also compared to those extracted from previous work as reported in Table 2, which confirmed the sensing ability of the proposed sensor towards detection of TP and CAF.

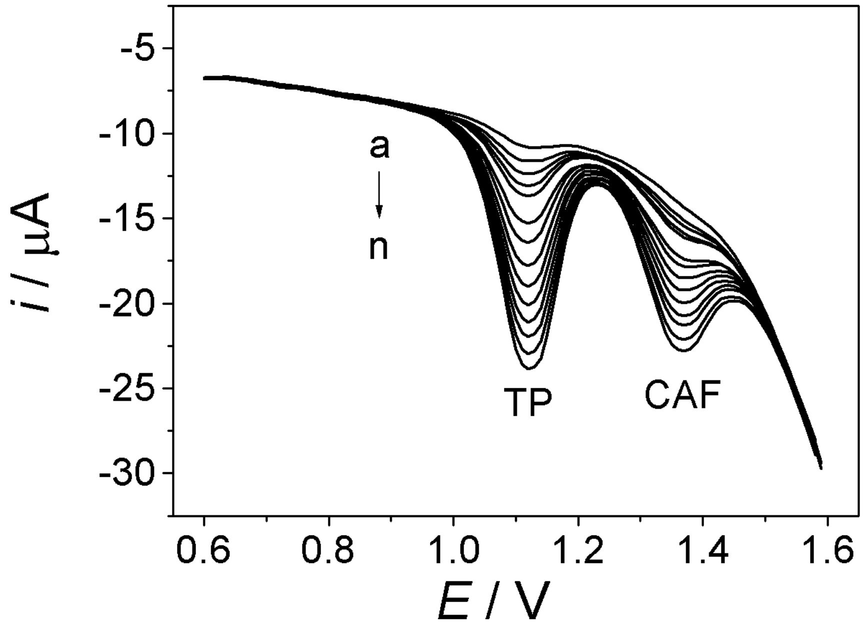

Figure 7. DPVs obtained for the simultaneous concentration increment of TP and CAF at the modified electrode. TP or CAF concentrations: a→n: 2.0, 4.0, 6.0, 8.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 μmol L^{-1}; Pulse amplitude: 50 mV; Pulse width: 100 ms;

Table 1. Analytical characteristics for simultaneous determination of TP and CAF by the proposed method

Analyte	Linear range (μM)	Linear regression equation (i: μA, C: μM)	Correlation coefficient	Detection limit (μM)
TP	0.2~10	i_{pa} = 0.3425C+0.02949	0.9996	0.03
	10~100	i_{pa} =0.1045C+2.549	0.9990	
CAF	1.0~10	i_{pa} =0.1262C-0.2000	0.9996	0.08
	10~160	i_{pa} =0.05144C+0.7195	0.9990	

Table 2. Comparison of the efficiency of some electrochemical sensors used in the determination of TP and CAF

Electrode	Linear range (μM)	Detection limit (μM)	Ref.
dsRNA / GNPs / GCE	TP: 2.0~50	TP: 1.2	[11]
CdSe / GCE	TP: 1.0~700	TP: 0.4	[12]
MWCNTs/GCE	TP: 0.3~10	TP: 0.05	[13]
Poly(safranine T) /GCE	CAF: 3.0~400	CAF: 0.1	[14]
Sol-Gel Film / GCE	CAF: 7.5~400	CAF: 0.22	[15]
MWCNTs/GCE	CAF: 10~500	CAF: 3.5	[16]
Mesoporous carbon /Nafion / GCE	TP: 0.08~180 CAF: 1.3~230	TP: 0.37 CAF: 0.47	[17]
Poly(FA)/ GR /GCE	TP: 0.2~100 CAF: 1.0~160	TP: 0.03 CAP:0.08	This work

3.6 Interferences

The intermolecular effects between TP and CAF were studied by two different experiments. In each experiment, the concentration of one specie was increased linearly in the presence of fixed concentrations of the other one. As can be seen in Figure 8, the peak currents and potentials of CAF had no obvious changes when increasing the concentration of TP. Similarly, fixing the concentration of TP, the oxidation peak current of CAF increased linearly with increasing its concentrations, and its peak potentials remained constant. All the results demonstrated that the oxidation of TP and CAF at the modified electrode are independent processes, and no intermolecular interference can be observed for the simultaneous determination of the two species [30].

Under optimal experimental conditions, possible interference for the detection of TP and CAF was evaluated. The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error less than ±5.0% at a concentration of 10 μmol L^{-1} of TP and CAF [17]. The results showed that 20-fold excess of xanthine, cysteine and dopamine, 60-fold excess of uric acid, acetaminophen and ascorbic acid, 300-fold excess of histidine and phenylalanine, 500-fold excess of sodium citrate, glutamic acid and glucose had no effect on the peak current of TP and CAF. These results suggested that the simultaneous detection of the two compounds in food and biological samples using the proposed sensor was not significantly affected by the most common interfering species.

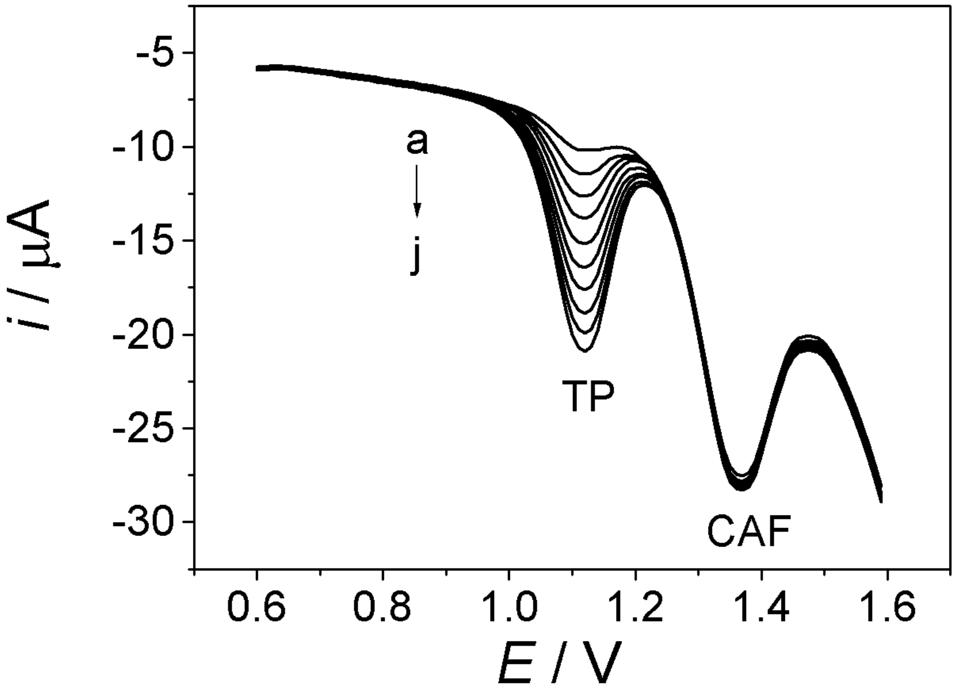

Figure 8. Differential pulse voltammograms for different concentrations of TP with in the present of 300 μmol L^{-1} CAF at PFA/GR/GCE. TP concentrations: a→j: 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0 μmol L^{-1}. Other conditions as in the Fig.7.

3.7 Stability and reproducibility

In order to investigate the stability of the modified electrode, the DPV for 50 μmol L^{-1} TP in PBS was recorded to periodically measure the response sensitivity. The results showed that the current response of TP decreased about 2% in 2 week and 5% in 3 weeks, indicating that the PFA/GR/GCE had good long-term stability. To ascertain the reproducibility of the results further, the mixture solution containing 50 μmol L^{-1} of the two species was measured for ten times. The relative standard deviations (RSD) of the peak currents for TP and CAF were 1.01% and 2.45 %, respectively. The same solutions were also determined with six electrodes made independently, and the interelectrode RSD for TP and CAF were 2.13% and 3.22%, respectively. Therefore, the above results indicated that the present modified electrode was extremely stable and highly reproducible.

3.8 Simultaneous determination of TP and CAF in food samples

In order to evaluate the practical application of this method, the modified electrode was used to determine TP and CAF in some tea and drink samples. The treated sample solution was diluted with pH 4.5 phosphate buffer solution and differential pulse voltammograms was recorded under the optimal experimental conditions. Meanwhile, in order to calculate the recovery, TP and CAF standard solutions was added. The results were listed in Table 3, which were average values of six repeated determinations. The recovery was in the range of 97-102%, indicating that the sensor might be sufficient for practical applications.

Table 3. Determination of TP and CAF in food samples (n=6).

Samples	Original(µM)		Added (µM)		Found (µM)		Recovery (%)	
	TP	CAF	TP	CAF	TP	CAF	TP	CAF
Black tea	1.94	19.25	6.0	10.0	8.18	29.1	102.9	99.3
Green tea drink	—	14.52	4.0	30.0	4.01	44.72	100.1	100.4
Coca cola drink	—	9.95	15.0	10.0	15.27	19.37	97.1	101.8
Red bull drink	—	33.19	30.0	30.0	29.74	62.19	99.1	98.5

4. CONCLUSION

The proposed novel PFA/GR composite modified glassy carbon electrode exhibits highly electrocatalytic activity to the oxidations of TP and CAF. The electrochemical sensor displays high sensitivity and good selectivity in voltammetric detection of TP and CAF. The proposed method provides a useful tool for the assay of theophylline and caffeine in food analysis, pharmaceutical analysis and clinical diagnosis.

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