

## A Graphene-Based Electrochemical Sensor for Sensitive Detection of Vanillin

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A simple and rapid electrochemical method was developed for the determination of trace-level vanillin, based on the excellent properties of graphene. The graphene film-coated glassy carbon electrode (GCE) was constructed and the electrochemical behavior of vanillin at the electrode was investigated in detail. The results indicated that graphene modified GCE exhibited efficiently electrocatalytic oxidation for vanillin with relatively high sensitivity and stability. The electrocatalytic behavior was further exploited as a sensitive detection scheme for the analyte determinations by differential pulse voltammetry (DPV). Under the optimized conditions, the oxidation peak current was proportional to vanillin concentration in the range of  $6.0 \times 10^{-7}$  to  $4.8 \times 10^{-5}$  mol L<sup>-1</sup> with the correlation coefficient of 0.9996 and the detection limit of  $5.6 \times 10^{-8}$  mol L<sup>-1</sup> (S/N = 3). The proposed method was successfully applied to biscuits samples with satisfactory results. The recovery of the method was in the range of 97.9-103.5%.

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**Keywords:** Graphene, Vanillin, Differential pulse voltammetric, Determination

### 1. INTRODUCTION

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the world's flavor extracts obtained primarily from *Vanillia planifolia*, a specie of tropical climbing orchid native to Mexico or Central America, but currently cultivated in many countries, principally in Madagascar, Indonesia, Tahiti, and Togna. Although more than 12,000 t of vanillin are produced each year, less than 1% of this is natural vanillin from Vanilla; the remainder is synthesised much more cheaply via chemical or biochemical processes [1]. Even though synthetic flavours are available at very low prices, consumers prefer natural ones. One of the major uses of vanilla is as a flavour in desserts and baked goods. The desirable flavour and aroma properties of vanilla have lead to its widespread use in confectionery, beverages,

pharmaceuticals, foods and perfumery. Vanillin also used as inhibition of the oxidation of human low density lipoproteins, which leading to lower rates of cardiac disease mortality [2]; and used as an antisickling effect in sickle cell anaemia sufferers [3]. So, controlling the quality of vanillin is of great importance.

Several methods for determining vanillin from several drink samples or vanilla extracts were described using UV spectrophotometry [4], GC–MS [5,6], high-performance liquid chromatography (HPLC) [3,7,8] and capillary electrophoresis (CE) [9,10]. These methods may be costly and involve time-consuming sample pretreatment processes. Recently, novel approaches for vanillin detection using electrochemical sensor have been reported, which are environmental friendly and using extremely low sample consumption as well [11-16]. Due to the simplicity, fast response, high sensitivity and online detection ability, electrochemical sensors have potential advantages to determine vanillin.

Owing to its large specific surface area, extraordinary electrical and thermal conductivities [17], good biocompatibility [18], and low manufacturing cost [19], graphene and its derivatives, including graphene oxide (GO), have attracted ever increasing attention in recent years as a novel class of 2D carbon-based nanomaterials. Like carbon nanotubes (CNTs), which have been widely used for the study of electrode materials, graphene provides a new avenue for fabricating electrochemical devices because it can facilitate electron transfer between electroactive species and electrodes. Moreover, they are more easily produced in mass quantities as compared with the CNTs. In the recent past graphene-modified electrodes have been used for the determination of a variety of electroactive species, such as NADH [20], hydrogen peroxide [21], dopamine [22], glucose [23] and azithromycin [24].

To our knowledge, voltammetric determination of vanillin using a graphene/GCE has not been reported yet. The objective of the present work is to develop a convenient and sensitive method for the determination of vanillin based on the unusual properties of graphene modified electrode. Here we report the electrochemical oxidation of vanillin on graphene modified GCE. The ability of the modified electrode for voltammetric response of selected compound was evaluated. Finally, this modified electrode was used for the analysis of vanillin in biscuits samples. The proposed sensor exhibits high sensitivity, rapid response, good reproducibility, long-term stability and freedom of other potentially interfering species.

## 2. EXPERIMENTS

### 2.1. Reagents and apparatus

Vanillin was purchased from Aladdin-Reagent Company (Shanghai, China). Vanillin was dissolved in double redistilled water to form a stock solution of  $0.012 \text{ mol L}^{-1}$  and stored at  $4 \text{ }^{\circ}\text{C}$ . Graphite powder (spectroscopically pure reagent) was obtained from Qingdao Hensen Graphite Co., Ltd. (China). Graphene was synthesized according to previous report [25]. Other reagents were of analytical grade and used as received. Aqueous solutions were prepared with double redistilled water.

The transmission electron microscope (TEM) image was obtained at JEOL-1200 EX TEM (Japan). The image of scanning electron microscope (SEM) was obtained at Hitachi S-4800 (Japan). Electrochemical experiments were performed with a CHI660D electrochemical workstation (Shanghai Chenhua Co., China) with a conventional three-electrode cell. A bare or graphene modified GCE ( $\phi=3\text{mm}$ ) was used as working electrode. A saturated calomel electrode (SCE) and a platinum wire were used as reference electrode and auxiliary electrode, respectively.

## 2.2. Preparation of the graphene-modified electrode

Before modification, the GCE surface (Alda Co. Ltd., China) was polished with  $0.05\ \mu\text{m}$  alumina slurry, and sonicated in redistilled water to give a clean surface. About 1 mg of graphene particles was dissolved in 1 mL of N,N-dimethylformamide (DMF) and then subjected to ultrasonication for about 30 min to facilitate the complete dissolution of graphene. Finally, the treated GCE surface was coated with  $5\ \mu\text{L}$  graphene suspension and dried under an infrared lamp. Prior to use, the modified electrode was carefully rinsed with water to remove the loosely attached graphene at the electrode, and dried under an infrared lamp.

## 2.3 Sample preparation

The analyzed samples were commercial biscuits purchased in a local super-market, containing vanillin. First of all, the solid samples were ground in a mortar with a pestle. About 0.4 g was weighed and 10ml of ethanol was added. The mixture was mechanically shaken for 1 h. After centrifugation at 3000 rpm for 10 min, the clear part of the solution in the tube was used for analysis.

## 2.4 Analytical procedure

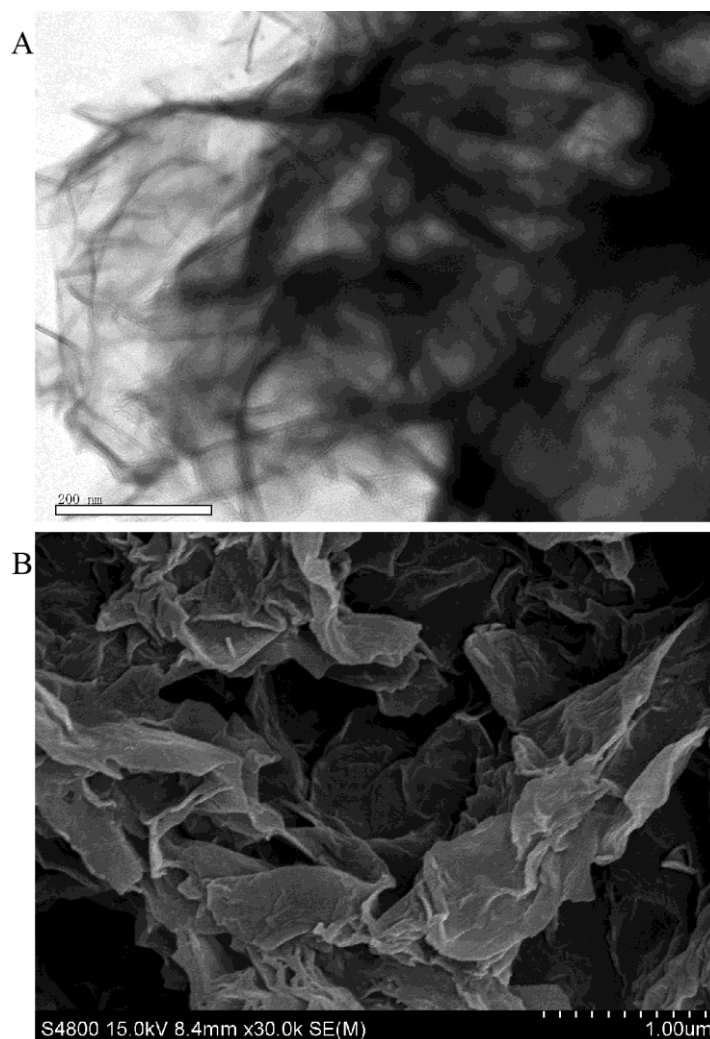
The pH 5.0  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} - \text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  buffer was used as the supporting electrolyte for vanillin. After 3 min of stirring, the DPV curves were recorded from 0.50 to 0.90 V, and the oxidation peak currents at 0.65 V was measured for vanillin. The pulse amplitude is 50 mV, the pulse width is 0.2 s.

# 3. RESULTS AND DISCUSSION

## 3.1. Characterization of graphene dispersed in DMF

Fig. 1A shows the TEM image of graphene sheets. As shown in this figure, the graphene exhibits transparent and wrinkled graphene sheets. This wrinkled nature of graphene is highly beneficial in maintaining a high surface area on the electrode since the sheets cannot readily collapse back to a graphitic structure [26, 27]. Fig. 1B shows the SEM image of graphene film on the surface of

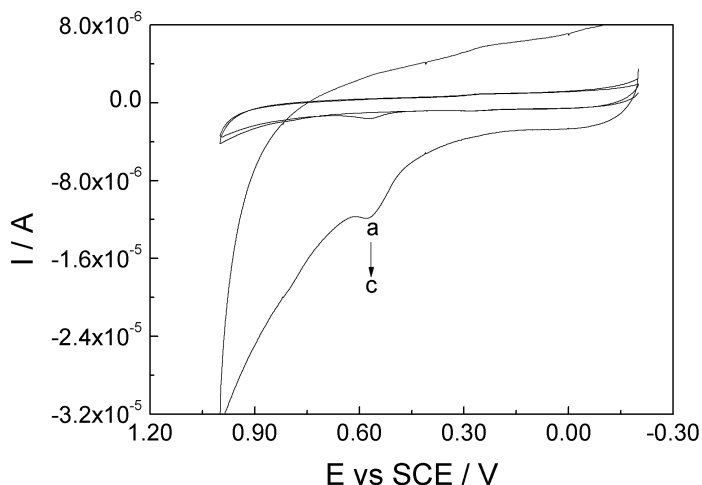
the GCE, revealing the typical crumpled and wrinkled graphene sheet structure on the rough surface of the film.



**Figure 1.** TEM (A) of graphene in DMF solvent and SEM (B) image of graphene-film modified GCE.

### 3.2. Electrochemical behaviour of vanillin

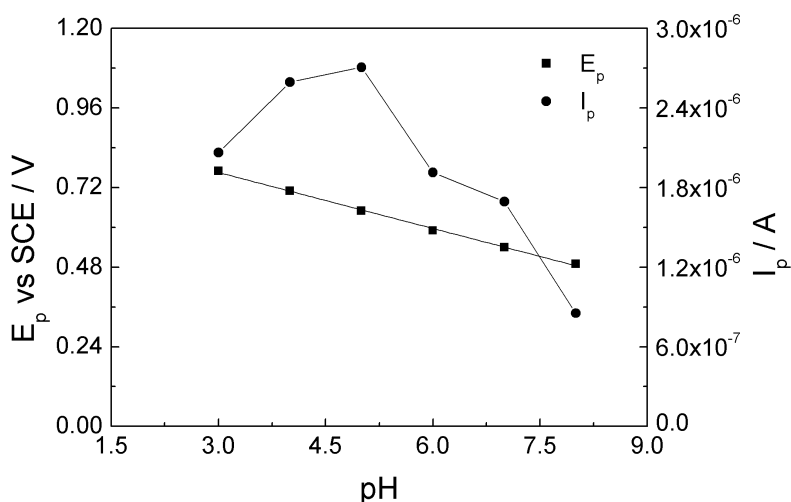
The voltammograms of vanillin at a bare GCE and graphene/GCE in  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} - \text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  buffer (pH 7.0) were shown in Fig. 2. It can be seen that the vanillin oxidation peak at the bare GCE was weak due to slow electron transfer, while the response was improved at the graphene/GCE. The peak potential at above two electrodes was almost the same, at about 0.57 V. The oxidation peak current value ( $I_p$ ) of vanillin at graphene/GCE was  $2.186 \times 10^{-6}$  A, which was 4.22 times larger than the one at the GCE. It also showed that no reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. The oxidation peak current was recorded for vanillin analysis in the following studies.



**Figure 2.** CVs recorded at a bare GCE without vanillin(a); bare GCE with  $2.4 \times 10^{-5}$  mol L<sup>-1</sup> vanillin(b); graphene/GCE with  $2.4 \times 10^{-5}$  mol L<sup>-1</sup> vanillin (c) in the buffer of Na<sub>2</sub>HPO<sub>4</sub> · 12H<sub>2</sub>O · C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> · H<sub>2</sub>O, pH 7.0, scan rate: 50 mV s<sup>-1</sup>.

### 3.3. Effect of pH value

The effect of solution pH on the electrochemical response of  $2.4 \times 10^{-5}$  mol L<sup>-1</sup> vanillin at graphene/GCE was investigated in the pH range from 3.0 to 8.0 by DPV. As can be seen in Fig. 3, the solution pH obviously influenced the oxidation peak current and the maximum current response was obtained at pH 5.0. Thus this pH was chosen for the subsequent analytical experiments.



**Figure 3.** Influence of pH on the oxidation peak current and potential of  $2.4 \times 10^{-5}$  mol L<sup>-1</sup> vanillin.

The peak potential was closely dependent on the pH of the solution. It was found that the values of peak potential shifted to more negative values with the increase of pH (as shown in Fig. 3),

indicating protons take part in electrode process, which is in agreement with that obtained by [14]. The peak potential ( $E_p$ ) moved in negative direction with pH rising and they showed such relationship as:  $E_p(\text{V}) = -0.058 \text{ pH} + 0.942$  ( $R = 0.9994$ ). The slope of  $-0.058 \text{ V pH}^{-1}$  demonstrated that the numbers of electron and proton transferred in the electrochemical reaction of vanillin were equal.

### 3.4 Effect of scan rate

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behaviors of vanillin at different scan rates were investigated on the surface of the graphene modified GCE by DPV. A linear relationship ( $I_p = -3.12 \times 10^{-8} v - 9.29 \times 10^{-7}$ ,  $n=13$ ,  $R=0.9975$ ) with a correlation coefficient of  $R = 0.9985$  was observed between the peak current and the scan rate in the range of 20–250 mV/s, which revealed that the oxidation of vanillin was an adsorption-controlled step.

The peak potential shifted to more positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as  $E_p(\text{V}) = 0.0152 \ln v(\text{V/s}) + 0.7682$  ( $n=13$ ,  $r=9956$ ). As for an irreversible electrode process, according to Laviron [28],  $E_p$  is defined by the following equation:

$$E_p = E^{\theta'} + \frac{RT}{\alpha nF} \ln v \quad (1)$$

where  $E_p$  is the peak potential (V vs. SCE),  $E^{\theta'}$  is the formal potential (V vs. SCE),  $R$  is the universal gas constant ( $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ ),  $T$  is the temperature (K),  $\alpha$  (alpha) is the charge transfer coefficient for the oxidation step,  $n$  is the number of electrons involved in the rate determining step, and  $F$  is the Faraday constant ( $96,485 \text{ C mol}^{-1}$ ). According to Bard and Faulkner [29], for a totally irreversible wave,  $E_p$  is a function of scan rate, shifting (for an oxidation) in a positive direction by an amount  $1.15RT/\alpha F$  (or  $30/\alpha \text{ mV}$  at  $25^\circ\text{C}$ ) for each ten fold increase in  $v$ . So, from this we get the value of  $\alpha$  to be 0.789. The number of electron ( $n$ ) transferred in the electro-oxidation of Syn was calculated to be 2.140 (approximately equal to 2). The conclusions are well consistent with previous report on the electrochemical oxidation of vanillin [16].

### 3.5 Chronocoulometry

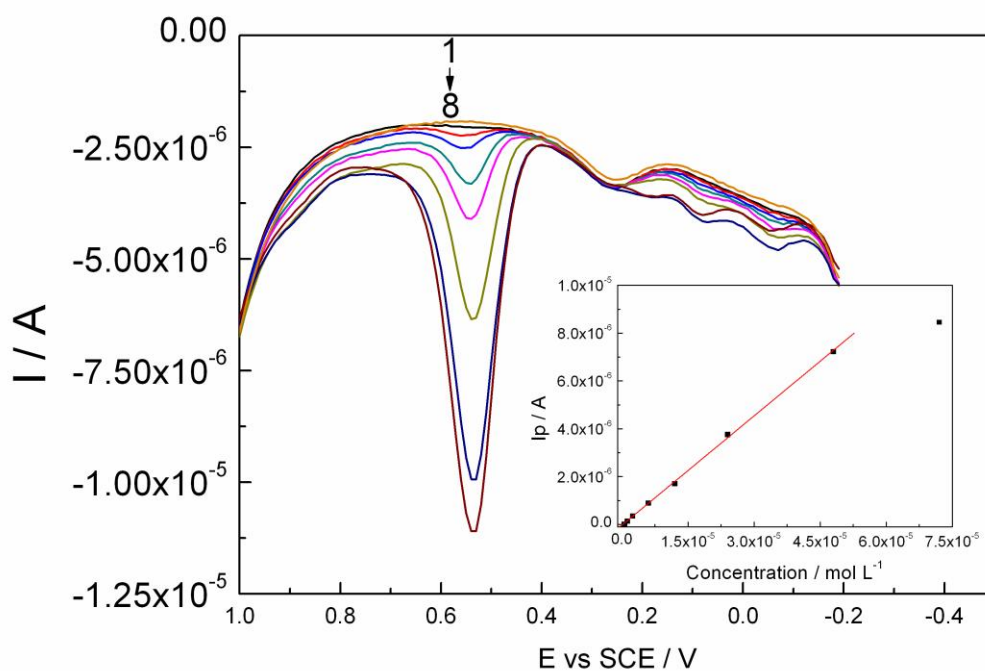
The electrochemically effective surface areas ( $A$ ) of GCE and graphene/GCE were investigated by chronocoulometry using  $1.0 \times 10^{-4} \text{ mol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$  as model complex (the diffusion coefficient of  $\text{K}_3[\text{Fe}(\text{CN})_6]$  in  $1.0 \text{ mol L}^{-1} \text{ KCl}$  is  $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  [30] based on Anson equation [31]:

$$Q(t) = \frac{2nFAcD^{1/2}t^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \quad (2)$$

where  $c$  is substrate concentration,  $D$  is the diffusion coefficient,  $n$  is electron transfer number,  $Q_{dl}$  is double layer charge which could be eliminated by background subtraction,  $Q_{ads}$  is Faradic charge. Other symbols have their usual meanings. Based on the slopes of the curves of  $Q$  versus  $t^{1/2}$ ,  $A$  was calculated to be  $0.087 \text{ cm}^2$  (GCE) and  $0.21 \text{ cm}^2$  (graphene/GCE), indicating that the electrode effective surface area was increased obviously after electrode modification, which would increase the electrochemical active site, enhance the electrochemical response, and decrease the detection limit.

### 3.6. Calibration curve

Under the optimal conditions, differential pulse voltammetry (DPV) was used to determine vanillin due to its higher sensitivity than cyclic voltammetry. Fig. 4 shows the differential pulse voltammograms of different concentrations of vanillin in pH 5.0. As can be seen in the insert of Fig. 4, the oxidation peak current was proportional to vanillin concentration in the range of  $6.0 \times 10^{-7}$  to  $4.8 \times 10^{-5} \text{ mol L}^{-1}$  with the linear regression equation of  $I \text{ (A)} = 0.1523c \text{ (mol L}^{-1}) - 2.8 \times 10^{-8}$  ( $R = 0.9996$ ). The detection limit was estimated to be  $5.6 \times 10^{-8} \text{ mol L}^{-1}$  ( $S/N = 3$ ). Analysis of vanillin by DPV at a GCE was investigated and was listed in Table 1. The comparison of graphene/GCE with other modified electrodes for vanillin determination was also listed in Table 1. It can be seen that the graphene/GCE offered reasonable linear range for vanillin detection and the detection limit was lower than some of previous reports. These results indicated that graphene/GCE is an appropriate platform for the determination of vanillin.



**Figure 4.** Differential pulse voltammograms of graphene/GCE in different concentrations of vanillin solutions. (1)–(8)  $0, 6.0 \times 10^{-7}, 1.2 \times 10^{-6}, 2.4 \times 10^{-6}, 6.0 \times 10^{-6}, 1.2 \times 10^{-5}, 2.4 \times 10^{-5}, 4.8 \times 10^{-5}, 7.2 \times 10^{-5} \text{ mol L}^{-1}$ . Insert: calibration curve.

**Table 1.** Comparison of different modified electrodes for vanillin determination

Electrode	Electrochemical techniques	Detection limit (mol L <sup>-1</sup> )	Linear range (mol L <sup>-1</sup> )	Correlation coefficient	Refs
PVC/graphite electrode	Amperometric	$2.9 \times 10^{-4}$	$6.6 \times 10^{-4}$ - $9.2 \times 10^{-3}$	0.9991	[15]
Cylindrical CFME	SWV*	$4.2 \times 10^{-6}$	$1.0 \times 10^{-5}$ - $7.0 \times 10^{-4}$	0.9990	[11]
GCE	SWV	$1.6 \times 10^{-7}$	$5.0 \times 10^{-5}$ - $3.0 \times 10^{-4}$	0.9987	[13]
Graphite electrode	SWV	$4.0 \times 10^{-7}$	$5.0 \times 10^{-6}$ - $4.0 \times 10^{-4}$	0.9995	[12]
MWCNTs-TAPcCo/GCE	SWV	$4.4 \times 10^{-7}$	$4.2 \times 10^{-6}$ - $5.0 \times 10^{-4}$	0.9993	[14]
CDA/Au-AgNPs/GCE	Amperometric	$4.0 \times 10^{-8}$	$2.0 \times 10^{-7}$ - $5.0 \times 10^{-4}$		[16]
GCE	DPV	$5.1 \times 10^{-7}$	$6.0 \times 10^{-6}$ - $2.6 \times 10^{-4}$	0.9966	Our work
graphene/GCE	DPV	$5.6 \times 10^{-8}$	$6.0 \times 10^{-7}$ - $4.8 \times 10^{-4}$	0.9996	Our work

\* SWV: square wave voltammetry

### 3.7. Reproducibility, stability and interferences

For investigating the fabrication reproducibility, a  $6.0 \times 10^{-6}$  mol L<sup>-1</sup> vanillin solution was measured by six modified electrodes prepared independently and the RSD of the peak current was 3.52%, revealing excellent reproducibility. After the electrode was stored for 15 days at 4°C in humidity environment, it could retain 92.35% of its original response, suggesting acceptable storage stability. In addition, 800-fold concentration of K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, glucose, sucrose, aminoacetic acid, VB1; 400-fold concentration of urea; 250-fold concentration of Ca<sup>2+</sup>; 200-fold concentration of Al<sup>3+</sup>, Fe<sup>3+</sup>; 150-fold concentration of Pb<sup>2+</sup>; 8-fold concentration of Vc; 6-fold concentration of Mn<sup>2+</sup>; 2.5-fold concentration of hypoxanthine do not interfere with the oxidation signal of  $6.0 \times 10^{-6}$  mol L<sup>-1</sup> vanillin (peak current change < 5%). These results indicated that graphene/GCE has an excellent selectivity for vanillin, and it might be applied to determine vanillin in real samples.

### 3.8. Analytical application

In order to test the practical application of the proposed method, the graphene/GCE was used to determine vanillin in two different commercial samples of biscuits. The results obtained by the proposed and reference procedures (Spectrophotometric method [4] for the assay of vanillin was



adopted ) were compared in Table 2 and were in good agreement. The recovery of the method was in the range of 97.9-103.5 %.

**Table 2.** Determination of vanillin in biscuit samples

Samples	reference method (mg g <sup>-1</sup> )	Proposed method (n=5)				
		Determination (mg g <sup>-1</sup> )	Added (mg g <sup>-1</sup> )	Founded (mg g <sup>-1</sup> )	RSD (%)	Recovery (%)
Sample 1#	2.99	2.91	1.50	4.47	2.45	103.5
Sample 2#	3.45	3.39	1.50	4.86	2.02	97.9

#### 4. CONCLUSIONS

An electrochemical sensor for rapid detection of vanillin was developed using graphene sensing film. Owing to large surface area and subtle electronic properties, the graphene film enhances the oxidation signals of vanillin. So, the sensitivity of detection of vanillin is greatly improved at graphene/GCE. The proposed method was applied to detect vanillin in real samples with detection limit ( $5.6 \times 10^{-8}$  mol L<sup>-1</sup>) and satisfied recoveries from 97.9% to 103.5%.

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