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Sensitive Voltammetric Determination of Vanillin with an Electrolytic Manganese Dioxide−Graphene Composite Modified Electrode

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The electrolytic manganese dioxide - graphene (EMDG) composite was used in the construction of a sensor, which showed strong electrocatalytic activity to the oxidation of vanillin in neutral solutions. Vanillin produces an anodic peak at about 0.67 V at this electrode. Several electrochemical parameters of these processes were further carefully calculated with the results of the charge transfer coefficient α as 0.703 and the electron transferred number n as 2. The electrocatalytic behavior was further exploited as a sensitive detection scheme for the vanillin determination by differential-pulse voltammetry (DPV). Under optimized conditions, the anodic peak current increased linearly with the vanillin concentration over the range from 1.0×10^{-7} to 4.5×10^{-5} mol L^{-1}. The detection limit was calculated as 3.2×10^{-8} mol L^{-1} (S/N=3). The proposed method was applied to the detection of biscuits and chocolates samples with satisfactory results.

Keywords: Electrolytic manganese dioxide Graphene Vanillin Differential pulse voltammetric Determination

1. INTRODUCTION

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the main aromatic compound in natural vanilla, and can be extracted from vanilla pods and beans. In the market, more than 99% of vanillin is synthesized much more cheaply via chemical or biochemical processes [1,2]. Vanillin is one of the most popular flavoring substances and it is widely used in confectionery, beverages, pharmaceuticals, foods and perfumery. However, Excessive ingestion of Vanillin can cause headaches, nausea and vomiting, and can affect potential damage to liver and kidney [3]. Hence, it is important and necessary to detect and control the content of vanillin in food products.

Several methods have been reported for the determination of vanillin which include spectrophotometry [3], fluorescence [4], capillary electrophoresis [5,6], HPLC [7,8], LC- MS and GC-MS [9,10]. These methods can be applied to the detection of vanillin in various samples, but they are generally time- consuming and/or complicated. Electrochemical sensors are becoming increasingly important for quantitative determination of vanillin due to their high sensitivity, fast response, cheap instrumentation and online detection ability, etc [11-16].

Owing to its good electrochemical properties, low toxicity, low cost, relative abundance, manganese dioxide (MnO_2) is widely used in catalysts[17], molecular-sieves[18], and electromagnetic materials 19]. In the past few decades, with the advances of nanoscience, the controlled synthesis of MnO_2 nano–and micro-crystals with desired shapes have been used for the detection of epinephrine [19], hydrogen peroxide [21-24], Ascorbic acid [25], catechol [26,27], dopamine [28] and heavy metal ions (such as Cd^{2+} and Pb^{2+}) [29] et al. These studies show that nano–and micro-structured MnO_2 leads to the improvement of its electrocatalytic activity. Recently, graphene - MnO_2 nanocomposite modified electrode was applied for analysis of rutin, due to the electrochemical performances of the modified electrode were greatly enhanced [30].

In the present work, a composite was prepared by mixing graphene, electrolytic manganese dioxide with 1% chitosan solution and modified glassy carbon electrode (EMDG/GCE). The electrochemical response of vanillin at EMDG/GCE in phosphate buffer solution (PBS, pH 7.0) was investigated in detail and a novel method for the determination vanillin with sensitive, simple and rapid characteristics was presented. Finally, this approach was used for the detection of vanillin in biscuits and chocolates with a satisfactory result.

2. EXPERIMENTS

2.1. Reagents and apparatus

Vanillin, hydrazine hydrate and chitosan were purchased from Aladdin-Reagent Company (Shanghai, China). The standard stock solution (0.01 mol L^{-1}) of vanillin was held at refrigerator temperatures (4 °C) before use. Graphite powder (spectroscopically pure reagent) for preparation of graphene was obtained from Qingdao Hensen Graphite Co., Ltd. (China). Electrolytic manganese dioxide was obtained as a generous gift from Guangxi Eramet Comilog Chemicals Company Limited. Graphene (Gr) was synthesized by the reduction of exfoliated graphite oxide with hydrazine hydrate [31], and graphite oxide was prepared from natural graphite powder through Hummers method [32]. Other reagents were of analytical grade and used without further purification. The water used throughout all experiments was double redistilled water.

The morphology of samples was examined by scanning electron microscopy (SEM; ZEISS EVO18, Germany). All the electrochemical tests were carried out on a CHI660D electrochemical workstation (Shanghai Chenhua Co., China) with a conventional three-electrode cell. A bare or EMDG modified GCE (φ=3mm) was used as working electrode. A saturated calomel electrode (SCE) as reference electrode and a platinum wire electrode (Pt) as the auxiliary electrode were used. Phosphate buffer solutions (PBS) was prepared by mixing KH_2PO_4, Na_2HPO_4, and the pH values were accurately adjusted using a pHS-25 pH-meter (Shanghai Leici Instrument Plant, China) at room temperature.

2.2. Preparation of the EMDG modified electrode

The EMDG composite film electrode was fabricated with the following procedures. GCE was polished with 0.05 μm alumina powders and rinsed thoroughly with water. It was sonicated in double redistilled water to give a clean surface and dried at room temperature. 1 mg graphene and 1 mg electrolytic manganese dioxide were added into 1 mL 1% chitosan (Chi) solution, followed by ultrasonication for 2 h to form homogenous EMDG dispersion. Finally, 5 μL EMDG suspension was cast on the treated GCE surface and dried under an infrared lamp.

2.3 Sample preparation

Several commercial food samples, such as biscuit and chocolate, were purchased from a local market, containing vanillin. First of all, the solid samples were ground in a mortar with a pestle. Then, about 0.5 g of this powder, 10 ml of absolute ethanol were place into a tube and shacked by a laboratory shaker 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 standard solution or sample solution of vanillin was added into PBS buffer solution (pH = 7.0). After 30 s stirring, the cyclic voltammograms (CVs) was recorded from -0.2 to 1.0 V at a scan rate of 0.1 V s^{-1}. The differential pulse voltammetry (DPV) was performed from 0.25 to 1.0 V with amplitude of 0.05 V, pulse width of 0.2 sec. All measurements were performed at room temperature (25 °C).

3. RESULTS AND DISCUSSION

3.1. Characterization of EMDG

The SEM images of graphene sheets and EMD are shown in Fig. 1. It can be seen from the figure, the graphene exhibits transparent and wrinkled structure morphology. These wrinkles are considered to be important for preventing aggregation of graphene and maintaining high surface area on the electrode.

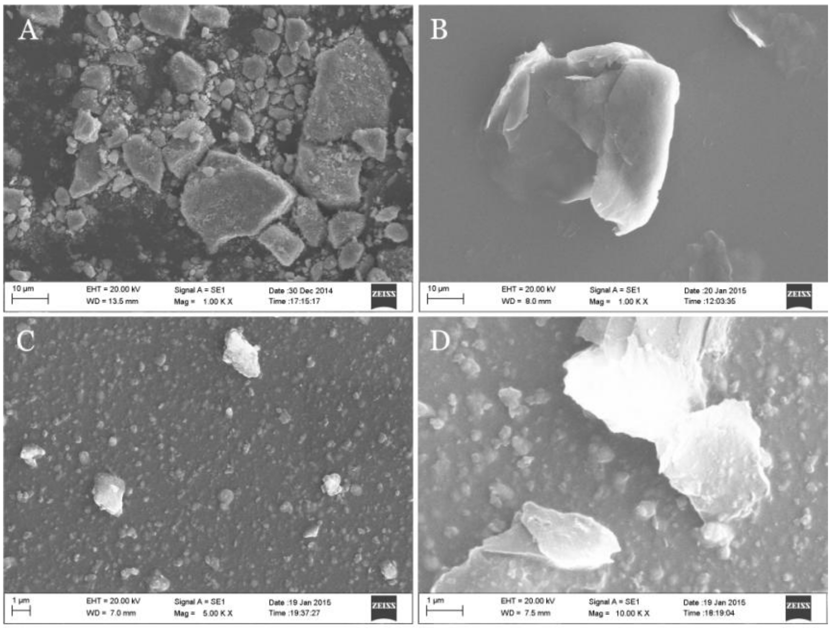


Figure 1. SEM of EMD (A), Gr (B), EMD(C), EMDG film modified GCE. The solution for A is DMF, else is 1% Chi.

3.2. Electrochemical behaviour of vanillin

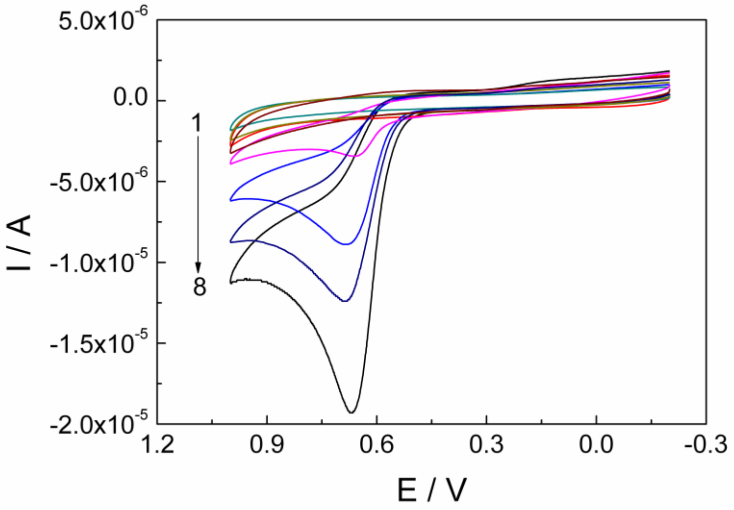


Figure 2. CVs recorded at GCE(line 1,5), Gr/GCE(line 2,6), EMD/GCE(line 3,7) and EMDG/GCE(line 4,8) with 1.0×10^{-3} mol L^{-1} vanillin in the buffer of PBS, pH 7.0, scan rate: 100 mV s^{-1}.

The voltammograms of vanillin at a bare GCE, Gr/GCE, EMD/GCE and EMDG/GCE in PBS (pH 7.0) were shown in Fig. 2. It showed that no cathodic peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. As can be seen, the oxidation peak potential of vanillin at bare GCE, Gr/GCE, MnO_2/GCE and EMDG/GCE are all 0.668 V. The oxidation peak current value (Ip) of vanillin at EMDG/GCE was 1.9×10^{-5} A, which is 8.9 times larger than the one at the GCE, 2.6 times larger than that of Ip at the Gr/GCE and 1.9 times larger than that of Ip at the EMD/GCE. The data obtained clearly show that the combination of Gr and EMD definitely improve the characteristics of vanillin oxidation. So EMDG could be substituted for the oxidation of vanillin.

3.3. Effect of pH value

The effect of solution pH on the electrochemical response of vanillin at EMDG/GCE was studied by DPV (Fig. 3). It was observed that the maximum current response was obtained at pH 7.0. Thus this pH was chosen for the subsequent analytical experiments. Accordingly, the pH increase also resulted in the formal potential shifted toward negative, which is in agreement with that obtained by Peng and Shang [11, 14]. The regression equation can be expressed as Ep(V) =−0.055 pH+ 0.922 (R = 0.9970). According to the following formula [33]: dEp/dpH = 2.303mRT/nF, in which, m is the number of proton, and n is the number of electron, m/n was calculated to be 0.93 for the oxidation process. It indicates that the same amounts of electrons and protons take part in the electrode reaction.

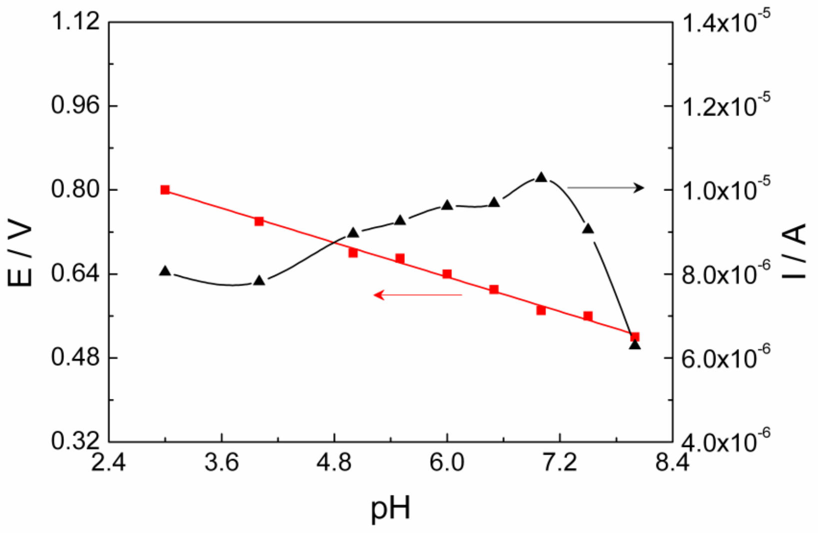


Figure 3. Influence of pH on the oxidation peak current and potential of 1.0×10^{-4} mol L^{-1} vanillin.

3.4 Effect of scan rate

The electrochemical behaviors of vanillin at different scan rates were investigated on the surface of the EMDG modified GCE. A linear relationship (I_p = -2.57×10^{-5} v-3.31×10^{-6}) with a correlation coefficient of R = 0.9985 was observed between the peak current and the scan rate in the range of 10–500 mV/s, indicating a typical adsorption-controlled process.

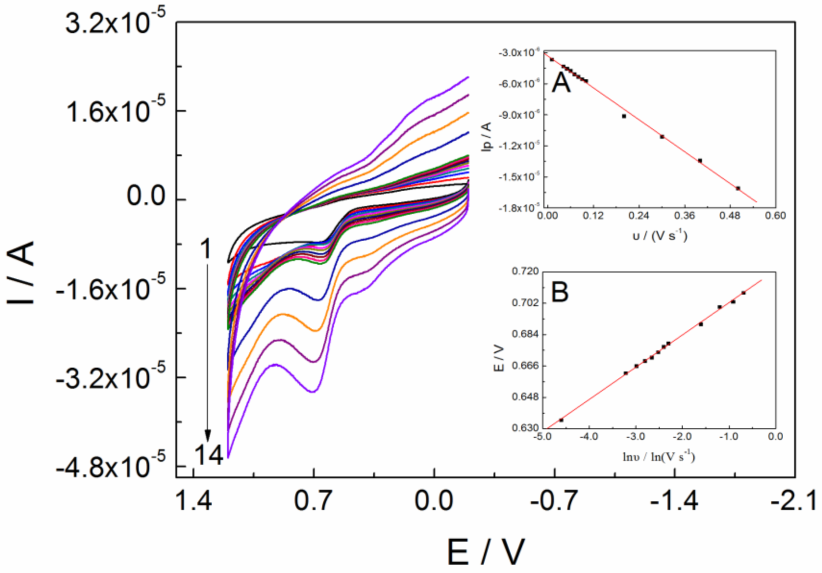
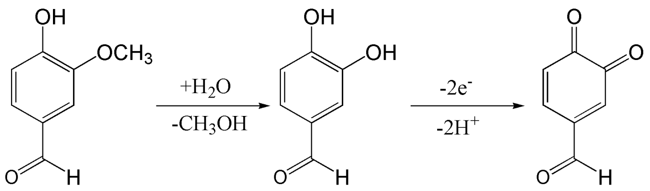


Figure 4. CVs of 1.0×10^{-4} mol L^{-1} vanillin in pH 7.0 PBS solution with different scan rates (from 1 to 14: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5 V s^{-1}); insert A is the *I*p vs υ plot; insert B is the *E*p vs lnυ plot.

The oxidation peak potential of vanillin positively shifted with the increasing scan rates. A linear relationship between peak potential and logarithm of scan rate was obtained as *E*p (V) = 0.0185 lnυ (V s^{-1}) + 0.7209 (n=12, r=9993) (Fig. 4 insert B). According to Laviron theory [34] for an irreversible electrode process, *E*p is defined by the following equation:

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

where $E^{\theta'}$ is the formal potential, *α* is the charge transfer coefficient for the oxidation step, *n* is the number of electrons involved in the rate determining step. *T*, *R* and *F* have their usual meaning. According to Bard and Faulkner [35], E_p positively shifted by an amount *1.15RT/αF* (or *30/α*mV at 25°C) for each ten fold increase in *v*. Thus the value of *α* was easily calculated to be 0.703 and the number of electron *n* transferred in the oxidation of vanillin 1.97 (approximately equal to 2). This conclusion is consistent with previous report on the electrochemical oxidation of vanillin at Gr/GCE [11]. As indicated above, two electrons and two protons take part in the oxidation process. The proposed mechanism on the EMDG /GCE may be expressed with Scheme 1.



Scheme 1. Proposed mechanism for the electrochemical behavior of vanillin on the surface of EMDG/GCE.

3.5. Calibration curve

The relationship between the oxidation peak current and the concentration of vanillin was examined by DPV, and the results are shown in Fig. 5. Under the optimized working conditions, the oxidation peak currents were linearly proportional to vanillin concentrations in the range from 1.0×10^{-7} to 4.5×10^{-5} mol L^{-1} with the linear regression equation of I (A) $=-1.01\times10^{-1}$ c (mol L^{-1})$-$ 9.08×10^{-8} (R = 0.9989). The detection limit was calculated to be 3.2×10^{-8} mol L^{-1} at a signal-to-noise ratio of 3.

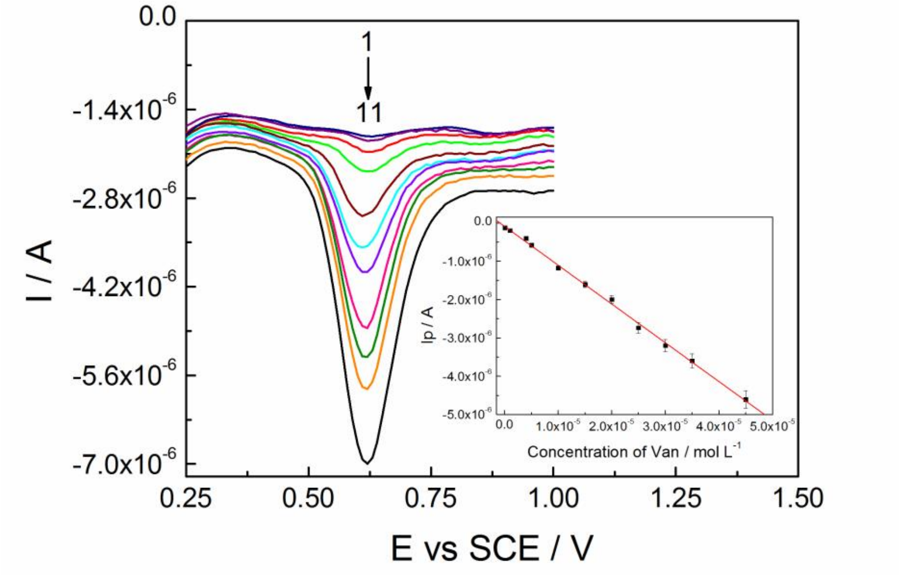


Figure 5. Differential pulse voltammograms of EMDG/GCE in different concentrations of vanillin solutions. (1)–(11) 1.0×10^{-7}, 1.0×10^{-6}, 4.0×10^{-6}, 5.0×10^{-6}, 1.0×10^{-5}, 1.5×10^{-5}, 2.0×10^{-5}, 2.5×10^{-5}, 3.0×10^{-5}, 3.5×10^{-5}, 4.5×10^{-5} mol L^{-1}. Insert: calibration curve.

3.6. Reproducibility, stability and interferences

To characterize the fabrication reproducibility, a 1.0×10^{-5} mol L^{-1} vanillin solution was measured by five modified electrodes prepared independently and the RSD of the peak current was 3.27%, revealing excellent reproducibility. The stability of EMDG/GCE sensor was evaluated by examining the current responses of vanillin after the electrode was stored for 5 days in a refrigerator at 4℃ in humidity environment. The peak currents of vanillin decreased to 91.57% of its original response, suggesting acceptable storage stability. Possible interferences for the detection of vanillin at the EMDG/GCE were investigated by adding various foreign species into the PBS (pH 7.0) containing 1.0×10^{-5} mol L^{-1} vanillin. The results indicated that 500-fold concentration of K^+, Na^+, Ca^{2+}, NH_4^+, Cl^-, NO_3^-, SO_4^{2-}, urea, glucose, sucrose; 300-fold concentration of Al^{3+}, Mg^{2+}, Cu^{2+}, aminoacetic acid; 200-fold concentration of Zn^{2+}, V_{B1}, V_c; 10-fold concentration of $Pb^{2+}$and Fe^{2+} have not significantly influenced the height of the peak currents (peak current change < 5%). These results indicated that the determination of vanillin at EMDG/GCE is not significantly affected by the most common interfering species and thus this method is selective in real samples.

3.7. Analytical application

In order to evaluate the validity of the proposed method, the EMDG/GCE was used to determine vanillin in commercial biscuit and chocolate (shown in Table 1). As can be seen, the RSD values of the measurements were not greater than 3.41%, and the recovery of the method was in the range of 95.5-102.5 %. The results obtained by the proposed and reference procedures (HPLC) were in good agreement.

Table 1. Determination of vanillin in biscuit and chocolate samples

Samples	reference method* (mg g^{-1})	Proposed method (n=5)				
		Determination (mg g^{-1})	Added (mg g^{-1})	Founded (mg g^{-1})	RSD (%)	Recovery (%)
biscuit	1.71	1.65	2	3.7	3.41	102.5
chocolate	2.75	2.65	2	4.56	2.76	95.5

* HPLC

4. CONCLUSIONS

EMD and Gr nanosheet were mixed together to get an EMDG composite, which was modified on GCE and further used for sensitive determination of vanillin. The significant increase of oxidation peak currents was observed at the EMDG/GCE, which clearly demonstrated that EMDG could be used as an efficient promoter to enhance the kinetics of the electrochemical process of vanillin. Electrochemical behaviours of vanillin on EMDG/GCE were carefully investigated with the electrochemical parameters calculated. The proposed method could be an extremely promising candidate applicable for vanillin analysis.

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