Electrochemical Determination of Vitamin C on Glassy Carbon Electrode Modified by Carboxyl Multi-walled Carbon Nanotubes

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A new sensor was constructed by using a glassy carbon electrode (GCE) modified with carboxyl multi-walled carbon nanotubes (MWCNTs) dispersed in N,N-dimethylfomamide (DMF) media. Compared with bare GCE, the resultant DMF/carboxyl/MWCNTs/GCE was used to detect vitamin C (VC) in samples with higher sensitivity, lower detection limit and better repetitiveness. Under the optimal conditions, the obtained sensor presented linear response to VC in the range of 1.0×10^{-6} to 1.0×10^{-3} mol/L, the linear regression equation was $i_{pa}(10^{-5}A) = 0.226\ c\ (10^{-4}\ mol/L) + 1.351$ with the linear correlation (r): 0.999, the limit of detection(LOD): 2.5×10^{-7} mol/L. As tomato samples were detected by the DMF/carboxyl/MWCNTs/GCE, the recovery rate was in the range of 99%~105%. Relative Standard Deviation (RSD) was in the range from 3.18 to 4.21. The proposed electrode was used to detect VC concentration in real samples successfully.

1. INTRODUCTION

Vitamin C (VC), also known as ascorbic acid, is a water-soluble substance and a hexonic sugar acid. It is widely present in many vegetables, beverages, fruits. VC is an efficient reducing agent. Therefore, it is frequently used as an important antioxidant in the food industry. Also, as an important micronutrient, VC is a vital biological factor to maintain the body's normal life, it plays many physiological roles in intermediate metabolism and the immune system, biosynthesis and metabolism of certain compounds [1-4]. Lacking of VC would lead to gum bleeding, scurvy, and other symptoms and excessive intake of VC is harmful to human body. Moreover, VC can't be synthesis in the body

which can be got only from the outside. Due to the importance of VC, it is essential to develop the methods for detecting the concentration of VC. At the present, the methods for VC mainly included high-performance liquid chromatography[5-8], spectrophotometry[9-11], titrimetry[12-14], fluorometry[15-16], electrochemistry[17-19], chromatography[20-21]. Among these methods, electrochemical method has attracted many researchers' attention owning to its simple operation, low cost, fast response, and high sensitivity.

VC could be detected directly by electrochemical method due to its direct oxidation on electrodes. But, to the bare glassy carbon electrodes (GCE), electron transfer was sluggish because of surface fouling and lower electrochemical activity. In order to overcome above problems, the electrode modified with functional matierials was essential. Among the modified substances, multi-walled carbon nanotubes (MWCNTs) were molecular-scale wires with low impedance, extremely high mechanical modulus and strength [22]. At the surface of the MWCNTs, small diameter, high surface energy, and lacking of coordination make its surface atomic activity highly. So it was easy to transfer electron with other around materials and MWCNTs was widely used as modified materials [23-25]. Nevertheless, the unpretreated MWCNTs modified on the electrode surface directly could aggregate easily, and make electrode passivated [26-29]. Therefore, as the modified materials, MWCNTs need to be pretreated functionally.

In this work, MWCNTs was pretreated by using carboxyl method with strong mixed acid and ultrasonic dispersing in N,N-dimethylfomamide (DMF) media to form DMF/carboxyl/MWCNTs. A novel sensor was proposed and established for rapid detecting VC, using a GCE modified with the above functional materials, namely DMF/carboxyl/MWCNTs/GCE. The fabricated electrode exhibited enough selectivity, simplicity, and sensitivity to detect VC in real samples.

2. EXPERIMENTAL

2.1. Reagents and Apparatus

Vitamin C (VC) and N,N-dimethylfomamide (DMF) were purchased from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China). Multi-walled carbon nanotubes (MWCNTs) were supplied by Beijing Gaoke Technology Material Co., Ltd. (Beijing, China). The PBS buffer solution was prepared by mixing sodium dihydrogen phosphate, disodium hydrogen phosphateand and sodium chloride. Unless otherwise indicated, all other reagents were analytical grade.

The experiments were performed on an electrochemical potentiostat (CHI660E, Shanghai Chen Hua Instrument Co., China) with a saturated calomel electrode (SCE) as the reference electrode, a platinum wire as the counter electrode, and a bare or modified GCE as the working electrode.

2.2. Fabrication of the working electrode

Prior to modification, 45 mL concentrated H_2SO_4 and 15 mL concentrated HNO_3 were mixed to form a strong acid solution. Then 60 mg MWCNTs were added into the mixed acid solution and vibrated ultrasonically for 24 h. Then the carboxyl products were washed until the filtrate was neutral
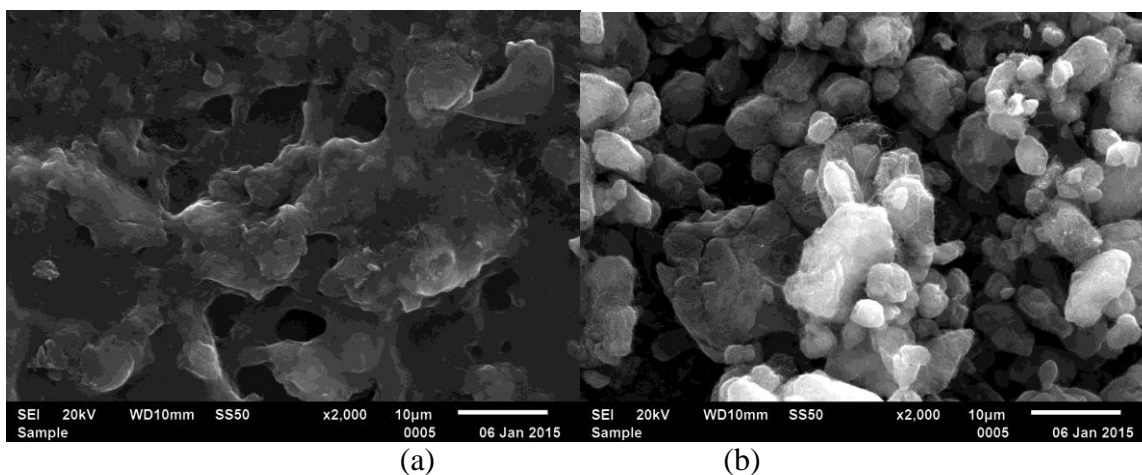
and dried to form the carboxyl MWCNTs. Afterwards, 3 mg carboxyl MWCNTs was dispersed in 10 mL DMF by ultrasonic for 24 h. The bare GCE was successively polished with 1.0, 0.3, 0.05 μm graininess Al_2O_3 emulsion priorly, and then washed ultrasonically by using acetone, double distilled water sequentially and dried. 2μL of the DMF/carboxyl/MWCNTs suspension was dropped on a bare GCE and dried under the infrared lamp. Thus, the DMF/carboxyl/MWCNTs/GCE was constructed.

2.3. Voltammetric analysis procedure

To prepare the supporting electrolyte, 20 mL aliquot of the PBS buffer (pH 7.0) was transferred into a clean and dry voltammetric cell. The DMF/carboxyl/MWCNTs/GCE was put into PBS solution. After the electrolyte was bubbled with highly pure nitrogen gas (≥99.999%) for 10 min, cyclic voltammetry (CV) was then performed from -0.50 V to 0.50 V at a scan rate of 100 mV/s. The added VC concentration was confirmed by detection of the oxidation peak current at 0.01 V. Each experiment was repeated for three times under the same conditions.

3. RESULTS AND DISCUSSION

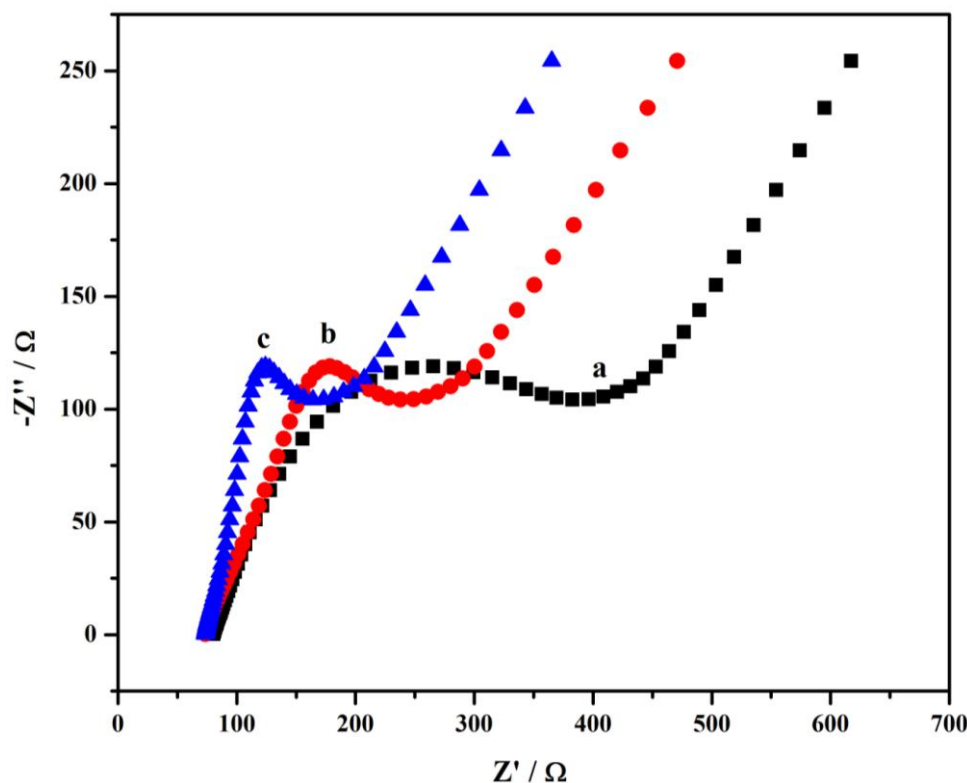### 3.1. Morphology of modified electrodes

Figure 1. Morphology images of MWCNTs (a) and DMF/carboxyl/MWCNTs (b).

The morphology of the MWCNTs and the DMF/carboxyl/MWCNTs on the surface of GCE was investigated by scanning electron microscopy (SEM) as shown in Fig. 1. At the surface of MWCNTs/GCE (Fig. 1a), it was obvious that the flakes layer of carbon powder was presented irregularly and scattered with each other. Whereas, DMF/carboxyl/MWCNTs dropped on the surface of GCE, the resultant film had a visualized three-dimensional structure (Fig. 1b), and presented a rather small, compact and homogeneous nano-particle morphology. The presence of DMF/carboxyl/MWCNTs leaded to an increase in the surface area and improved electrochemical reactivity of VC.

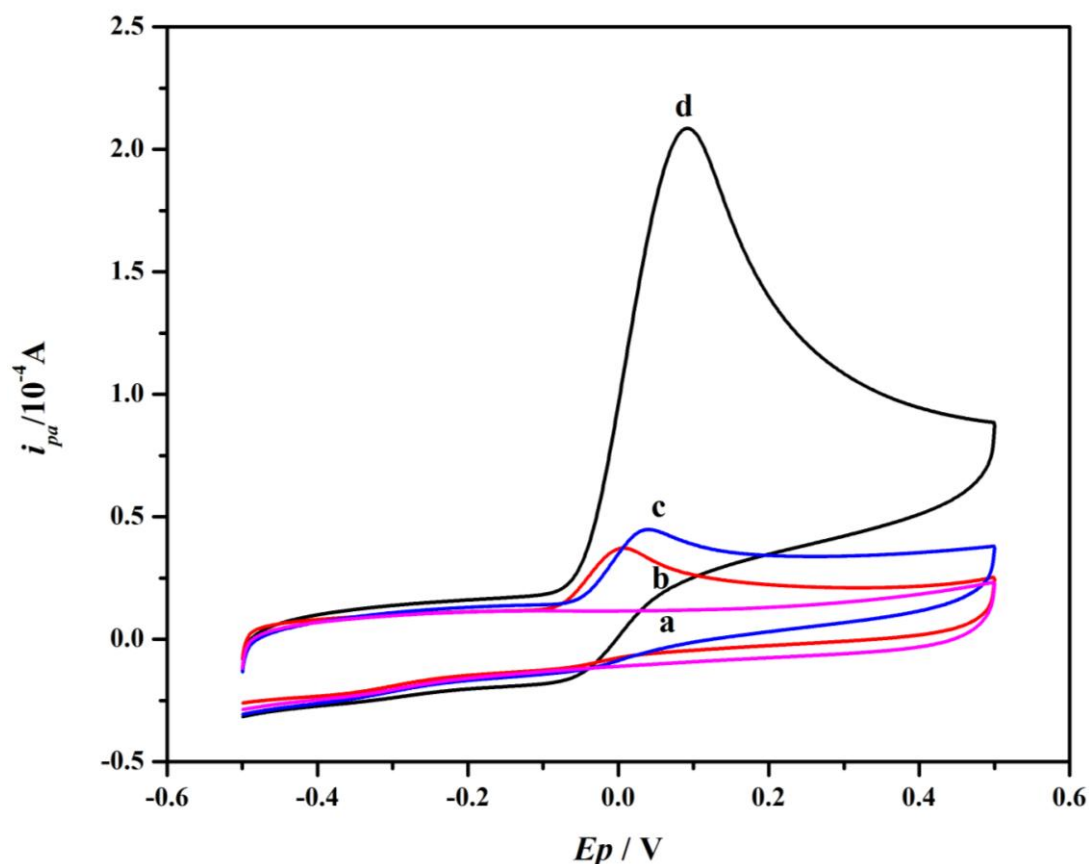*3.2. Electrochemical impedance spectroscopy of the electrodes*

        To investigate the properties of the bare GCE (a), DMF/MWCNTs/GCE (b) and DMF/carboxyl/MWCNTs/GCE (c) and choose the appropriate electrode for succedent study, it is essential to study the electron-transfer resistance (Ret) of these different electrodes.

Figure 2. Nyquist diagram for the bare GCE (a), DMF/MWCNTs/GCE(b) and DMF/carboxyl/MWCNTs/GCE(c) in a solution of 10 mM $[Fe(CN)_6]^{3-/4-}$ and 0.10 M KCl solution.

        The Nyquist plots of the prepared electrodes in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution including 0.10 M KCl were shown in Fig. 2. The fitted value of Ret for the bare GCE was 320 Ω. A remarkable decrease in the Ret for the DMF/MWCNTs/GCE (120 Ω) was observed compare with the Ret value for the bare GCE, suggesting a decrease in resistance and the conductivity of the electrode was promoted by DMF/MWCNTs drastically. The decrease in the Ret value of DMF/carboxyl/MWCNTs/GCE (70 Ω) reflected the improvement in the conductivity. During the carboxyl processes of MWCNTs, more active sites of MWCNTs were produced which were beneficial to interact with VC and enhanced the anodic peak current of VC. In addition, the DMF/carboxyl/MWCNTs could act as an electron transfer medium and increased electron transfer, so that the adsorbent VC would transfer electron much easily from VC to the electrode, indicating better electro-catalysis activity, which was well consistent with profiles described in literature [30-31]. Thereupon, the DMF/carboxyl/MWCNTs/GCE was used in subsequent experiments.
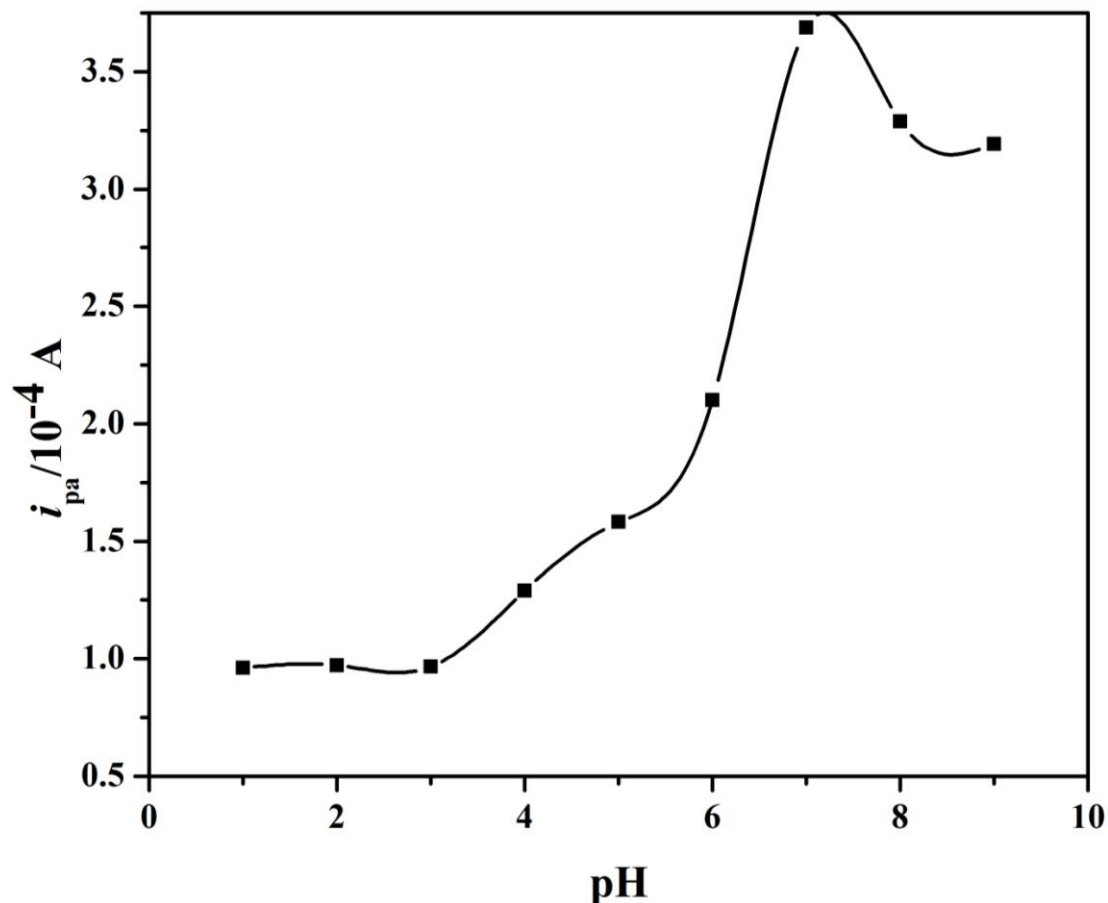
3.3. Electrochemical Behaviors of VC

Figure 3. Cyclic voltammograms of bare GCE in the absence of VC (curve a), bare GCE (curve b), DMF/MWCNTs/GCE (curve c) and DMF/carboxyl/MWCNTs/GCE (curve d) in the presence 5.0×10^{-3} mol/L VC in pH 7.0 PBS.

Fig. 3 exhibits CV responses of VC on the bare GCE, DMF/MWCNTs/GCE and DMF/carboxyl/MWCNTs/GCE respectively. The preconditioning process was carried out at the range of -0.50~0.50 V in the PBS (pH 7.0) solution containing 5.0×10^{-3} mol/L VC. It could be seen that there was no obvious anodic peak when the working solution had no VC based on the bare GCE (curve a). An oxidation peak current appeared at around 0.01 V when VC was added in (curve b). There was slightly increased peak measured with the DMF/MWCNTs/GCE (curve c). But significantly incremental peak for VC was measured with the DMF/carboxyl/MWCNTs/GCE (curve d). The three peak currents corresponding to the bare GCE (curve b), DMF/MWCNTs/GCE (curve c) and DMF/carboxyl/MWCNTs/GCE (curve d) could be identified 0.30×10^{-4} A, 0.45×10^{-4} A and 2.19×10^{-4} A respectively. The enhanced signals from the DMF/carboxyl/MWCNTs/GCE may be due to its strong absorptive ability towards the VC and the increased total surface area of the electrode. In addition, it may be because of the intercalation between DMF and carboxyl MWCNTs preventing MWCNTs from agglomeration and hence improving the electric conductivity of the electrode. The result was well agreeable to the SEM and EIS analysis.

3.4. Effect of pH

The effect of pH on electrochemical reactions of $5×10^{-3}$ mol/L VC was investigated on the DMF/carboxyl/MWCNTs/GCE by CV.
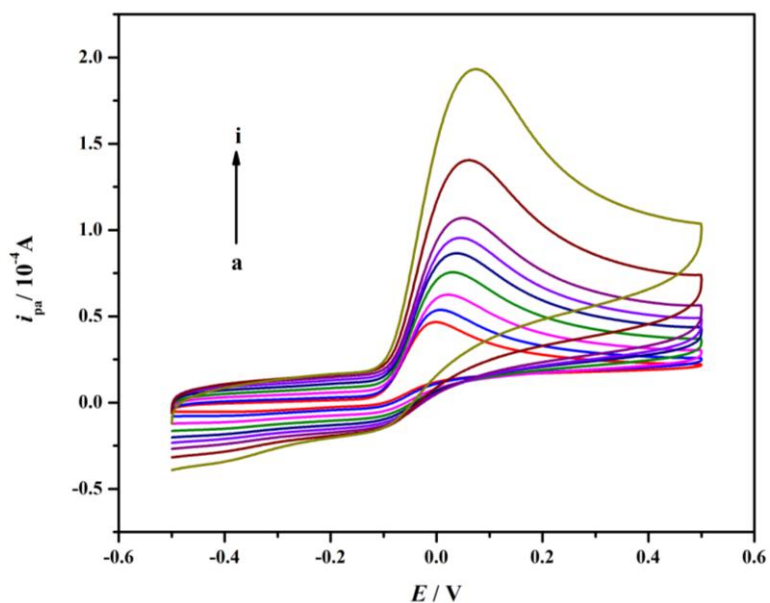

Figure 4. Influences of pH on anodic peak current of $5.0×10^{-3}$ mol/L VC.

From Fig. 4, the anodic peak current increased integrally from pH1.0 to pH7.0 due to the strong acid environment could prevent the reaction of VC with DMF/carboxyl/MWCNTs on the modified electrode and the activity of film enhanced with acidity decreasing. When pH value exceeded 7.0, the main factor was the hydrophobic interaction between the film and VC molecules. The surface of electrode had more negative allegation and protons participated in the electrode procedures decreased so that the peak current decreased obviously. Consequently, pH 7.0 was selected for further studies, which was consistent with that in previous reports [26, 32].
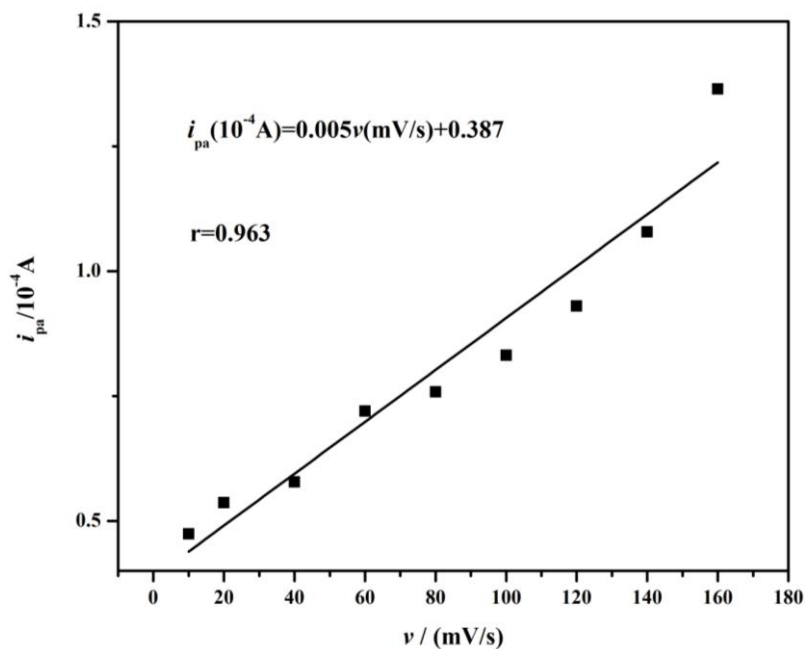
3.5. Effect of scan rates

In pH 7.0 PBS buffer containing $5.0×10^{-3}$ mol/L VC, the CVs of the DMF/carboxyl/MWCNTs/GCE at different scan rates were shown in Fig. 5. According to Fig. 5, there was a linear relationship between the anodic peak current of VC and the scan rates in the range of

10~160 mV/s, with the equation: i_{pa} (10^{-4}A) = 0.005 v (mV/s) + 0.387, r = 0.963, which representing that the electrochemical oxidation of VC was a surface adsorption controlled process [25,33].
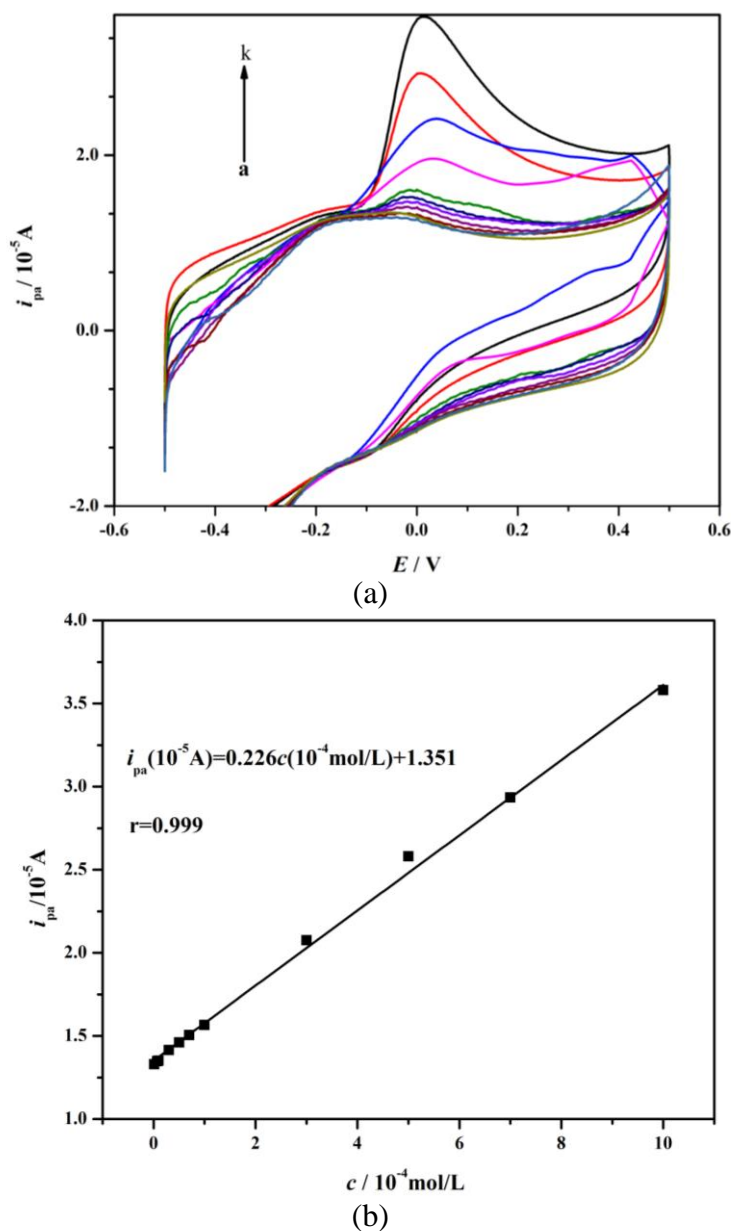

(a)

(b)

Figure 5. (a) Cyclic voltammograms of 5.0×10^{-3} mol/L VC on the DMF/carboxyl/MWCNTs/GCE in pH 7.0 of PBS buffer solution at various scan rates: a 10 mV/s; b 20 mV/s; c 40 mV/s; d 60 mV/s; e 80 mV/s; f 100 mV/s; g 120 mV/s; h 140 mV/s; i 160 mV/s. (b) Plot of anodic peak currents versus scan rates of 5.0×10^{-3} mol/L VC.

3.6. Determination of VC concentration

(a)

(b)

Figure 6. (a) Different cyclic voltammograms on the DMF/carboxyl/MWCNTs/GCE with the scan
rate of 100 mV/s in pH 7.0 PBS solution containing different VC concentrations: a 1.0×10^{-6} mol/L; b 7.0×10^{-6} mol/L; c 1.0×10^{-5} mol/L; d 3.0×10^{-5} mol/L; e 5.0×10^{-5} mol/L; f 7.0×10^{-5} mol/L; g 1.0×10^{-4} mol/L; h 3.0×10^{-4} mol/L; i 5.0×10^{-4} mol/L; j 7.0×10^{-4} mol/L; k 1.0×10^{-3} mol/L. (b) Plot of anodic peak currents versus VC concentrations.

Under the optimized experimental conditions, the corresponding cyclic voltammograms of varying concentrations of VC from 1.0×10^{-6} mol/L to 1.0×10^{-3} mol/L in PBS buffer at DMF/carboxyl/MWCNTs/GCE are illustrated in Fig. 6a. The peaks obtained were well-defined and showed good linear relationships with the concentrations of VC (Fig. 6b).

The equation of linear regression for VC was i_{pa} (10^{-5} A) $= 0.226$ c (10^{-4} mol/L) $+ 1.351$, with the correlation coefficient of 0.999. The limit of detection (LOD) was found 2.5×10^{-7} mol/L (signal-to-noise ration=3). Compared with previous literatures [34-36], this established method had a wider linear range and lower LOD to detect VC concentration. From these results, we could conclude that the constructed DMF/carboxyl/MWCNTs/GCE could be applicable for detecting VC with good sensitivity in a wide range of concentration.

3.7. Interference of coexisted substance

The effect of different foreign species which potentially interfere with the VC detection was studied to evaluate the selectivity of the proposed method. The major potentially interferents such as glucose, fructose were selected from the substances commonly stayed with VC in fruit and vegetable juice. Different quantities of foreign substances and 5×10^{-3} mol/L VC were prepared for investigating the influence of coexisting interferents. According to the results, 200-fold of glucose, fructose, K^+, Na^+, Ca^{2+} and 400-fold of Cl^- had no meaningful interference on the determination of VC at the DMF/carboxyl/MWCNTs/GCE. As a consequence, the proposed electrode could be applied on detection of VC for practical applications.

3.8. Analysis of VC in tomato samples

For purpose of exploring the feasibility of DMF/carboxyl/MWCNTs/GCE for practical analysis, the fabricated electrode was used to detect VC in tomato samples using standard addition method. Firstly, 15 g tomato samples was cut into small pieces and grounded in the mortar. Then the mashed samples were centrifuged at 6000 r/min for 10 min. 0.5 mL supernatant was collected and added in 10 mL PBS solution. After that, the resultant PBS containing different concentration of VC was based on triplicate measurements to evaluate the recovery.

Table 1. Recovery tests of VC in tomato samples

VC	Addition (10^{-3} mol/L)	Found (10^{-3} mol/L)	Recovery (%)	RSD (%, n=3)
	1.0	1.05	105	3.79
	2.0	1.98	99	4.21
	5.0	5.12	103	3.18

As shown in Table 1, the recoveries are ranged from 99% to 105%, and reveal that the DMF/carboxyl/MWCNTs/GCE has the properties of celerity, simplicity and high sensitivity on VC detection in practical applications.

4. CONCLUSIONS

In this paper, a novel GCE fabricated with DMF/carboxyl/MWCNTs was constructed for the determination of VC. Based on the modified material, the modified electrode revealed higher conductivity, sensitivity and faster electron transfer over bare GCE, suggesting that electro-catalysis of DMF/carboxyl/MWCNTs/GCE got improved dramatically. Ultimately, the fabricated electrode was used to detect VC in tomato simples and the results were satisfactory, which suggested that the DMF/carboxyl/MWCNTs/GCE would be an attractive candidate for VC determination in real simples.

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