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Electrochemical Determination of Cefotaxime Using Nafion-Graphene Oxide Modified Electrode

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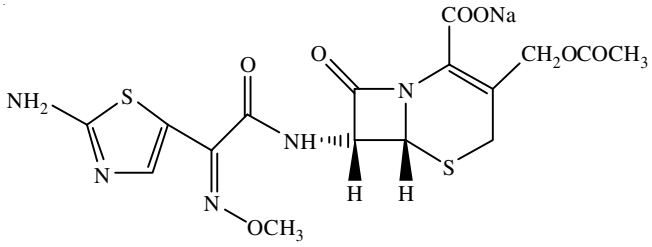
In this work, graphene was activated by oxidizing acid for improving its dispersity and characterized by scanning electron microscopy and Fourier transform infrared spectroscopy. Then direct electrochemistry of cefotaxime was studied by cyclic voltammetry based on composite film consisting of Nafion and oxygen-containing groups functionalized graphene modified glass carbon electrode. The results demonstrated that the Nafion-graphene oxide modified electrode exhibited obviously electrocatalytic oxidation of cefotaxime by lowering the anodic overpotential due to their excellent compatibility, high specific surface area and good conductivity. Under the optimum conditions, a linear dynamic range of 0.5 μM to 100 μM for cefotaxime was obtained in phosephate buffer solution at pH 2.0 with the detection limit of 2.6×10^{-7} M (S/N = 3). Moreover, the modified electrode possessed some advantages such as easy preparation, rapid response, good stability and high reproducibility. The proposed method was applied to determine cefotaxime in pharmaceutical preparations with satisfactory results.

Keywords: Cefotaxime, Graphene, Nafion, Modified electrode, Determination.

INTRODUCTION

Cefotaxime (CFX) is chemically named as (7-[α-(2-aminothiazol-4-yl)-α-(z)-methoxyimino-acetamido]-3-(1-methyl acetate)methyl-3-cephem-4-carboxylic or its sodium salt). It is a white crystalline powder, soluble in water and similar biopharmaceutical profile with proteins and peptides [1]. Cefotaxime belongs to a semisynthetic third-generation cephalosporin drugs used primarily for treatment of moderate and severe pneumonia of hospitalized patients. Like other antibiotic, the drug efficacy is related to the length of time and drug concentration [2]. Thus, it is important to quantify the cefotaxime in biological tissues.



Chemical structure of cefotaxime

Up to now, a variety of methods have been proposed for the measurement of cefotaxime, including high performance liquid chromatography (HLPC) [3], chemiluminescence [4], liquid chromatography (LC) [5,6], spectrophotometry [7], pulse-polarography [8] and fluorescence [9]. Meanwhile, electrochemical techniques are suited for the determination cefotaxime since they are simple, inexpensive, rapid and sensitive. The electrochemical behaviour of cefotaxime at activated glassy carbon electrode, platinum electrode and hanging mercury dropping electrode have been reported previously [10,11]. However, their voltammetric responses are not satisfactory due to slow heterogeneous electron transfer at the electrode and large overpotential required for the reduction or oxidation of cefotaxime. Therefore, there has been an increasing interest in the development of chemically modified electrodes in order to obtain better electrochemical cefotaxime sensors [12-14].

Recently, electrodes coated with graphene have attracted much attention because of its larger specific surface area, unique electrical properties, good thermal conductivity and mechanical properties [15]. Studies have indicated that the use of graphene as modifier can be utilized to rapid, sensitive and selective analysis of various important biological compounds and clinical species such as dopamine [16], glucose [17], hemoglobin [18] and nitric oxide [19]. Unfortunately, it is well known that the tendency of forming insoluble aggregates by van der Walls interaction and strong π-π stacking effect will limit the extensive research of original graphene. So treatment with oxidizing acids was an effective approach for improving the solubility of graphene [20]. In addition,

a water-soluble polyelectrolyte, Nafion was also needed to further improve disperse properties of graphene and reduce interferences of other surface-active species [21]. However, so far, no literature has been published about determination of cefotaxime based on the graphene-Nafion composite modified electrode.

In the present study for the first work, the graphene-Nafion composite modified glassy carbon electrode was prepared for determination of cefotaxime. Graphene showed significantly electrocatalytic activity towards cefotaxime. And this method was successful applied for determination cefotaxime of pharmaceutical preparations. This paper will amplify the applications of graphene-based materials in biosensor field.

EXPERIMENTAL

Graphene was purchased from Nanjing XianFeng Nano Co. Ltd, China. Cefotaxime was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and cefotaxime sample was purchased from Shanghai Xinya Pharmaceutical Co. Ltd, China. A 5 wt. % Nafion was obtained from Anhui Ruibang Science and Technology Co. Ltd, China. All other chemicals and reagents used in this work were of analytical grade and used without further purification. The electrolyte solution was 0.1 M phosphate buffer solution (PBS) at pH 2.0. Ultrapure water was obtained from a Milli-Q water purification system and used throughout the experiment.

The voltammetric curve were recorded with a CHI650D electrochemical workstation (Shanghai Chenhua Co. Ltd., China).The three-electrode system included a Nafion-graphene film working electrode, a saturated calomel reference electrode (SCE), a platinum wire counter electrode. Fourier-transform infrared spectrometric (FT-IR) analysis was carried out on a Perkin Elmer spectrum one spectrophotometer using a potassium chloride pellet technique. Scanning electron microscopy (SEM) was obtained using an S-4800 scanning electron microscope (Hitachi, Japan). All electrochemical experiments were performed at room temperature.

Preparation of Nafion-GO/GCE: 20 mg original graphene was dispersed in 8 mL HCl forming a black solution with the aid of ultrasonication for 6 h. After that, the centrifugal product was washed with ultrapure water to neutral. The dry powder was then refluxed in 15 mL HNO_3 for 3 h, followed by subsequently centrifuged and washed with water for three times. Then, the water was removed under vacuum and 1 mg as-synthesized graphene oxide solid was suspended in 1 mL ethanol solution containing 0.5 % Nafion with the aid of ultrasonication.

Glass carbon electrode was polished to mirror-like with 0.05 μm alumina powder suspension and followed by ultrasonication in 1:1 HNO_3, acetone, ethanol and distilled water for 3 min and dried in air. Then 10 μL Nafion-graphene oxide suspension was dripped onto the fresh surface of glassy carbon electrode and then naturally dried in the air for 0.5 h to form Nafion-graphene oxide flim.

Experimental procedures: Prior to cefotaxime determination, the modified electrode was placed in a blank phosphate buffer solution (pH 2.0) and the potential was swept between -0.5 and 1.5 V until a stable voltammetric response was obtained. The electrocatalytic activity toward cefotaxime was tested by cyclic voltammetry in the potential range of 0.40 and 1.20 V in deoxygenated phosphate buffer solution at a scan rate of 50 mV/s. Finally, voltammetric detection of cefotaxime was recorded by differential pulse voltammograms (DPV) containing 10 mL phosphate buffer solution under nitrogen atmosphere.

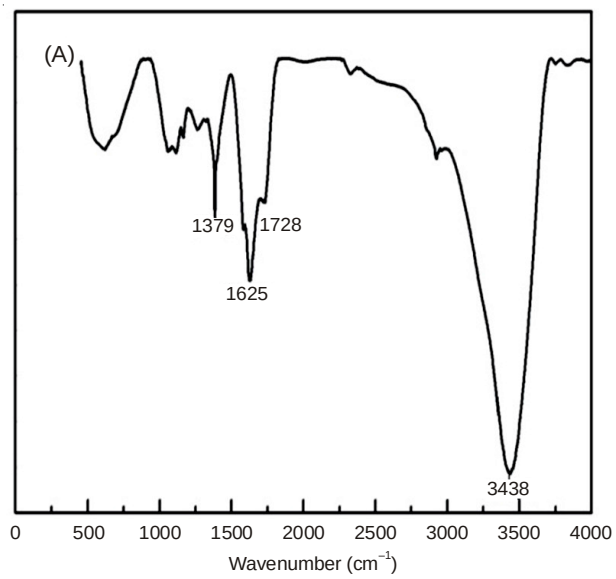
RESULTS AND DISCUSSION

Characterization of the graphene oxide: The composition of graphene oxide was investigated by FT-IR. As shown in Fig. 1a, comparing with original graphene, the presence of the peaks at 1728 cm^{-1} ν(C=O), 1365 cm^{-1} ν(C-OH), 1250 cm^{-1} ν(C-O-C) and 1060 cm^{-1} ν(C-O) indicated that there were many oxo-groups on the surface of graphene after treatment by nitric acid.

The typical micrograph of the graphene treated with oxidizing acid has been investigated by SEM (Fig. 1b). Numerous transparent and irregular carbon nano sheets on the surface of graphene oxide can be observed. Moreover, the edges of these fragments had slightly distorted. It was reported [22] that carboxylic group generated by strong acid prevented further agglomeration of graphene and retained its high surface area.

Electrochemical behaviours of cefotaxime: Fig. 2 shows the cyclic voltammograms for 1.0×10^{-4} M cefotaxime in a phosphate buffer solution with pH 2 on the glassy carbon electrode (dashed line) Nafion-graphene oxide modified electrode (solid line) at a potential sweep rate of 50 mV s^{-1}. It can be seen that a relatively broad and weak anodic wave for the electro-oxidation of cefotaxime on the surface of unmodified electrode due to large activation overpotential or sluggish electrochemical process. However, using Nafion-graphene oxide modified electrode, a well-defined and very sharp anodic wave with a slight shift of the oxidation potential toward the negative direction was obtained. In acidic solution, cefotaxime molecules can combine the Nafion disperse agent possessing negative charge easily due to protonation of the oxime and imino groups. Because of large specific surface area and strong adsorption capacity of graphene oxide, this modified electrode showed an excellent electrocatalytic activity for the oxidation of cefotaxime by lowering the anodic overpotential and enhancement of the anodic peak current. For cefotaxime, in the potential range of 0.4-1.2 V, no cathodic peak was observed on the reverse sweep. This confers to us a fact that there is an irreversible process for its oxidation on the surface of modified electrode.

The electrochemical behaviour of cefotaxime at different scan rates were investigated at Nafion-graphene oxide modified electrode (Fig. 3). The peak potential shifted positively with the increase of the scan rates. A linear relationship [i_p (μA) = -8.332-0.3474ν (mV s^{-1})] (eqn. 1) with a coefficient of r = 0.9940 was observed in the range of 10 to 125 mV s^{-1}, which revealed that the oxidation of cefotaxime was adsorption-controlled process. In this experiment, the relationship between the oxidation peak potential and scan rate can also be described as $E_p = 0.89 + 0.061 \log \nu$ (mV s^{-1}), r = 0.9951. Based on Laviron's equation [23]: $i_p = nFQ\nu/4RT$ (eqn. 2), Q was the



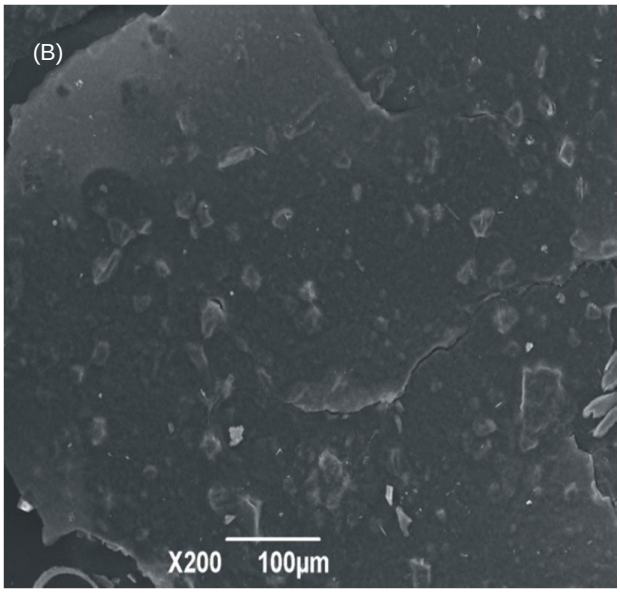


Fig. 1. FI-IR spectra of graphene oxide (A) and the SEM of graphene oxide (B)

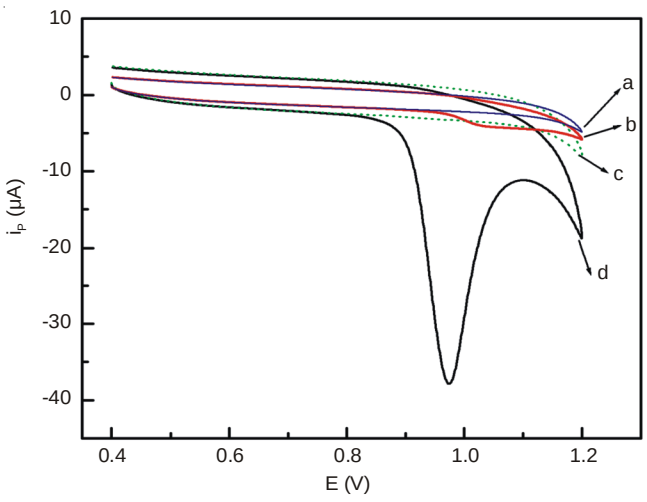


Fig. 2. Cyclic voltammograms of the bare GCE and Nifion-GO/GCE in 0.1 M phosphate buffer solution (pH 2.0) in the absence of cefotaxime (a, c) and in the presence of 1.0×10^{-4} M cefotaxime (b, d) at a scan rate of 50 mV s^{-1}

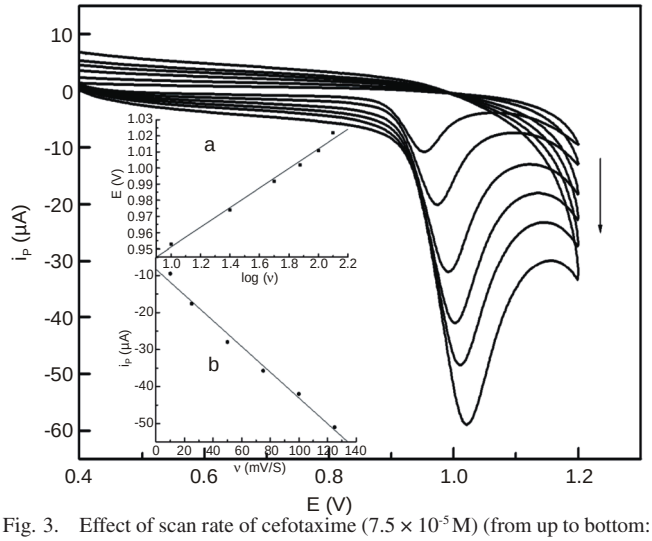


Fig. 3. Effect of scan rate of cefotaxime (7.5×10^{-5} M) (from up to bottom: 10, 25, 50, 75, 100, 125 mV/s). Inset a and b are the relationship between the peak potential and current with the san rate

charge transfer in the reaction and the F, R, T were constants. Integrating above eqn. 1 with eqn. 2, obtained the average value of n was 2.3. This result suggested that two electrons were involved in the oxidation process of cefotaxime on the Nafion-graphene oxide modified electrode, which was consistent with the reported value [10,24].

Effect of the amount of suspension: The amount of suspension has a large influence on the anodic peak current of cefotaxime. It is found that the peak current increase with the amount of modifier within 0-10 μL and then decrease when the amount of the modifier was increased further. The results shows that with further increase the modifier film thickness, the resistance of modified electrode becoming larger, so as to hinder mass transfer of cefotaxime and the electron exchange reaction on the surface of the electrode. Therefore, suspension of 10 μL was selected for all the voltammetric experiments.

pH effect: The phosphate buffer solution buffers (pH 2-10) were prepared by adjusting the ratio of 0.1 M Na_2HPO_4 and NaH_2PO_4 solution. Then the electrochemical behaviour was studied in phosphate buffer solution of different pH values in the range of 2-10 containing 5×10^{-5} M cefotaxime. Fig. 4 showed the effect of pH on the peak current and potential at Nafion-GO/GCE. It was demonstrated that the peak current decreased with pH from 2 to 5 and then increased slightly when the pH exceeded 5. Therefore, the phosphate buffer solution buffer with pH 2 was used as the supporting electrolyte in all experiment. Meanwhile, the oxidation peak potential shifted negatively with the increase of pH value due to the impediment of oxidation at low pH. The relationships between oxidation peak potential (E_p) and pH (2.0-10.0) can be expressed by the equation: E_p/V = 1.066-0.0514 pH (r = -0.9935), the slope of 0.0514 mV/pH, closed to the ideal value of -0.059 mV/pH, indicating the participation of the same protons and electrons in the electrochemical process. The oxidation reaction of cefotaxime is similar with Zhang *et al.* [25].

Effect of accumulation potential and time: The accumulation potential had no influence on the oxidation peak current, so experiment selected open circuit potential as accumulation potential.

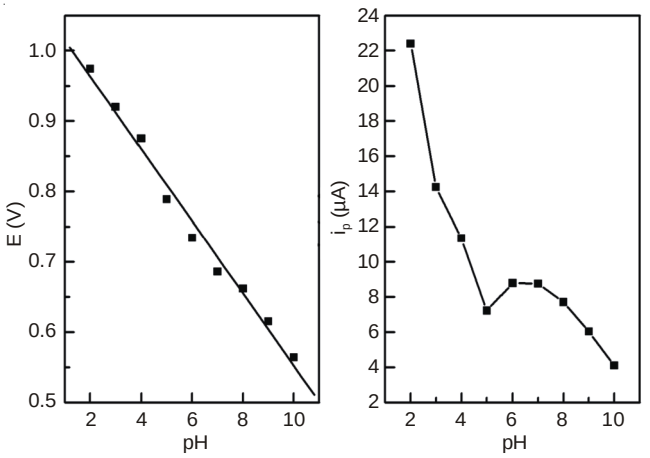


Fig. 4. Influence of solution pH on anodic peak potential and peak current

At the open circuit potential, as accumulation time increasing, the oxidation peak current also increased accordingly. When the accumulation time exceeded 30 s, the peak current increased slowly and tend to stability. So the accumulation time was set as 30 s.

Differential pulse voltammograms determination, stability and reproducibility: Under the above optimum conditions, a differential pulse voltammetry method was performed for determination cefotaxime and studied the relationship between the peak current and the concentrations of cefotaxime. The results were shown in Fig. 5. It was found that the oxidation peak current increased linearly with the concentration of cefotaxime range from 0.5 μM to 100 μM. The linear regression equation was: i_p (μA) = -2.556-0.3622C (μM) (r = -0.9974) and the detection limit was 2.6×10^{-7} M. These results show that the method has more wider linear range and higher sensitivity than other measurement methods on the detection of cefotaxime [4,10].

With the same electrode the determination of the cefotaxime (7.5×10^{-5} M) was performed for eleven times, the relative standard deviation was 3.86 %, indicating that the modified electrode has a good reproducibility. Storing this modified electrode at 4 °C for 48 h, the peak current of differential pulse voltammetry measurement can still reach 92.1 % of the initially current, showing that the modified electrode is stability.

Interference: It is well known that Nafion of negative charge not only acts as an effective disperse agent for graphene, in favour of combination protonated of cefotaxime in acidic solution, but also as an antifouling coating to reduce the interference of the surface-active macromolecules [21]. Therefore, in order to decrease the effect of other species frequently found with cefotaxime in pharmaceutical formulations, Nafion was embedded in the graphene film. Then the effect of some inorganic ions and organic compounds on the determination of

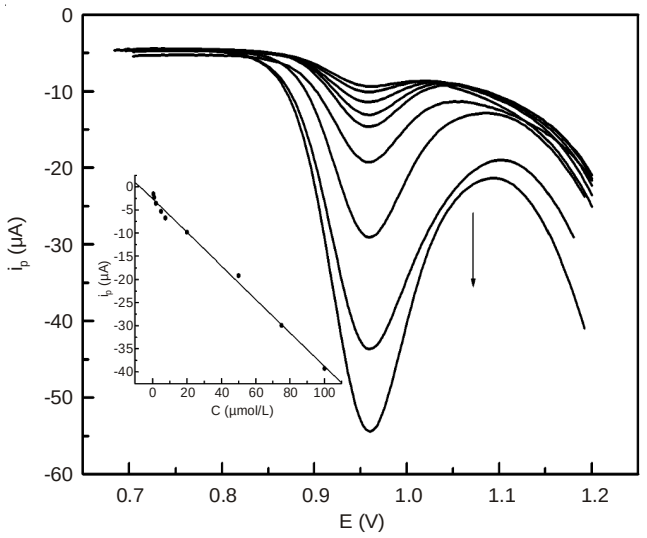


Fig. 5. Differential pulse voltammograms of cefotaxime determination (from up to down: 5×10^{-7}, 1×10^{-6}, 2×10^{-6}, 5×10^{-6}, 7.5×10^{-6}, 2×10^{-5}, 5×10^{-5}, 7.5×10^{-5}, 1×10^{-4}M), Inset was the calibration curve of cefotaxime with different concentration

cefotaxime was performed. The result showed that 500-fold of K^+, Na^+, Fe^{3+}, Co^{2+}, Cu^{2+}, Cl^-, Br^-, SO_4^{2-}, NO_3^-, glucose, 100-fold of Mg^{2+}, Zn^{2+}, Mn^{2+}, citric acid, ascorbic acid, urea, uric acid, L-histidine, L-tryptophan had no interference with the detection of 7.5×10^{-5} M cefotaxime. Only L-tyrosine caused tiny change in the cefotaxime electrochemical signal. But the interference of L-tyrosine can be neglected since they don't appear in same drugs.

Sample analytical: The preparation of 0.01 M sample solution was obtained from 0.2387 g cefotaxime sodium for injection dissolved in 50 mL volumetric flask and injected 50 μL sample solution to 10 mL volumetric flask and diluted to the mark with phosphate buffer solution buffer solution (pH = 2.0). The modified electrode was used for determination and recovery of cefotaxime in drug sample by standard addition method. The results were showed in Table-1. The recoveries between 96 and 104 % were received. The RSD was lower than 4 %, indicating that the method is reliable and practical.

Conclusion

Graphene is an ideal modified electrode material due to its large specific surface area and unique electrical properties. This paper used functionalized of graphene contain Nafion immobilized electrode to determine cefotaxime. This modified electrode performed excellent electro-oxidation of cefotaxime since it increased the oxidation peak current and lowered the oxidation overpotential of cefotaxime. Moreover, the modified electrode showed other electrochemical advantages such as nice stability, good reproducibility, high sensitivity, wide linear

TABLE-1
DETERMINATION AND RESULTS OF RECOVERY TEST OF CEFOTAXIME SODIUM INJECTION (n = 6)

Samples	Labeled (μM)	Determined (μM)	RSD (%)	Added (μM)	Found (μM)	Recovery (%)
Cefoaxime sodium for injection	50	52.7	3.3	25	77.6	103.5
	50	51.3	3.3	30	78.2	97.7
	50	49.0	3.3	35	86.4	101.4
	50	50.8	3.3	40	86.5	96.1
	50	48.0	3.3	45	93.4	98.3

range and lower limit detection and this sensor was applied for determination of cefotaxime in pharmaceutical with successful result. Consequently, the sensor provided a novel, simple, fast electrochemical method to determination of cefotaxime.

ACKNOWLEDGEMENTS

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