Electrochemical Behaviors of Ceftazidime at Gr/GCE and its Interaction with DNA Studied by Fluorescence and CV Method

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The electrochemical behaviors of CFD (ceftazidime) at a graphene modified electrode (Gr/GCE) were investigated by cyclic voltammetry. Compared with a bare glassy carbon electrode, the Gr/GCE exhibited a significantly enlarged irreversible oxidation peak due to the excellent electron transfer function and catalytic effect of graphene. The peak currents were proportional to CFD concentration of 0.25~10.0 µΜ and 10.0~75.0 µΜ, with the detection limit of 0.1 µΜ. Furthermore, the interaction between CFD and double stranded DNA was studied by electrochemical and fluorescence methods. The results showed that the peak current of CFD decreased remarkably and the fluorescence intensity quenched significantly with the increasing of DNA concentration, which indicated that the CFD molecule intercalated into DNA to form a CFD-DNA complex. In addition, the number of electrons transferred in the electrochemical reaction was calculated based on the electrochemical results to assess the possible interaction mechanisms.

Keywords: Ceftazidime detection; Electrochemical sensor; Fluorescence; Graphene; Interaction between CFD and DNA

1. INTRODUCTION

Since penicillin was discovered more than half a century ago, antibiotics have become a powerful weapon against bacterial infections. With the rapid development of medical technology, the variety of antibiotics that have been widely used in humans and animals is increasing day by day [1, 2]. However, the unreasonable use and even abuse of antibiotics brought by this kind of convenience cannot be ignored. On the one hand, antibiotics can be accumulated in body through food chain, causing damage to health even at a low concentrations, such as toxicity to organs, hearing loss, etc[3]. On the other hand, the overuse of antibiotics leads to a shorter period of drug resistance, which may render the treatment of the disease ineffective[4]. Therefore, the maximum residue limits (MRL) has been established in many
countries aimed at the control of antibiotic residues in foodstuffs of animal origin, and the development of methods for antibiotics residue detection is grimly needed[5].

In recent years, large analytical instruments such as high performance liquid chromatography (HPLC)[6], chromatography-mass spectrometry (LC-MS/MS, HPLC-MS/MS)[7, 8] have been widely used to detect antibiotics. Though results are relatively satisfactory, there are some apparent shortcomings in these analytical methods, such as complex sample pretreatment, large amount of solvent required, expensive equipment and time-consuming, which limits their wider application. Hence, it is urgent to develop other simple, convenient, rapid and sensitive technologies.

Compared with separation methods above, electrochemical sensing technology possesses the advantages of high selectivity, rapid detection and no separation which have made it become a reliable alternative or supplementary analysis tool for antibiotics detection[9-11]. And specially, graphene modified electrode is one of the attention focuses. Graphene, an allotrope of carbon, has the structure of one atom-thick planar sheets of sp²-bonded carbon atoms that are densely packed in a honeycomb crystal lattice. Due to its extraordinary structure and properties, graphene has a large specific surface area, excellent conductivity, and strong mechanical strength[12, 13]. Therefore, it can performs strong electrocatalytic activity towards many molecules and has been used to prepare a new generation of electrodes for electrochemical studies[14-16].

DNA, the basic genetic material, is an important macromolecule in biological organism, which plays an important role in the translation, transcription and reproduction of the genetic code. The study of DNA is an important subject in molecular biology technology, biological science, chemical and electrochemical sciences[17]. Among them, the interaction between DNA and other small molecules, especially drug molecules, has been a relatively active research field, enabling people to understand the pathogenesis of some diseases and provides important guidance to design reasonable and effective drugs[18, 19].

At present, main methods to study the interaction of small molecules and DNA are spectroscopy[20-22], electrochemical analysis method[23, 24] and the molecular simulation, etc. The combination of spectroscopy and electrochemistry is of great significance for deep understanding of the mechanism of interaction between DNA and drug molecules. CFD is an important class of third-generation cephalosporin antibiotic, which has the characteristics of wide antibacterial spectrum, strong antibacterial activity and enzyme resistance (Figure 1) [25]. The founded of detection methods for CFD is helpful for the reasonable use of CFD, and the study of its interaction with DNA can contribute to understanding the function mechanisms of CFD and providing theoretical basis for the design of antibiotics with better effect.

In the present work, a graphene modified glassy carbon electrode (Gr/GCE) for the determination of CFD was fabricated. The electrochemical behaviors of CFD at the modified electrode were investigated in detail by cyclic voltammetry and the electrode showed an excellent electrocatalytical effect on the reaction of CFD. A novel voltammetric method for the determination of CFD has been established and is validated, which provides a new electrochemical approach for antibiotics detection.
2. EXPERIMENTAL

2.1 Material and apparatus

CHI660E electrochemical workstation (Shanghai Chenhua Instrument co., LTD.); KQ-600 ultrasonic cleaner (Kunshan Ultrasonic Instrument co., LTD.); PHS-3G precise pH meter (Shanghai Precision Science Instrument co., LTD.); F-280 fluorescence spectrophotometer (Tianjin Gangdong Technology Development co., LTD.); Graphene (Shandong Yuhuang Chemical Co., Ltd); Ceftazidime (Aldrich, USA); Herring sperm DNA (dsDNA, Beijing Jingke Reagent Company). Other reagents used in the process were all analytical pure. All solutions were made up with ultrapure water.

The Graphene solution of 0.5 mg·mL⁻¹ was obtained by dispersing Graphene in N, N-dimethyl formamide (DMF). CFD was dissolved in ethanol and diluted to 10⁻³ mol·L⁻¹ by ultrapure water and the stock solution was received.

2.2. Fluorescence spectrometric method

Firstly, 1 mL 2.5×10⁻⁵ mol·L⁻¹ CFD solution and DNA solutions of different concentration was added into the beakers. And then, the volume was fixed to 10 mL using pH=4.0 PBS buffer, mixed and left for 20 min. Finally, Fluorescence emission spectra (excitation wavelength of 337 nm, excitation and emission slit width of 5 nm) were recorded on a fluorescence photometer.

2.3. Preparation of the modified electrode (Gr/GCE)

A glassy carbon electrode was grounded with gold sand paper and 0.05 μm Al₂O₃ powder firstly, and rinsed with ultrapure water. Then, the electrode was sonicated successively in HNO₃ solution (1: 1), absolute ethanol and ultrapure water. After that, 6 μL 0.5 mg·mL⁻¹ graphene solution was dropped onto the glassy carbon electrode and dried under an infrared lamp.
2.4. Electrochemical measurements

A three-electrode system with the saturated calomel electrode (SCE) as the reference electrode, platinum wire electrode as counter electrode, and glass carbon electrode (GCE) as the working electrode was employed in all electrochemistry experiments.

3. RESULTS AND DISCUSSION

3.1 Fluorescence spectrometry study of the interaction between CFD and DNA

In order to investigate the mechanism of interaction between CFD and DNA, the fluorescence characteristics of CFD solution before and after DNA addition were studied. In the presence of different concentrations of DNA, the fluorescence emission spectra of $2.5 \times 10^{-5}$ M CFD (pH 4.0) in the range of 290-460 nm with the fixed excitation wavelength at 337 nm was recorded (Figure 2). The result showed that the fluorescence intensity of CFD decreased obviously with the addition of DNA. The reason may be that CFD embed into the DNA molecules and quenched its fluorescence[26, 27].

![Graph showing fluorescence spectra](image_url)

**Figure 2.** Emission spectra of $2.5 \times 10^{-5}$ M CFD in the absence (a) and the presence of DNA 0.02 (b), 0.08 (c), 0.1 (d), 0.15 (e), 0.2 mg·mL$^{-1}$ (f).

3.2 Electrochemical behavior of CFD at Gr/GCE and electrochemical study of the interaction between CFD and DNA

3.2.1 Electrochemical behavior of CFD at Gr/GCE

Figure 3 shows the cyclic voltammetry curves of different electrodes in solutions. As can be seen from the voltammetry curves, the electrochemical reaction process of CFD has an obvious oxidation...
peak, indicating that its electrochemical process is completely irreversible. On account of the excellent catalytic characteristic of graphene, the current signal on the Gr/GCE is apparently amplified compared with that on bare electrode. However, when the solution of 0.16 mg·mL\(^{-1}\) DNA was added, significantly lower oxidation peak current of CFD was observed and peak potential moved slightly at the same time. It may be that the formation of a kind of supramolecular compounds between CFD and DNA molecules reduced CFD equilibrium concentration and so made the peak current decrease[28].

Figure 3. Cyclic voltammograms of different electrode (pH=4.0, \(v=80\) mV·s\(^{-1}\)) a: bare electrode in 2.5×10\(^{-5}\) M CFD solution; b: Gr/GCE electrode in 2.5×10\(^{-5}\) M CFD solution; c: b+0.16 mg·mL\(^{-1}\) dsDNA;

3.2.2 The selection of electrolyte solution and optimal pH value

The electrochemical behaviors of CFD were studied when Britton-Robinson, HAc-NaAc, Na\(_2\)HPO\(_4\)-NaH\(_2\)PO\(_4\), Tris-HCl solutions were used as electrolytes respectively. It was found that CFD had best peak shape, maximum peak current and good sensitivity in the PBS buffer solution composed of Na\(_2\)HPO\(_4\)-NaH\(_2\)PO\(_4\). So, PBS buffer was selected as the supporting electrolyte.

To get more information about reaction mechanism, the influence of pHs on the electrochemical behaviors of CFD was also investigated by cyclic voltammetry method. It was found that within the range of pH 3.0~8.0, the oxidation peak potentials shifted negatively with the increase of pH, and the oxidation peak current firstly increased and then decreased. The current reached the maximum value at pH 4.0 (Figure 4) which was selected for the tests. It was also found that the oxidation peak potential presented a linear relationship with pH (the inset) and obeyed the linear regression equation: \(E_{pa}(V)=1.1137-0.05803pH\), \(r=0.9971\), with a slope of 58 mV/pH, indicating that the oxide reaction process of CFD involved the protons[29, 30].
Figure 4. Cyclic voltammograms of 2.5×10⁻⁵ mol·L⁻¹ CFD at Gr/GCE in different pHs (3.0, 4.0, 5.0, 6.0, 7.0, 8.0) with 80 mV·s⁻¹ scan rate. The inset is the plot of peak currents versus pHs.

3.2.3 The influence of scanning rate on electrochemical behavior of CFD

The effect of scan rate on the electrochemical behavior of CFD at Gr/GCE was investigated (Figure 5). Results showed that the oxidation peak current gradually increased with the increase of scanning rate and the oxidation potentials shifted to the positive directions. The same change trend was observed in the presence of DNA with a slightly lower peak current.

There is also linear relationship between the peak currents of CFD and scanning rates in the range of 10~160 mV·s⁻¹ whether DNA existed or not (Figure 6: A). The linear fitting equation are: $i_{pa}(\text{mA})=0.04517 + 0.00215 v$ (mV·s⁻¹) $(r = 0.9946)$ and $i_{pa}(\text{mA})=0.01885 + 0.0013 v$ (mV·s⁻¹) $(r= 0.9967)$, respectively. The excellent linear relationship shows that CFD at the Gr/GCE is an adsorption process.

In addition, the oxidation peak potential of CFD showed a linear positive shift with $\ln v$ in two conditions (Figure 6: B), and corresponding fitting lines can be obtained as: $E_{pa} = 0.02854 \ln v + 0.7314$, $r = 0.9949$ and $E_{pa} = 0.0245\ln v + 0.7605$, $r = 0.9962$. According to Laviron equation[31, 32]:

$$E = E_o + \frac{RT}{anF} \ln \frac{RTk_s}{anF} - \frac{RT}{anF} \ln v \quad (1)$$

where $R$ is the universal gas constant (8.314 J·K⁻¹·mol⁻¹), $T$ is Kelvin temperature (298K), $F$ is the Faraday constant (96,487 C·mol⁻¹), $\alpha$ is the transfer coefficient, $k_s$ is the rate constant of the electrochemical reaction, $n$ is the numbers of electrons transferred in the whole reaction, $E_o$ is the standard potential for the redox reaction. In most systems, $\alpha$ values range from 0.3 to 0.7 and are approximately 0.5 under ideal conditions, so $n$ can be obtained from the linear relationship between $E_{pa}$ and $\ln v$. When DNA was not added, $n=1.8$; when dsDNA was added, $n=2.1$, and the similar electron numbers suggest that DNA is not involved in the electrochemical process.
3.2.4 The linear range of CFD at Gr/GCE

The relationship between oxidation peak currents and the concentrations of CFD was studied by cyclic voltammetry under the optimal conditions. As can be seen in Figure 7, two linear relationships between oxidation peak currents and the concentrations of CFD were obtained from 0.25~10.0 μM and 10.0~75.0 μM, and the linear equations are \( i_{pa}(mA) = 2.0853c(CFD) + 4.79 (r=0.9931) \) and \( i_{pa}(mA) = 0.4476c(CFD) + 21.22 (r=0.9971) \) respectively, which can be applied to the quantitative analysis of CFD. The electrode has a wonderful detection limit of 0.1 μM that was measured by a successive subtraction method.
Figure 7. Cyclic voltammograms of different CFD solutions on Gr/GCE in pH 4.0 PBS with scan rates of 80 mV·s⁻¹ ((a→h: 0.25, 1.0, 1.5, 5.0, 10, 25, 50, 75 μM)). Inset: the plot of the oxidation peak currents versus concentration of CFD.

3.2.5 Comparation of diffusion coefficient

Figure 8. Dependence of charges on the square root of time: (a) $2.5 \times 10^{-5}$M CFD and (b) $2.5 \times 10^{-5}$M CFD mixing with 0.16 mg·mL⁻¹ DNA.

Two-step timing coulomb method was used to study the interaction of CFD and DNA. The relation between the square root of the coulomb charge $Q$ and time $t$ in the two-step process conforms to the Anson equation[33]:

$$Q = \frac{2nFADt}{\pi^{1/2}} + Q_0 + Q_{ads} \quad (2)$$
It can be seen that if the electrode area $A$ and the concentration $C$ of the electroactive substance are known, the slope of the linear relationship between $Q$ and $t^{1/2}$ is related to the diffusion coefficient. As shown in Figure 9, the linear equations are:

$$Q \text{ (mC)} = -18.82 t^{1/2} + 1.163, r = 0.9998 \text{ (curve a)}; \quad Q \text{ (mC)} = -14.02 t^{1/2} + 1.157, r = 0.9989 \text{ (curve b)}.$$

The slope of the line $Q \sim t^{1/2}$ of CFD decreased significantly after DNA was added, which indicated that the diffusion coefficient of the complex CFD-DNA was less than that of the free CFD. It can be concluded that the formation of CFD-DNA results in the lower diffusion rate than that of CFD.

4. CONCLUSIONS

In the paper, the electrochemical behaviors of CFD at Gr/GCE were investigated detailly. The interaction between CFD and DNA was studied by electrochemical and fluorescence methods. Based on the experimental results, the electrochemical oxidation of CFD at Gr/GCE is an irreversible process involved protons, and the electrode reaction mechanisms of CFD at Gr/GCE in the absence and presence of DNA were proposed. Importantly, the interaction of CFD with DNA was studied, which is helpful for us to understand the pharmacology of CFD. Therefore, we can conclude that this method can be used to detect such variety of antibiotics and study the interactions between antibiotics and DNA.

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