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A Novel Electrochemical Method for Sensitive Detection of Anticancer Drug Picoplatin with Graphene Multi-walled Carbon Nanotubes Modified Glassy Carbon Electrode

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In this paper, graphene dispersed multi-walled carbon nanotubes composite modified glassy carbon electrode (GR-MWNTs/GCE) was prepared and the GR-MWNTs/GCE was employed for the electrochemical determination of anticancer drug picoplatin. A pair of well-defined redox peak of picoplatin was observed at the GR-MWNTs/GCE in 0.05 mol L^{-1} KCl solution (pH 7.4) and electrode process is adsorption-controlled. The result indicates that the GR-MWNTs composite materials can effectively improve the sensibility and performance of the electrode for picoplatin. A differential pulse voltammetry (DPV) method for the determination of picoplatin was proposed based on the GR-MWNTs/GCE. Under the optimal conditions, the proposed method exhibited linear ranges from 2.13×10^{-6} mol L^{-1} to 3.46×10^{-5} mol L^{-1}. It can be used to detect as low as 2.10×10^{-7} mol L^{-1} of picoplatin with a recovery of 96.0~99.0% and RSD ranges of 0.73%~1.60%. The proposed method was used for the determination of picoplatin in this work.

Keywords: Picoplatin; Graphene; Multi-walled carbon nanotubes; Cyclic voltammetry; Differential pulse voltammetry.

1. INTRODUCTION

Over the past 30 years, platinum-based drugs, notably cisplatin and carboplatin, have dominated the treatment of various cancers by chemical agents.[1-2] However, because these drugs

cause serious side effects, chemists are looking to other platinum complexes as potential anticancer agents.[3] Picoplatin (Fig. 1) is a new generation platinum-based analog designed to overcome platinum resistance,[4-5] which is one of the major limitations of other platinum-based therapies. Preclinical data demonstrated that picoplatin overcomes platinum resistance in lung and colon cancer. Furthermore, picoplatin has shown clinical efficacy in phase II studies in mesothelioma, ovarian cancer, non-smallcell lung cancer, SCLC, prostate cancer, and breast cancer, suggesting the potential for broad use in platinum-resistant as well as platinum-sensitive cancers.

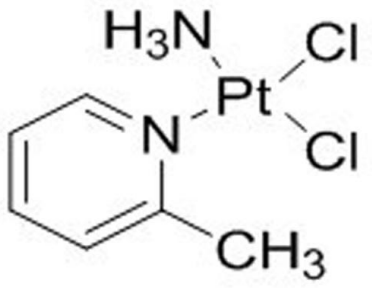

Figure 1. The structure of picoplatin.

HPLC method have been used for the determination of picoplatin.[6-7] And electrochemical methods represent the suitable techniques for determination of trace amounts of elements including platinum-based drugs.[8-12] The electrochemical determination of cisplatin at dropping mercury,[13] modified carbon paste and platinum electrodes,[14] electrochemical determination of carboplatin and cisplatin using a DNA-modified glassy carbon electrode,[15] and differential pulse voltammetry determination of carboplatin and oxaliplatin using hanging mercury drop electrode have been reported.[16] Electrochemical methods have good application prospect in the determination of platinum-based drugs due to their simplicity, low cost, high sensitivity and rapid response, however, few reports are reported for the electrochemical determination of picoplatin.

Graphene (GR) and carbon nanotubes (CNT) have been extensively used as electrode materials due to their unique physical and chemical properties such as superior toughnes,[17] good electrical conductivity and thermal stability. Recently, researcher found that carbon nanotubes can be combined wih graphene oxide (GO) to form a CNT-GO composite material through π-π role.[18-20] But to the best of our knowledge, the application of graphene-carbon nanotubes composite material in electrochemical analysis has been reported barely.

In this work, a novel graphene dispersed multi-walled carbon nanotubes composite modified electrode (GR-WMCNTs/GCE) was prepared. The electrochemical behavior of picoplatin at GR-WMCNTs/GCE was investigated and a sensitivity electrochemical analysis method was developed for the determination of picoplatin.

2. EXPERIMENTAL

2.1. Reagents and chemicals

The carboxyl functionalized multi-walled carbon nanotubes (MWNTs, purity>95%), purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences; Picoplatin reference (content≥99.9%, Kunming Guiyan Pharmaceutical Co. Ltd), a certain concentration of stock solution was prepared by 0.05 mol L^{-1} KCl and saved at 4^{o}C. Picoplatin injection (Kunming Guiyan Pharmaceutical Co. Ltd).

Other reagents used were of analytical-reagent grade. Twice-distilled water was used throughout all experiments.

2.2. Apparatus

ZAHNER Zennium IM6 Electrochemical Workstation (ZAHNER-elektrik GmbH & Co. KG, Kronach, Germany). A three-electrode system, including a GR-MWNTs/GCE working electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode.

2.3. Experimental Methods

2.3.1. Synthesis and Characterization of Graphene

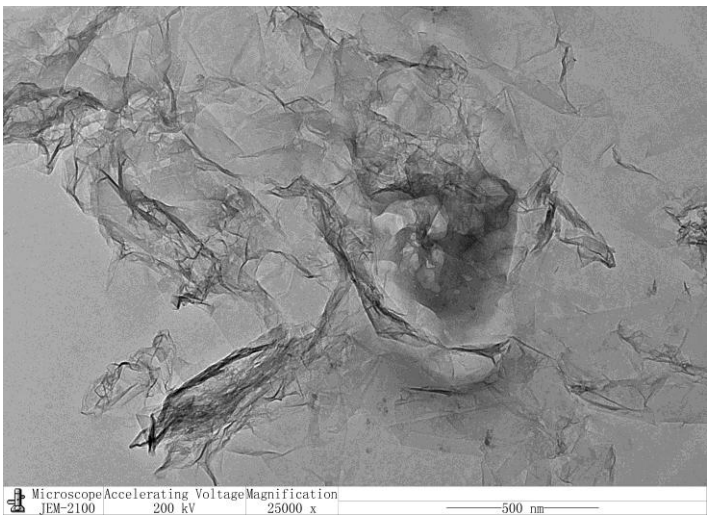

Figure 2. TEM image of GR

Graphene oxide (GO) was firstly synthesized from graphite according to the Hummers and Offeman method.[21] Then the GO was reduced and followed a typical procedure: the resulting GO dispersion (100 mL) was mixed with 70 μL of hydrazine solution (50 wt.% in water) and 0.7 mL of ammonia solution (28 wt.% in water). The mixture was stirred for 1 h at the temperature of 95°C.

Finally, black hydrophobic reduced graphene oxide (GR) sheets were obtained by filtration and dried in vacuum.[22]

The as-prepared GR was characterized by TEM as shown in Fig. 2.

2.3.2. Preparation of modified glassy carbon electrode

GR-MWNTs suspension was obtained by dispersing the treated GR (10 mg) and MWNTs (10 mg) into 10.0 mL distilled water with the aid of intensive sonication (120 W, 40 KHz, 3 h).

Glassy carbon electrode (3 mm in diameter) was polished to a mirror-like surface with metallographi sand paper and 0.05 μm Al_2O_3 suspension, respectively. After rinsed thoroughly with doubly distilled water between each polishing step, the electrode was subjected successively with 50% nitric acid, ethanol and doubly distilled water in ultrasonic bath, and dried in air. The electrode was then electrochemically cleaned in 0.5 mol L^{-1} H_2SO_4 by cycling potentials between -1.4 and +2.0 V at 0.1 V s^{-1} until a steady cyclic voltammogram was obtained. Graphene-multiwalled carbon nanotubes modified electrode (GR-MWNTs/GCE) was prepared by casting 10 μL of the GR-MWNTs suspensio onto the GCE surface and dried under an infrared lamp.

2.4. Electrochemical analysis

Cyclic voltammetry (CV) and differential pulse voltammeter (DPV) were performed in the three-electrode cell in pH 7.4 KCl solution between the potential range of -0.8 V and +0.8 V at a scan rate of 0.05 V s^{-1}. The DPV conditions were: pulse width 100 ms, pulse amplitude 40 mV and pulse interval 150 ms.

3. RESULTS AND DISCUSSION

3.1. Influence of supporting electrolyte and pH

Different supporting electrolyte such as 0.05 mol L^{-1} disodium hydrogen phosphate-citric acid buffer (pH 7.0), 0.05 mol L^{-1} sodium hydrogen phosphate-sodium dihydrogen phosphate buffer (pH 7.0), 0.05 mol L^{-1} Tris-HCl buffer (pH 7.0) and 0.05 mol L^{-1} KCl solution (pH 7.0) was tested at GR-MWNTs/GCE. Well-defined CV response with high redox peak of picoplatin was obtained in 0.05 mol L^{-1} KCl solution. The influence of pH on the differential pulse voltammetry (DPV) response of picoplatin at GR-MWNTs/GCE was then investigated in 0.05 mol L^{-1} KCl solution (pH 2.0 to 9.0). The results shows in Fig. 3, that the oxidation peak current of picoplatin increase as the pH increases from 3.5 to 7.4, and decrease as pH increases above 7.4. This phenomenon may be due to the following reason: there is a negative carboxyl group in grapheme oxide, and it can combine with H^+ more easily under acidic conditions, which leads the decreasing of the amount of picoplatin active substance reaching the surface of the electrode, and gains a small current response. Under alkaline conditions, picoplatin lost H^+ turning to an anion, which repel with the negative carboxyl group in the

electrode surface, causing the peak current decreasing. A maximum of oxidation peak current with the lowest background current was obtained at pH 7.4. 0.05 mol L^{-1} KCl solution (pH 7.4) was therefore chosen as supporting electrolyte for subsequent experiments.

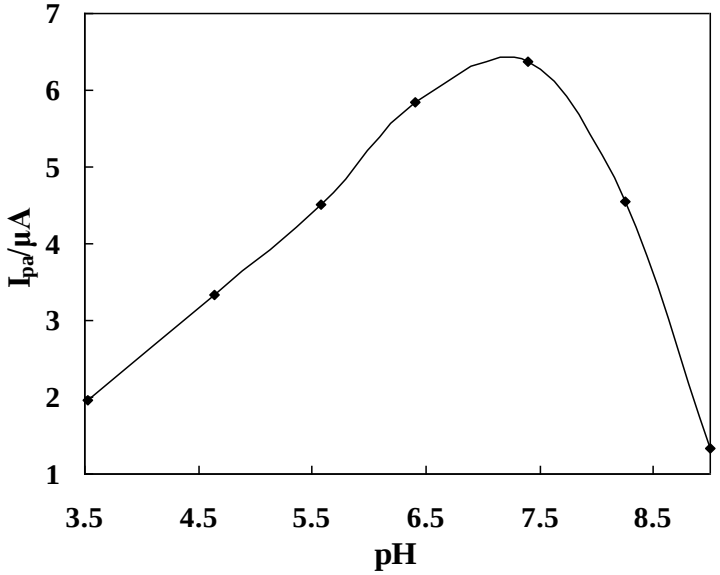

Figure 3. The influence of pH on peak current

3.2. Influence of the scan rate

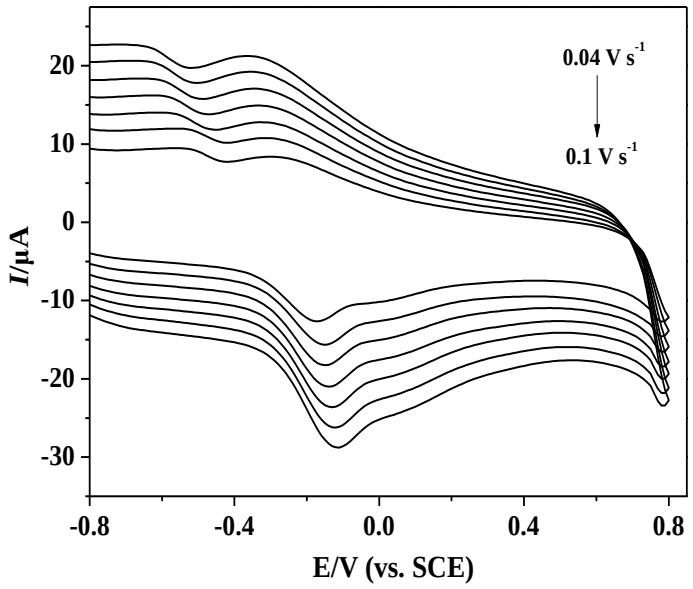

Figure 4. The cyclic voltammetry curves of picoplatin at different scan rates

Fig. 4 shows the effect of scan rate on the CV response of picoplatin at GR-MWNTs /GCE in KCl solution. It is found that both the oxidation peak current (I_{pa}) and reduction peak current (I_{pc}) are linear to the scan rate (υ) in the range of 0.01 to 0.1 V s^{-1} shows as Fig. 5, with the linear regression equation as I_{pa}(μA)= 267.6υ+2.1543 (r=0.9997) and I_{pc}(μA)= 223.7υ-0.0449 (r=0.9998), respectively,

indicating that the electrochemical process of picoplatin at GR-MWNTs/GCE is adsorption-controlled.[23] The maximum achievable peak signal-to-noise ratio for picoplatin was obtained at the scan rate of 0.05 V s^{-1}. The scan rate of 0.05 V s^{-1} was therefore selected in this work.

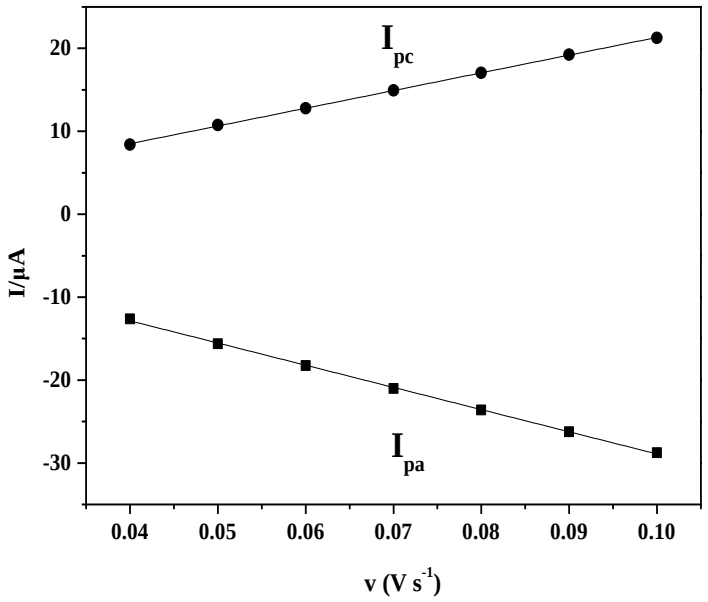

Figure 5. The linear relationship between the peak currents (I_{pa}, I_{pc}) and the scan rate(v).

3.3. The cyclic voltammetry behaviors of picoplatin

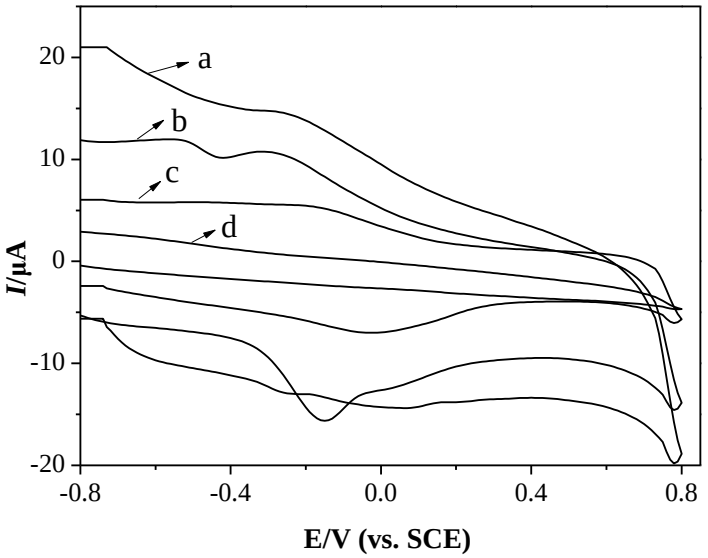

Figure 6. Cyclic voltammograms of picoplatin

Fig. 6 displays the CV curves of picoplatin in the 0.05 mol L^{-1} KCl solution (pH 7.4) at GCE (d), GR/GCE (a), MWNTs/GCE(c) and GR-MWNTs/GCE (b). A pair of redox peak was obtained at GR/GCE, MWNTs/GCE and GR-MWNTs/GCE, while the oxidation and reduction peak currents of picoplatin at GR-MWNTs/GCE is much higher than that at GR/GCE and MWNTs/GCE as fig. 4

shown. A well-defined CV response with much low background was observed, as compared with that at GR/GCE (a), which means that GR-MWNTs composite material can effectively improve the sensibility and performance of the electrode for the determination of picoplatin.[24]

The oxidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) of picoplatin were -0.15 V and -0.32 V (vs. SCE), respectively, $\triangle E$＝0.17 V (vs. SCE). The ratio of oxidation peak current (I_{pa}) and reduction peak (I_{pc}) was 1.45, implying that the electrode process of picoplatin at GR-MWNTs /GCE is quasi-reversible

3.4. Sensor Stability and Repeatability

The stability of the GR-MWNTs/GCE was tested by continuous scanning of the electrode potential between -0.8 and +0.8 V for 10 h in pH 7.4 KCl solution containing 2.98×10^{-5} mol L^{-1} picoplatin, no significant change of the peak current and potential were observed. The repeatability of the GR-MWNTs/GCE was detected by five intermittent determinations of a picoplatin solution (2.98×10^{-5} mol L^{-1}), RSD was found to be 1.23%. The reproducibility of the method was evaluated by analyzing seven replicates of picoplatin, RSD was found to be 0.93%.[25] These experiments demonstrate that the GR-MWNTs/GCE has good stability, repeatability and reproducibility for the determination of cisplatin.

3.5. Analytical performance characteristics of the method

Well-defined oxidation peak DPV response with high peak current of picoplatin was observed at GR-MWNTs/GCE. A good linear relationship was obtained between the oxidation peak current and the concentration of picoplatin within the range from 2.13×10^{-6} mol L^{-1} to 3.46×10^{-5} mol L^{-1}, with the linear regression equation $I_{pa}(\mu A)= 4\times10^{-6}c-8\times10^{-6}$ (n=9, r=0.9993). According to the method recommended by IUPAC, detection limit (CL)=$3S_b/S_x$, where, 3 is confidence factor, S_b is background noise standard deviation, S_x is the measurement sensitivity (The slope of the standard curve), as the result, the detection limit for picoplatin can reach to 2.10×10^{-7} mol L^{-1}.

3.6. Analytical Applications

For verifying the practically and reliability of the proposed method, the GR-MWNTs/GCE was applied to the determination of picoplatin in picoplatin injection by standard addition method as shown in Table 1. The average relative standard deviation (RSD. %) was found to be 1.08% and the average recovery was 97.6%, the determination result of picoplatin was in good agreement with that specified by the manufactures.

Table 1. Determination results of picoplatin in samples (n=5)

Picoplatin Injection Sample	By this method		Added / mg g^{-1}	Found / mg g^{-1}	Recovery (%)	By HPLC / mg g^{-1}
	Average / mg g^{-1}	RSD (%)				
1	197.9	0.73	100	293.9	96.0	200
2	198.5	1.60	150	345.2	97.8	200
3	196.7	0.92	200	394.7	99.0	200

4. CONCLUSIONS

A novel sensitive electrochemical method for the determination of anticancer drug was proposed in this work based on the graphene and multi-walled carbon nanotubes composite modified electrode (GR-MWNTs/GCE). The sensibility and performance of the electrode for the determination of picoplatin was improved by graphene and multi-walled carbon nanotubes composite material as compared with graphene or carbon nanotubes modified elecrode. And a well-defined CV response with much low background and much high peak current was observed at GR-MWNTs/GCE. The quantitative analysis of picoplatin with differential pulse voltammeter was demonstrated good analytical performance with a detection limit of 2.10×10^{-7} mol L^{-1}, a average RSD of 1.08% and average recovery of 97.6%.

ACKNOWLEDGEMENTS
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