Determination of Promethazine in Pharmaceutical Samples by Using Graphene Modified Electrode

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A glass carbon electrode modified with functionalized graphene has been fabricated due to its elusive two-dimentional structure and unique electronic properties. Electrochemical experiments indicated the modified electrode showed a preferable electrocatalytic activity for the oxidation of promethazine. Under the optimum conditions, the increase of response current is proportional to the promethazine concentration in the range of 2.0×10^{-6} and 1.0×10^{-3} M, with the detection limit of 4.2×10^{-7} M. In addition, the developed sensor shows acceptable stability and reproducibility. These results demonstrate the viability of the use of graphene modified electrode as a potential amperometric sensor for pharmaceutical and clinical measurements.

Keywords: Graphene, Promethazine, Modified electrode, Measurement.

INTRODUCTION

The rapid and accurate analysis of promethazine hydrochloride, N,N-dimethyl-(10*H*-phenthiazine-10-yl) propan-2-amine hydrochloride, is of great significance in pure form or pharmaceutical preparations. It is because that promethazine hydrochloride is commonly known as neuroleptic tranquilizer and used as psychotropic, local anaesthetic anti-allergic and antivomiting drugs[1]. Conventional techniques such as spectrophotometry[2,3], oxidation titrations[4], chemiluminescence[5], capillary electrophoresis[6] and chromatography[7,8] have been reported in the literatures. However, many of the mentioned methods are generally time-consuming manipulation steps or difficult for an automated detection. Therefore, electroanalytical technique has been aroused attention for the determination of promethazine hydrochloride due to their simple, rapid and cost effective properties[9-12]. On the other hand, graphene, a single layer of carbon, is well recognized for the excellent electrocatalytic potential toward the detection of many important analytes due to its extraordinary mechanical properties and high electrical conductivity[13-15]. Unfortunately, the strong tendency for monolayered graphene sheets to agglomerate into multilayered graphite seriously hampered its application. Recently, some efforts have been focused on the functionalization of graphene by using different methods in order to modify the hydration properties of the surface of graphene. Among these approaches, moderate funtionalization of sidewalls of graphene in oxidizing acids is an effective way to preserve the sp^2 plane structure and thus its electronic charac-

teristics[16]. After oxidation, graphene containing oxygen groups, which is similar to carbon nanotubes[17,18], can disperse in some solvents forming suspension without the use of dispersant.

In the present work, we report on the utilization of functionalized graphene as novel immobilization matrix for the oxidation of promethazine. In comparison with bare glass carbon electrode, the graphene modified electrode showed an excellent catalytic activity in the electrochemical oxidation of promethazine, leading to remarkable enhancements of peak currents and lowering the peak potential. Moreover, the prepared voltammetric sensor exhibited many advantages, such as high sensitivity, wide dynamic range and long-time stability. Therefore, it indicated graphene could be good candidate material for determine promethazine and thus fabricating the new drug sensors.

EXPERIMENTAL

Promethazine hydrochloride were purchased from Alfa Pharma (Belgium). Graphene was obtained from Nanolab (Brightm, MA. USA). The buffer solutions (0.1 M) were prepared from NaH_2PO_4 and Na_2HPO_4 and regulated with 0.1 M H_3PO_4 and NaOH. The stock solutions of promethazine (0.5 mM) was prepared by dissolving in 0.1 M phosphate buffer solution and was stored at 4 °C. All other chemicals with analytical reagents grade were from Shanhai Chemical Company. Deionized water was used throughout this study.

Scanning electron microscopy (SEM) was conducted by Hitachi S-4800 (Hitachi, Japan) for surface morphology observations. FT-IR spectra were recorded by an FT-IR spectropho-

tometer (PE950, Japan) with KBr pellets. All the voltammetric experiments were performed on CHI660E Electrochemical analyzer (Chenhua Instrumental Co., Shanghai) in a three-electrode configuration: graphene modified glass carbon electrode as a working electrode and a dual Ag/AgCl reference and platimum auxiliary electrode.

Synthesis of graphene suspension: The graphene powder was suspended in a concentrated nitric acid and reflux for 6 h prior to filter. After the oxidized graphene was thoroughly rinsed with water, many oxide functional groups (carbonyl and carboxylate) would produce at the defect sites located at the ends of graphene and hence develop the soluble properties of graphene. Then a suspension of the modifier was prepared by dispersing 2.5 mg of functionalized graphene in 5 mL N,N-dimethylformamide by sonication for 0.5 h, which result a black homogeneous mixture.

Fabrication of graphene modified electrode: The glass carbon (GC, 3 mm, indiameter) electrode was carefully polished to a mirror-like with 0.3 and 0.05 µm alumina slurry and was placed in ethanol and nitric acid (1:1) to sonication for 5 min in turn. Substantially, 30-cycle scans were carried out in the potential range of -1.5 to 1.5 V in a solution of 0.5 M H$_2$SO$_4$ prior to the modification. Then 12 µL of graphene N,N-dimethylformamide solution (0.5 mg/mL) was cast on the surface of GC electrode and dried under an infrared camp to form a graphene modified electrode. Before the voltammetric determination, the pretreatment of activation was performed in the potential range of -1.0 to 1.2 V with a scan rate of 100 mV/s to reach steady state. Furthermore, solutions were deaerated by purging with nitrogen through them prior to the experiments. All electrochemical experiments were carried out at the ambient temperature of 25 °C.

RESULTS AND DISCUSSION

Material characterization: It is well known that mono-layered graphene sheets tend to agglomerate into multilayered graphite. Therefore, it is reasonable that functionalize graphene for suspension without the use of a dispersant. The SEM image depicted in Fig. 1A show the general morphology of the graphene that was used in this work. It can be found that many prepared graphene oxide wrinkled the flake-like structures. FT-IR spectroscopy was also used to investigate the functionalized graphene material. As show in Fig. 1B, compared with original graphene, the presence of additional peaks at 1639 cm^{-1} (C=O), 1385 cm^{-1} (C-OH), 1121 cm^{-1} (C-O) of graphene oxide demonstrate the produce of oxo-groups on graphene after oxidation.

Electrocatalysis of promethazine at the graphene modified electrode: The electrochemical behavior of promethazine was investigated by cyclic voltammetry. As can be seen dotted line in Fig. 2, no redox peaks were obtained at bare glassy carbon graphene in the absence of promethazine (green). However, for graphene modified electrode, when a potential sweep was cycled between 0.2 to 1.0 V, a pair of stable peaks were observed and oxidation peak and reduction peak potential were at 0.32 and 0.36 V, respectively (blue). It is suggested that carboxylic groups of oxidized graphene performed quasi-reversible transfer reaction. That the background of graphene modified electrode was larger than that of bare glassy carbon
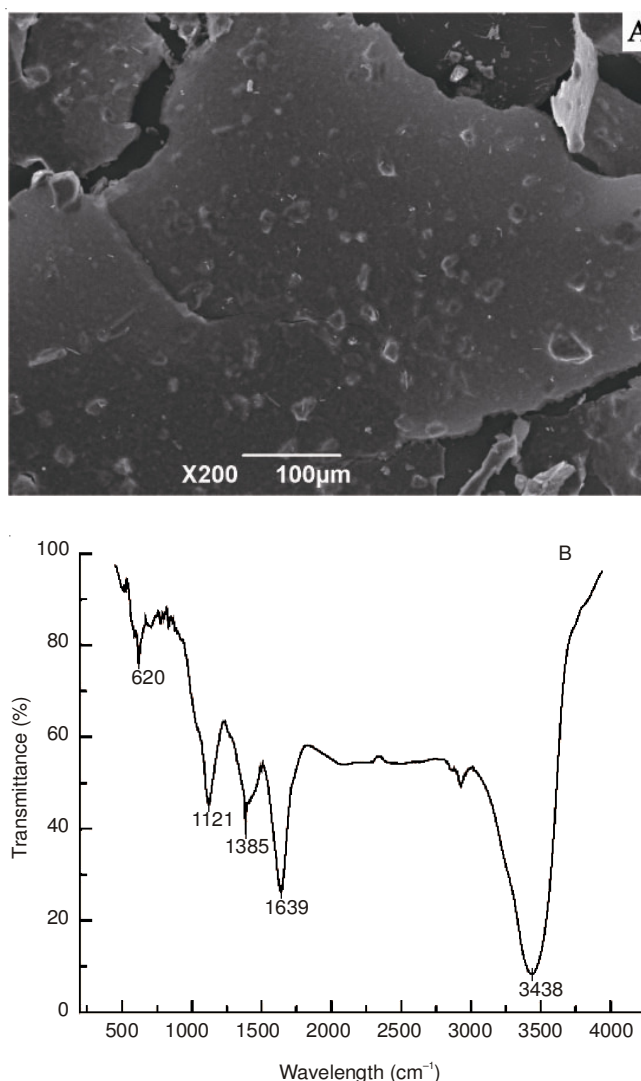

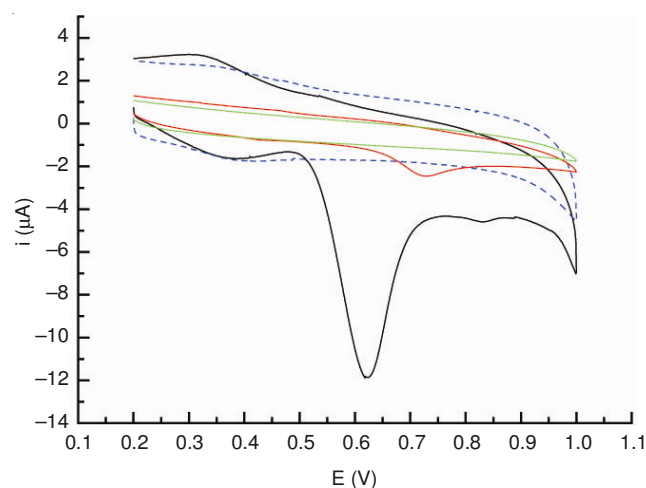Fig. 1. SEM image (A) and FTIR spectra (B) of acid-treated graphene material

Fig. 2. CVs of the bare GC electrode and graphene doped electrode in 0.1 M phosphate buffer solution (pH 7) in the absence of promethazine (green, red) and in the presence of 2.0 × 10^{-5} M promethazine (blue, black) at a scan rate of 50 mVs^{-1}

electrode can be attributed to the large specific area and good conductivity of graphene. After added promethazine, in the

case of bare GC electrode, the oxidation of promethazine resulted in a broad peak with the peak potential of 0.76 V (red). However, the graphene modified electrode yielded a significant enhanced voltammetric current with the oxidation peak potential at 0.62 V and an unobvious oxidation peak with a potential of 0.83 V (black). In subsequent experiments, the peak at 0.62 V was applied to the determination of promethazine with a view to sensitivity. In comparison with Xiao's work[19], the graphene modified electrode can provide a better performance for analysis than multi-walled carbon nanotubes coated gold electrode due to lower promethazine oxidation potential. Therefore, it is concluded that the unique structure and properties of graphene are the key points of electrocatalytic activity for oxidation of promethazine.

The effect of the scan rate on the electrocatalytic property of graphene modified electrode was studied (Fig. 3). With the scanning rate increasing, the potential of oxidation peak shifted positively. It is clear that the anodic peak current is linearly proportional to the scan rate between 10 and 250 mV s^{-1}. The regression equation is: i_{pa} (μA) = -1.1473-0.1332 ν (ν: mV s^{-1}, r = 0.9905). This is expected for a surface-controlled electrochemical reaction (inset of Fig. 3).
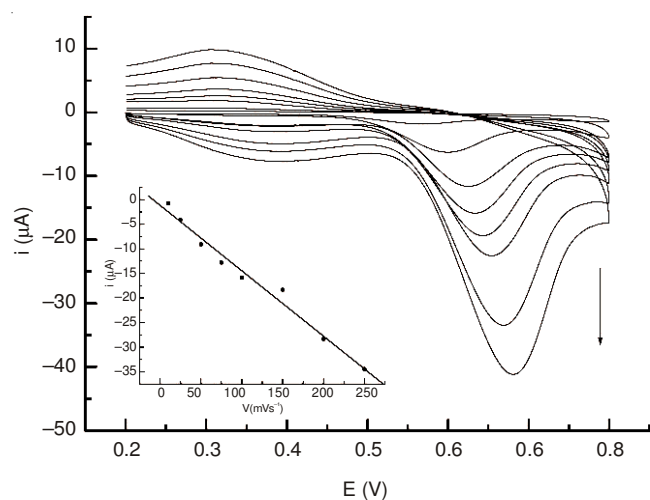

Fig. 3. Scan rate dependence of peak current of graphene modified electrode. Inset: the plot of anodic peak current *vs*. scan rate (from top to bottom scan rate is: 10, 25, 50, 75, 100, 150, 200, 250 mV s^{-1})

Influence of pH on the developed sensor response: The study of pH of the supporting electrolyte on the analysis signal is useful for the goal of optimization condition. In this work, it was found that a relatively poor electrochemical response with broadened wave shape was observed in acid media. The oxidation current increased from pH 3 and reached a maximum at pH 7 with the more negative E_{pa} values. With further enhancing the pH of solution, the E_{pa} shifted back to positive values with smaller peak current. Therefore, the phosphate buffer solution of pH 7 was selected as a suitable analytical medium.

Influence of amount of modifier: It was found that the amount of graphene doped on the GC electrode surface affects the efficient modification and electrochemical response of promethazine. In order to study the optimum amount of graphene, dispersion of different amount graphene in the range of 0 to 20 μL were casted on the electrode surface. It can be observed

that the oxidation peak was enhanced between 0 to 12 μL and then decrease when the amount of the modifier was increased further. This behavior occurs may be due to an increase of the thickness of film and consequent reduction of the conductive electrode area. Therefore, 12 μL graphene suspension was chosen as the best amount of the modifier.

Calibration curve: Under the optimal conditions, differential pulse voltammetry (DPV) was used to determine promethazine due to its higher sensitivity than cyclic voltammetry. Fig. 4 displays the DPVs of different concentration of promethazine in pH 7 phosphate buffer solution. As can be seen in the insert of Fig. 4, the oxidation peak current was proportional to promethazine concentration in the range of 2.0×10^{-6} to 1.0×10^{-3} M with the detection limit of 4.2×10^{-7} M (S/N = 3). The linear regression equation is i_{pa} (μA) = -0.7458c (μM) -7.8226 (R = 0.9933). It can be found that the performances of the developed graphene modified electrode are superior to those of other methods reported in the literatures[20-22], especially the detection limit or linear range. Eleven successive determination of 5.0×10^{-5} M promethazine at one graphene modified electrode resulted in a relative standard deviation (RSD) of 3.1 %, suggesting a high reproducibility. The stability of modified electrode was examined by discontinuous amperometric measurement after stored in 4 °C for 48 h and retained about 90 % of its initial value to promethazine.
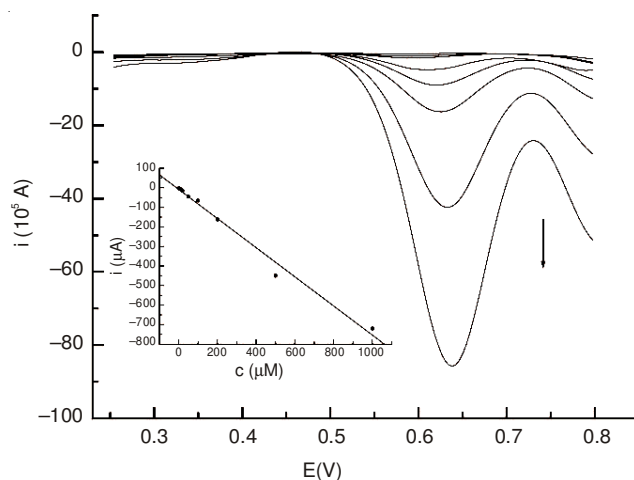

Fig. 4. DPVs of different concentration of promethazine on graphene modified electrode in the phosphate buffer solution (pH = 7). (from top to bottom concentration is 2, 10, 20, 50, 100, 200, 500, 1000 μM), Inset: linear relationship between the oxidation peak current and the concentration of promethazine

Interference study in determination of promethazine: The influence some inorganic ions on the determination of promethazine was studied. The results suggested that 500-fold of Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cl^-, SO_4^{2-}, NO_3^-; 200-fold of Fe^{3+}, Zn^{2+}, Cu^{2+}, F^-, CO_3^{2-}, PO_4^{3-}; 100-fold of Mn^{2+}, Co^{2+}, Al^{3+}, Br^-, do not interfere with the oxidation signal of 5.0×10^{-4} M promethazine. In addition, the present modified electrode could resolve completely the voltammetric response of promethazine from some other biological compounds (such as ascorbic acid, glucose, perazine, sucrose, lactose, fructose, acetyl salicylic acid, formaldehyde). Promazine and thioridazine were found to effect the determination. Hence, it is demonstrated that

		TABLE-1				
		RESULTS OF ANALYSIS OF PROMETHAZINE IN REAL SAMPLES				
Samples	Labeled (μM)	Determined (μM)[c]	Added (μM)	Found (μM)[c]	Recovery (%)	RSD (%)[c]
1[a]	77.9	79.3	20	99.6	101.5	3.1
	77.9		50	127.6	96.6	2.3
2[b]	77.9	76.8	20	95.8	95.0	3.9
	77.9		50	127.9	102.2	2.6

[a] Promethazine hydrochloride injection purchased from Xi'an pharmaceutical Co. Ltd. (Labeled content: 50 mg/2 mL)
[b] Promethazine hydrochloride injection purchased from Northeast pharmaceutical group shenyang No. 1 pharmaceutical Co. Ltd. (Labeled content: 25 mg/1 mL)
[c] Average of six determinations

graphene modified electrode has an excellent selectivity for analysis of promethazine in real samples.

Analytical application: In order to evaluate the validity of graphene modified electrode for the determination of promethazine in pharmaceutical formulations, the developed method was applied to detect promethazine in two commercial promethazine hydrochloride injections by the standard addition method. The pharmaceutical samples was performed as follows: 10 mL from promethazine hydrochloride injection solution (specified content of promethazine is 7.79×10^{-2} M) was diluted to 1000 mL with distilled water and then this diluted solution was diluted to volume of 10 mL with phosphate buffer solution (pH 7) and then was placed in the electrochemical cell. The determination results are shown in Table-1. As can be seen, the recoveries were acceptable, suggesting that the proposed method could be efficiently used for the determination of trace amounts of promethazine in pharmaceutical preparations.

Conclusion

In this work the preparation and usefulness of the functionalized graphene modified electrode were investigated. This electrochemical sensor showed a short response time, a wide linear range, excellent stability and repeatability and little interferences in the process of analysis of promethazine. In addition, it has been applied successfully to determine promethazine in real sample. Therefore, above results demonstrate that graphene can be developed a promising material of novel drug sensor.

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