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A Sensitive Chlorpromazine Voltammetric Sensor Based on Graphene Oxide Modified Glassy Carbon Electrode

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A glassy carbon electrode modified with graphene oxide (GO/GCE) is proposed as a novel electrochemical platform for detection of chlorpromazine. The electrochemical activity of GO/GCE towards chlorpromazine was investigated using cyclic voltammetry (CV) experiments in 0.1 M phosphate buffer solution (PBS). The overpotential for the oxidation of chlorpromazine decreased significantly and its oxidation peak currents increased dramatically at GO/GCE. The potential utility of the sensor was demonstrated by applying it to the analytical determination of chlorpromazine concentration using differential pulse voltammetry (DPV). These results are beneficial for real sample analysis. The sensor worked linearly in the range of 0.05-200.0 μM and had a detection limit of 42.0 nM using DPV. The fabricated sensor was successfully applied to the detection of chlorpromazine in real samples. The experiments illustrate that graphene oxide is a worthy electrode material that offers a large surface-to-volume ratio and improves the sensitivity. Here, a new sensor is introduced that is simple, rapid, sensitive and cost-effective for quantitation of chlorpromazine.

Keywords: Chlorpromazine, Voltammetry, Graphene oxide, Glassy carbon electrode

INTRODUCTION

Chlorpromazine (α-2-chloro-10-(3-dimethylamino-propylidine)-phenothiazine is the significant compound in the great group of phenothiazine derivatives. It is widely used in the treatment of psychotic conditions [1]. The discovery of antipsychotic effect of chlorpromazine in the early 1950s and the advent of an even more powerful phenothiazinic psychopharmacological agent showed a turning point in the history of medical and psychiatric science [2]. Chlorpromazine is useful for schizophrenic patients for controlling agitation, excitement and other psychomotor disturbances and reduces the manic phase of manic-depressive conditions [3]. It is reported that the possible of risk of cancer is higher in patients undergoing continuous treatment with chlorpromazine [4]. Therefore, the accurate and reliable determination of chlorpromazine in pharmaceuticals and biological fluids would be a great significance for effective therapy and controlling its side effects. Currently, quantitative determination of chlorpromazine in biological fluids has been extensively accomplished by high performance liquid chromatography, chemiluminescence, spectrophotometry, spectrofluorimetry, gas chromatography and phosphorimetry [5-11]. However, most of the above techniques are time-consuming and often need the pretreatment step, sample preparation, sophisticated instruments and expert operator [11]. However, electrochemical methods as alternative methods have also received much interest due to possess such advantages as higher selectivity, simplicity, lower cost, quick response, and ease in automation [12-15]. Therefore, electrochemical methods have become of considerable importance for determination of chlorpromazine [16,17].

Nanotechnology is seen as one of the key technologies

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of the 21st Century. Nowadays, nanotechnology has occupied a unique position in both science and technology sharing knowledge: tools, techniques, and information with electrochemistry and electroanalysis [18-20]. In order to improve their electrochemical performance, glassy carbon electrode surfaces have been modified and activated with nanosized materials [21-24]. Nanostructures modified electrodes have been good electrochemical activity, sensitivity, and selectivity; they have also a low detection limit compared to unmodified electrodes [25-32].

Graphene, a single layer of sp^2-hybrirdized carbon atoms packed in a honeycomb crystal lattice, has attracted considerable attention in recent years due to high specific surface area, extraordinary electronic properties and electron transport capabilities, unprecedented pliability and impermeability, strong mechanical strength and excellent thermal and electrical conductivities [33,34]. These unique physicochemical properties suggest a great potential for graphene to provide new approaches and critical improvements in the field of electrochemistry. For example, the high surface area of electrically conductive graphene sheets can give rise to high densities of attached analyte molecules. This in turn can facilitate high sensitivity and device miniaturization. Facile electron transfer between graphene and redox species opens opportunities for sensing strategies based on direct electron transfer rather than mediation. The oxygenated groups in graphene oxide (GO, oxidative derivative of graphene) can strongly affect its electronic, mechanical, and electrochemical properties. Underpinning the significance of GO in electrochemistry are the very specific properties such as facile synthesis, high dispersibility in a range of solvents, capability of coupling electroactive species onto the surface, and unique optical properties [35,36]. Therefore, GO has recently attracted great attention from the electrochemical society.

According to the previous points, it is important to create suitable conditions for analysis of chlorpromazine in biological fluids. In this study, we describe application of graphene oxide nanoplates as a nanostructure sensor for voltammetric determination of chlorpromazine. The proposed sensor showed good effects on chlorpromazine electrochemical oxidation. Eventually, we evaluate the analytical performance of the suggested sensor for chlorpromazine determination in real samples.

EXPERIMENTAL

Chemicals and Apparatus

To perform the electrochemical experiments an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was used and the system was controlled using a general purpose electrochemical system software. A conventional three-electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the GO/GCE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was employed for pH measurements.

Chlorpromazine and all other reagents were in analytical grade, and were obtained from Merck (Darmstadt, Germany), and the orthophosphoric acid and its salts were used to prepare buffers in the pH range of 2.0-9.0.

Preparation of the Electrode

The bare glassy carbon electrode was coated with graphene oxide nanoplates as follows. A stock solution of GO in 1 ml aqueous solution was prepared by dispersing 1 mg GO with ultrasonication for 1 h. A 5 µl aliquot of the GO/H_2O suspension solution was dropped on the working electrodes, and the solvent was evaporated in room temperature.

The microscopic areas of the GO/GCE and the bare GCE were obtained by CV using 1mM $K_3Fe(CN)_6$ as a probe at different scan rates. For a reversible process, the Randles-Sevcik formula is used:

$$i_{pa} = 2.69 \times 10^5 n^{3/2} A C_0 D_R^{1/2} v^{1/2} \quad (1)$$

where i_{pa} refers to the anodic peak current, n the electron transfer number, A the surface area of the electrode, D_R the diffusion coefficient, C_0 the concentration of $K_3Fe(CN)_6$ and v is the scan rate. For 1 mM $K_3Fe(CN)_6$ in the 0.1 M KCl electrolyte: n = 1 and $D_R = 7.6 \times 10^{-6}$ cms^{-1}, then from the slope of the i_{pa} - $v^{1/2}$ relation, the microscopic areas were calculated. In bare GCE, the electrode surface was found to be 0.041cm^2 and for GO/GCE the surface was nearly 4.0-4.5 times greater.

Preparation of Real Samples

Five chlorpromazine tablets (labeled 100 mg per tablet,

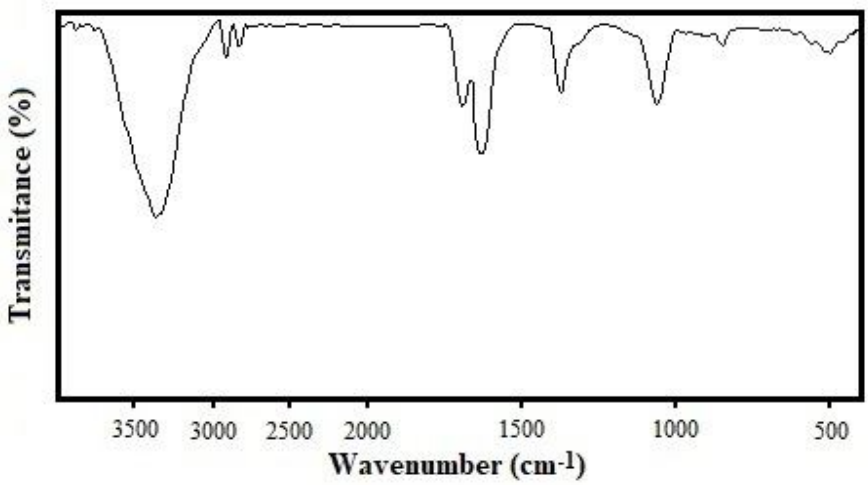

Fig. 1. FT-IR spectra of graphene oxide.

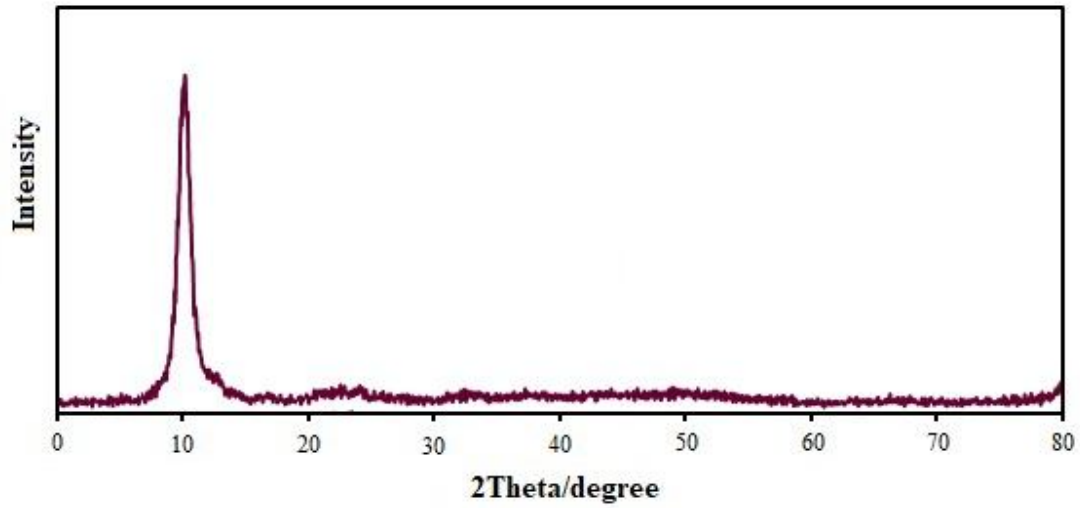

Fig. 2. XRD Patterns of graphene oxide.

Tehran Chemie Pharmaceutical Company, Iran) were ground. Then, the tablet solution was prepared by dissolving 500 mg of the powder in 25 ml water by ultrasonication. Then, different volume of the diluted solution was transferred into a 25 ml volumetric flask and diluted to the mark with PBS (pH 7.0). The chlorpromazine content was analyzed by the proposed method using the standard addition method.

The urine specimens were kept in a refrigerator after sampling. To prepare the test samples, 10 millilitres of urine specimens were taken or centrifuged at 2000 rpm for 15 min. After filtering the supernatant with a 0.45 µm filter, different volumes of it were diluted in 25 ml volumetric flasks using PBS (pH = 7.0). The diluted urine sample was spiked with different amounts of chlorpromazine.

RESULTS AND DISCUSSION

FT-IR, XRD and SEM Characterization

Figure 1 shows the FT-IR spectra of graphene oxide. FT-

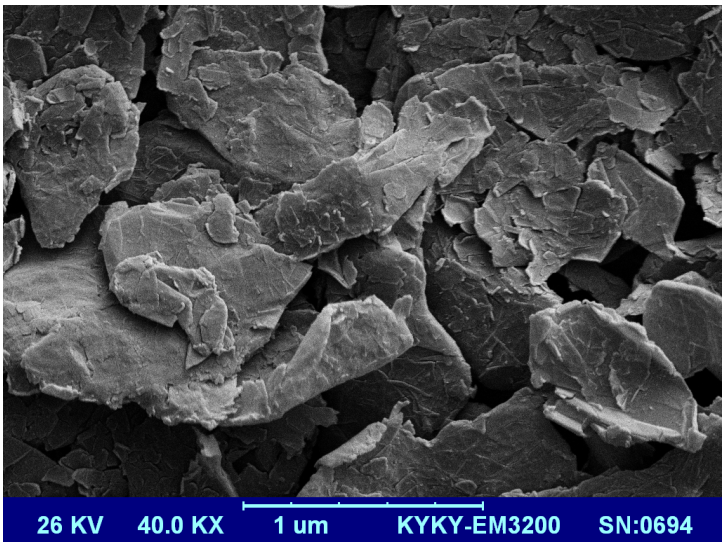

Fig. 3. SEM image of graphene oxide.

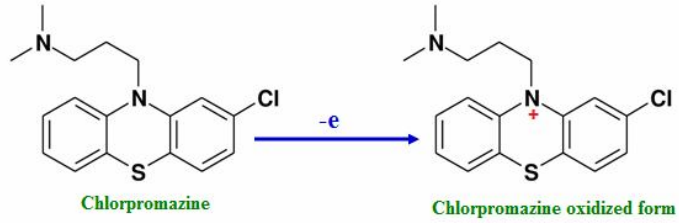

Fig. 4. Electro-oxidation mechanism of chlorpromazine at GO modified electrode.

IR analysis provides evidence for the presence of oxygen containing functional groups. As shown, fairly broad and intense peak in the region around ~3700 to 3000 cm^{-1} is responsible for stretching vibrations of hydroxyl group, where the hydroxyl groups may be from absorbed water molecules or phenolic OH or OH from carboxylic groups [37]. The two small peaks near ~2929 cm^{-1} and ~2866 cm^{-1} can be observed in graphene oxide, corresponding to the hydrogen bonded OH groups of dimeric COOH groups and intra-molecular bonded O-H stretching of alcohols, respectively [38]. The peak in the low frequency region close to 1623 cm^{-1} is attributable to O-H vibrations of water. Another intense band near ~1710 cm^{-1} corresponds to the C=O stretching vibrations of conjugated acid especially in the form of dimmer appeared in graphene oxide. In fact, corresponding IR absorption peaks for functional groups such as C-OH (1378 cm^{-1}), and C-O (1035 cm^{-1}) can be clearly observed in graphene oxide [39].

XRD is a very important tool for the characterization of graphene oxide since it gives the idea about the interlayer spacing and corresponding diffraction angle. Figure 2 shows the XRD pattern of graphene oxide indicating an intense peak at 2Θ = 10.1° (due to 0 0 2 crystal plane) with a spacing of 8.7 Å, that is typical for graphene oxide [40]. Also, Fig. 3 shows SEM image of graphene oxide.

pH Effect

In general, pH is one of the variables that commonly influences on the current and shape of voltammograms. It is important to investigate the effects of pH on electrochemical systems. DP voltammograms of solution containing 100.0 µM chlorpromazine were recorded in phosphate buffer

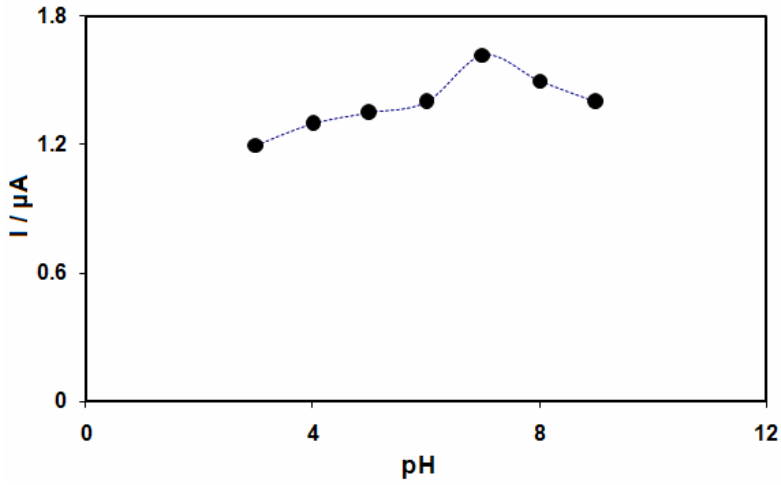

Fig. 5. Effects of solution pH on the Ip at the GO/GCE in 0.1 M PBS containing: 100.0 µM chlorpromazine.

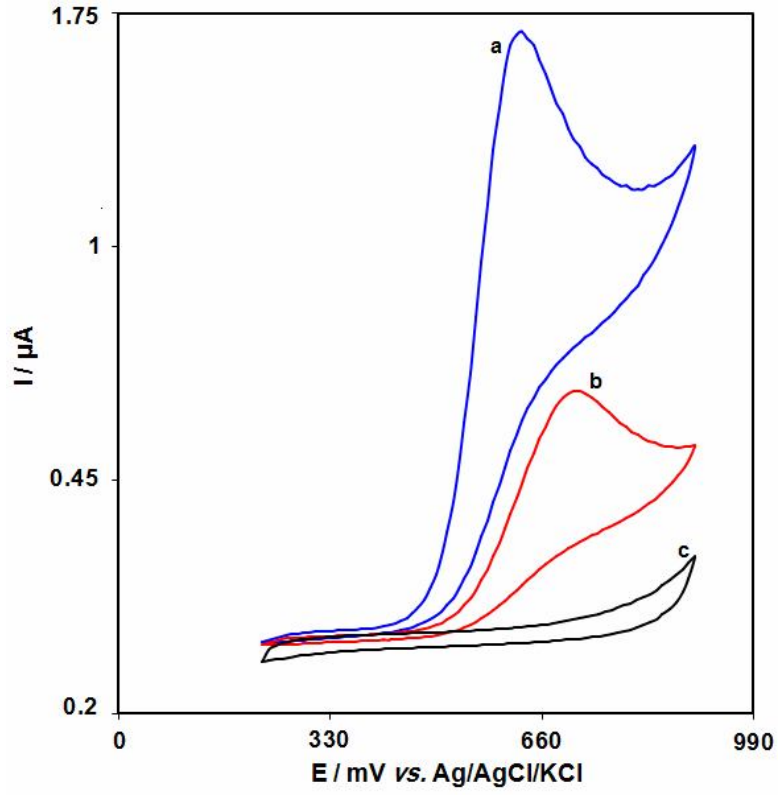

Fig. 6. CVs of (a) GO/GCE, and (b) bare GCE in 0.1 M PBS (pH 7.0) in the presence of 100.0 µM chlorpromazine, and (c) GO/GCE in 0.1 M PBS (pH 7.0) in the absence of chlorpromazine at the scan rate 50 mV s^{-1}.

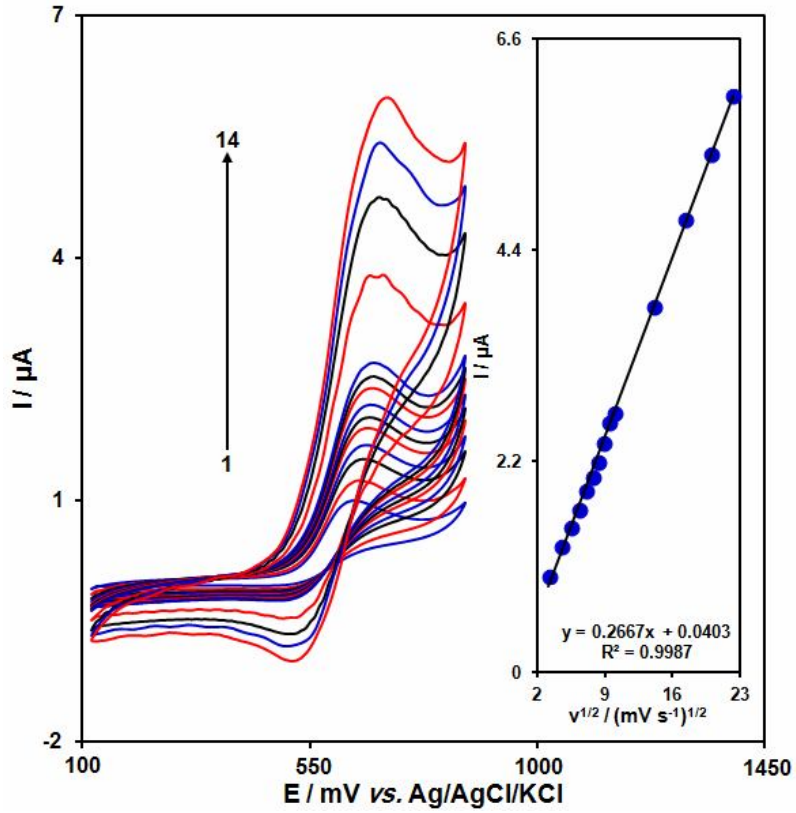

Fig. 7. CVs of GO/GCE in 0.1 M PBS (pH 7.0) containing 150.0 µM chlorpromazine at various scan rates; numbers 1-14 correspond to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400 and 500 mV s^{-1}, respectively. Inset: variation of anodic peak current (Ipa) *vs.* $v^{1/2}$.

solution at different pH values (pH 3.0-9.0). A stable peak was obtained for chlorpromazine in different pH values. Oxidation peak potential of the chlorpromazine at GO/GCE is independent of pH, meaning that the voltammetric behavior of chlorpromazine is not a proton transfer process under experimental conditions (Fig. 4). Furthermore, the peak current was found to be dependent on the pH and higher peak currents are observed at pH 7.0. Therefore, phosphate buffer with pH = 7.0 was selected and used as the supporting electrolyte in all voltammetric determinations (Fig. 5).

Electrochemical Profile of the Chlorpromazine on the GO/GCE

Figure 6 illustrates the cyclic voltammograms of a 100.0 µM chlorpromazine obtained using the GO/GCE (Curve a) and an unmodified GCE (Curve b). It can be noticed that Ep for chlorpromazine occurs at 640 mV in the case of GO/GCE that is around 80 mV more negative than that observed in the case of the unmodified GCE. These results show that GO can act very well in modification of the electrode surface. The increasing in the current shows increasing in the surface area of the electrode. Also, negative shift in oxidation potential shows acceleration in electron transfer at the surface of GO/GCE. Also, curve c shows the GO/GCE in 0.1 M PBS in the absence of chlorpromazine. As can be seen, GO/GCE in the absence of chlorpromazine did not show any peak.

Effect of Scan Rate on the Results

Figure 7 illustrates the effects of potential scan rates on the oxidation currents of chlorpromazine, indicating that increasing the scan rate increased the peak currents. Also, based on the fact that the plot of Ip against the square root

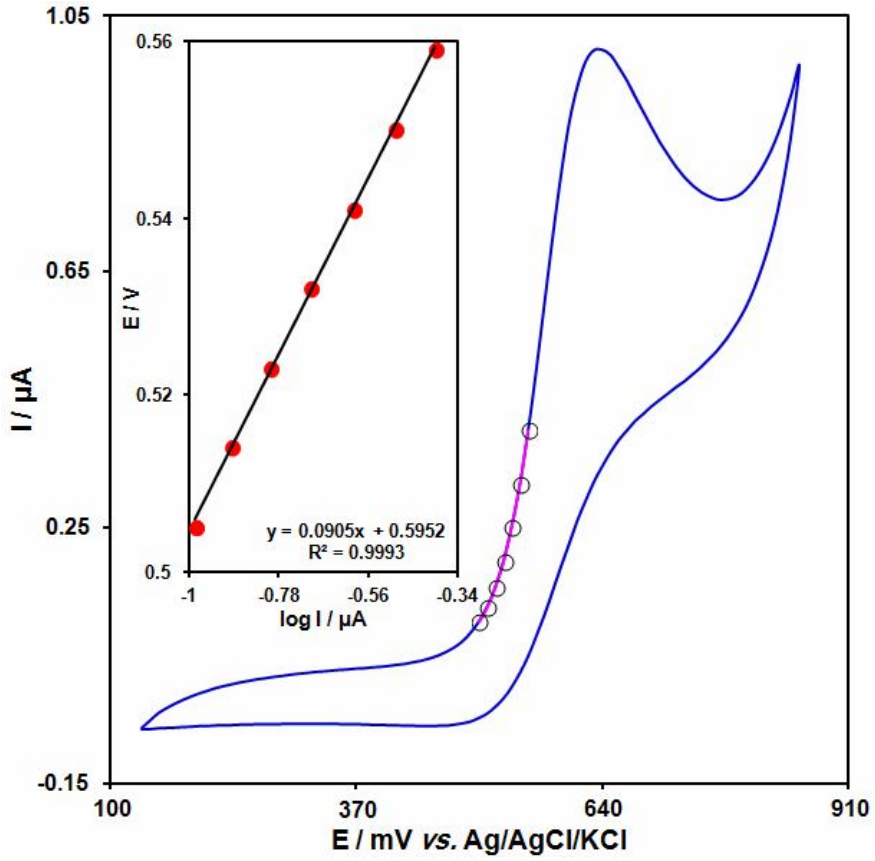

Fig. 8. CV (at 10 mV s^{-1}) of electrode in 0.1 M PBS (pH 7.0) containing 150.0 µM chlorpromazine. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the CV.

of the potential scan rate ($v^{1/2}$) for chlorpromazine was linear, it was concluded that the oxidation process is diffusion controlled.

Further Tafel curve of chlorpromazine was plotted using the data from the rising sections (*i.e.*, the Tafel regions) of the current-voltage curves obtained at 10 mV s^{-1} (Fig. 8). The Tafel regions of the current potential curves are influenced by the electron transfer kinetics of the electrode reactions. The results showed Tafel slope of 0.0905 V, indicating one electron (Fig. 5) rate determining step (RDS) for the electrode process [41] for charge transfer coefficient (α) of 0.35.

Chronoamperometric Analyses

The chronoamperometric analyses of the chlorpromazine samples using the GO/GCE were performed at 0.7 V *vs.* Ag/AgCl/KCl (3.0 M) and the results obtained for the different chlorpromazine samples in PBS (pH 7.0) are illustrated in Fig. 9. For chronoamperometric analysis of electroactive materials under mass transfer limited conditions, the Cottrell equation [41]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

where D is the diffusion coefficient (cm^2 s^{-1}), and C_b is the bulk concentration (mol cm^{-3}). Experimental plots of *I vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of chlorpromazine (Fig. 9A). The slopes of the resulting straight lines were then plotted *vs.* chlorpromazine concentration (Fig. 9B). From the resulting

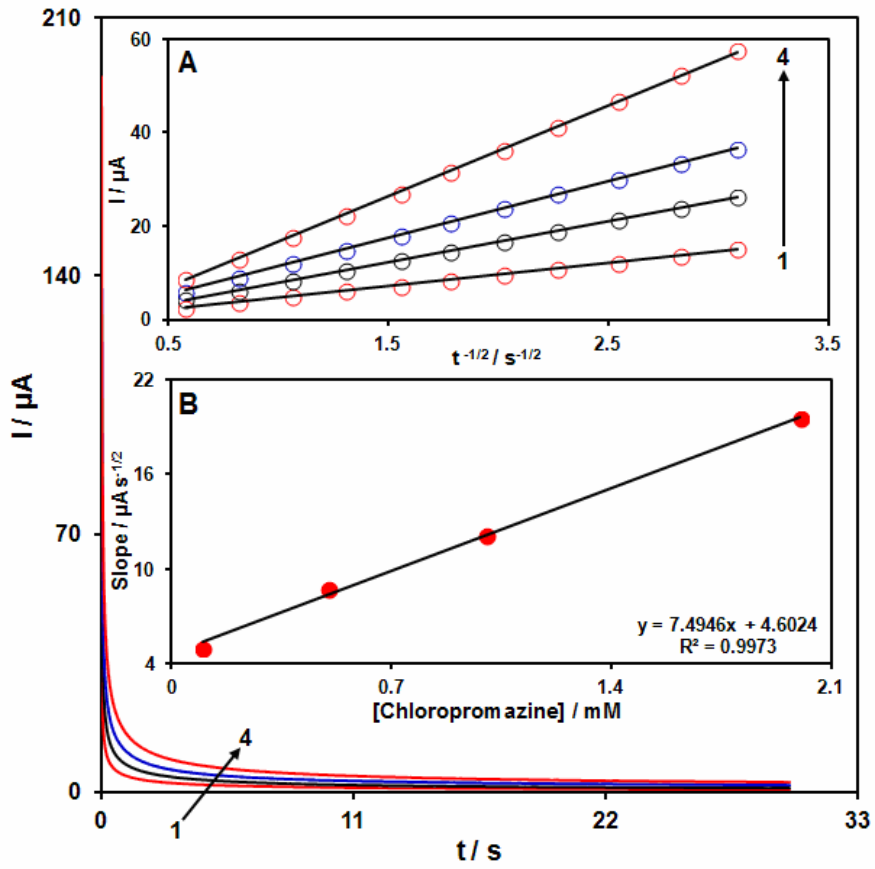

Fig. 9. Chronoamperograms obtained at GO/GCE in 0.1 M PBS (pH 7.0) for different concentrations of chlorpromazine. The numbers 1-4 correspond to 0.1, 0.5, 1.0 and 2.0 mM of chlorpromazine. Insets: (A) Plots of I *vs.* $t^{-1/2}$ obtained from chronoamperograms 1-4. (B) Plot of the slope of the straight lines against chlorpromazine concentration.

slope and Cottrell equation the mean value of the D was found to be 1.92×10^{-5} cm^2 s^{-1}.

Calibration Curves

The peak currents obtained for chlorpromazine using the GO/GCE were used for the quantitative analysis of chlorpromazine in PBS. Given the advantage of differential pulse voltammetry (DPV) in terms of improved sensitivity and better characteristics for analytical applications, the modified electrode was used as the working electrode in DPV (Initial potential = 0.4 V, End potential = 0.8 V, Step potential = 0.002 V, Modulation Amplitude = 0.02505 V) analyses in the range of chlorpromazine solutions in 0.1 M PBS and the results (Fig. 10) show that there is a linear relation between the peak currents and concentrations of chlorpromazine over the concentration range of 0.05-200.0 µM (with a correlation coefficient of 0.9989) and a detection limit (3σ) of 42.0 nM was obtained. These values are comparable with those reported by other research groups for the determination of chlorpromazine at the surface of modified electrodes (see Table 1).

The Repeatability and Stability of the GO/GCE

The long term stability of the GO/GCE was tested over a 3 week period. When CVs were recorded after the modified electrode was stored in atmosphere at room temperature, the peak potential for chlorpromazine oxidation was unchanged and the current signals showed less than 2.5% decrease

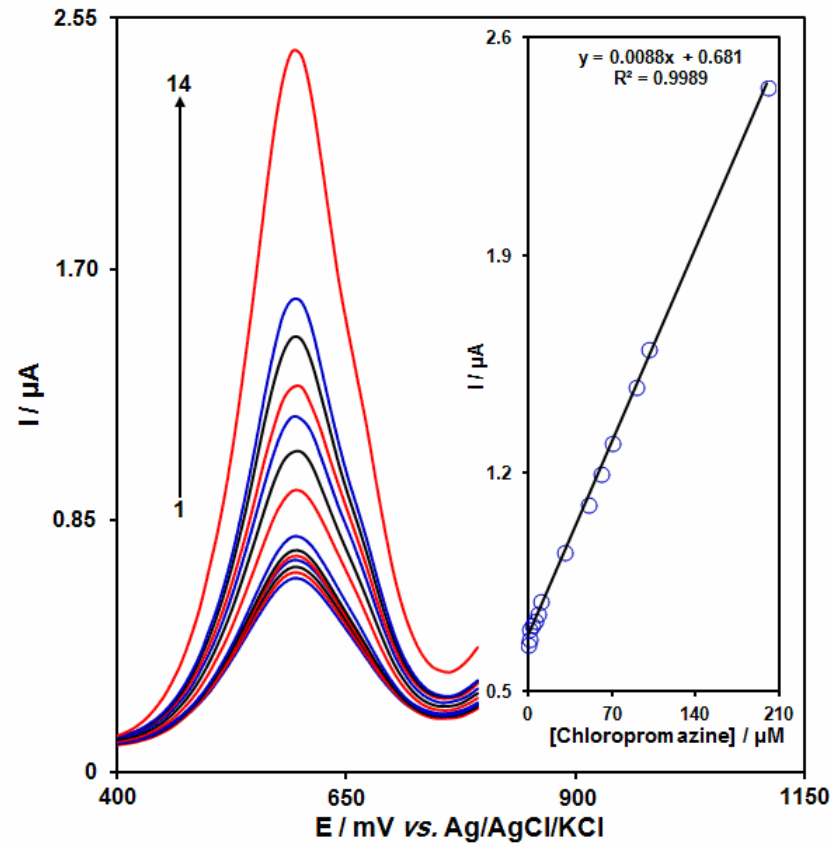

Fig. 10. DPVs of GO/GCE in 0.1 M (pH 7.0) containing different concentrations of chlorpromazine. Numbers 1-14 correspond to 0.05, 0.1, 1.0, 2.5, 5.0, 7.5, 10.0, 30.0, 50.0, 60.0, 70.0, 90.0, 100.0 and 200.0 µM of chlorpromazine. Inset: Plot of the peak current as a function of chlorpromazine concentration in the range of 0.05-200.0 µM.

relative to the initial response. The antifouling properties of the modified electrode toward chlorpromazine oxidation and its oxidation products were investigated by recording the CVs of the modified electrode before and after use in the presence of chlorpromazine. CVs were recorded in the presence of chlorpromazine after having cycled the potential 17 times at a scan rate of 50 mV s^{-1}. The peak potentials were unchanged and the currents decreased by less than 2.3%. Therefore, at the surface of the GO/GCE, not only does the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decrease.

Interference Studies

In some cases, the interference of foreign compounds can be overcome by using the oxidation peak for determination. The effects of inorganic ions and organic compounds commonly existed in pharmaceuticals and biological samples on the determination of 20 µM chlorpromazine were studied. The tolerance limit was defined as the concentration ratio of additive/ chlorpromazine causing less than ±5.0% relative error.

According to the results, Mg^{2+}, Fe^{2+}, Co^{2+}, Cl^{-}, SO_4^{2-}, Br^{-}, glycine, glucose, sucrose, fructose, valine, aspartic acid, urea, aspirin, uric acid, acetaminophen, cysteine, ascorbic acid and saturated starch solution did not show interference in determination of chlorpromazine.

Analysis of Real Samples

To assess the applicability of the modified electrode for the determination of chlorpromazine in real samples, the

Table 1. Comparison of the Efficiency of some Methods Used in Detection of Chlorpromazine

Method	LOD	LDR	Ref.
Electrochemistry	0.0018 μM	0.03-967.6 μM	[4]
Electrochemistry	0.6 nM	0.002-1.0 μM	[17]
Electrochemistry	0.4 μM	2.0-1000.0 μM	[42]
Electrochemistry	0.03 μM	1.0-130.0 μM	[43]
Electrochemistry	5.16 μM	10.0-500.0 μM	[44]
Electrochemistry	3.1 nM	0.01-3.0 μM	[45]
Electrochemistry	0.1 μM	0.6-10.0 μM	[46]
Fiber liquid phase microextraction followed by high-performance liquid chromatography	0.5 μg l^{-1}	1-500 μg l^{-1}	[47]
Tandem dispersive liquid–liquid microextraction followed by high performance liquid chromatography	1.0 ng ml^{-1}	5-3000 ng ml^{-1}	[48]
Electrochemistry	0.042 μM	0.05-200.0 μM	This work

Table 2. The Application of GO/GCE for Determination of Chlorpromazine in Chlorpromazine Tablet and Urine Samples (n = 5). All Concentrations are in μM

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Chlorpromazine tablet	0	5.0	-	3.2
	2.5	7.6	101.3	1.8
	7.5	12.2	97.6	2.9
	12.5	18.1	103.4	2.4
	17.5	22.3	99.1	3.1
Urine	0	-	-	-
	5.0	4.9	98.0	3.4
	10.0	10.3	103.0	1.9
	15.0	15.2	101.3	2.3
	20.0	19.8	99.0	2.8

described method was applied to the determination of chlorpromazine in chlorpromazine tablets and urine samples. For the purpose of this analysis the standard addition method was used and the results are given in Table 2. The observed recoveries of chlorpromazine were satisfactory and the reproducibility of the results was demonstrated based on the mean relative standard deviation (R.S.D.).

CONCLUSIONS

Glassy carbon electrode was modified with graphene oxide (GO/GCE). This electrode has shown very good activity towards the generation of electrochemical oxidation signal of chlorpromazine because of unique physicochemical properties of graphen oxide such as high specific surface area and extraordinary electronic properties. The anodic peak currents (I_{pa}) showed good linear correlation with the concentration of chlorpromazine. Spike recoveries test of chlorpromazine was successfully carried out in real samples.

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