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Amperometric determination of promethazine in tablets using electrochemically reduced graphene oxide modified electrode

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Abstract

Graphene films were prepared on a glassy carbon electrode for amperometric determination of promethazine hydrochloride in pharmaceutical products. This modified sensor was prepared by chemical oxidation of graphite powder followed by product exfoliation in ultrapure water by ultrasonic bath. Then, resulting graphene oxide was electrochemically reduced in 0.10 mol L^{-1} acetic acid/sodium acetate (pH = 5.0) on a glassy carbon electrode surface. The proposed sensor exhibited reproducible amperometric responses for wide linear range from 1.99 x 10^{-6} to 1.03 x 10^{-3} mol L^{-1} at +0.78 V (*vs.* Ag/AgCl). Low detection and quantification limits (1.99 x 10^{-7} mol L^{-1} and 6.63 x 10^{-7} mol L^{-1}, respectively) were achieved. This method was applied to the analyses of commercial tablet samples and all results were in good agreement with those obtained using spectrophotometry and high-performance liquid chromatography.

Introduction

Graphene is composed by a two-dimensional structure with a single atomic layer of sp^2-hybridized carbon atoms and has the same thickness as a one carbon atom, with crystalline hexagonal configuration.[1] It was firstly isolated by mechanical exfoliation of graphite and visualized under an optical microscope by Geim *et al.* in 2004.[2]

This new form of carbon arrangement has attracted enormous attention from different scientific fields due to its exceptional properties, such as high surface area, chemical inertness, optical transmittance, high current density and high electrical and thermal conductivities.[3] Because of its ability to promote fast electron transfer, it provides new opportunities to be used as an electrode material. However, the development of strategies for large-scale graphene production with high quality has been challenging since its discovery. Among some, the most reported involves soft chemistry routes focused on the oxidation and exfoliation of graphite followed by a reduction step which are typically used to prepare graphene-related materials such as graphene oxide (GO) and reduced graphene oxide (rGO).[1] GO exhibits an excessive number of oxygen-containing functional groups which makes it an electrically insulating material. In this matter, a reduction step is necessary to eliminate some oxygenated functionalities and restore the conjugation to graphene structure.

Among all methods used to produce rGO films from graphene oxide the electrochemical reduction is very simple, fast and low cost, while other methodologies have been using toxic reducing agents or high temperature operating routes.[4] Moreover, the use of high negative potentials along the reduction process can overcome the energy barriers for the reduction of oxygen functionalities found on the basal plane and the

edge.[11] As a consequence, GO can be efficiently reduced and subsequently applied for electrochemical quantification of different analytes.[5-10]

From the viewpoint of the electron and molecular structure perspective, it is a consensus that compounds of the phenothiazine group can be easily oxidized at graphene film surface. These drugs have a tricyclic aromatic ring with sulfur and nitrogen atoms, and different substituents attached at the 2 and 10 or 3 and 7 positions.[12] There are different electrode surfaces which are already been used as detectors for phenothiazine derivatives detection, such as boron-doped diamond,[13] nanodiamond modified with Ag particles,[14] gold,[15,16] carbon nanotubes,[17-19] glassy carbon,[20] FTO modified with $SiPy^{+}Cl^{-}$ and CuTsPc film,[21] and modified carbon paste electrodes.[22,23] To the best of our knowledge, the amperometric detection of promethazine hydrochloride using a rGO modified sensor is reported here for the first time.

Promethazine hydrochloride, (2RS)-N,N-dimethyl-1-(10H-phenothiazin-10-yl)propan-2-amine hydrochloride, is a neuroleptic agent which has sedative and antipsychotic effects.[13] In the following sections, the use of rGO modified glassy carbon electrode as an amperometric sensor for promethazine hydrochloride determination in pharmaceutical products is described. The proposed sensor was prepared just by a simple electrochemical reduction of GO on the electrode surface.

Experimental

Reagents and Solutions

Graphite oxide was prepared from natural graphite (powder with 99.80 % purity from Nacional Grafite Ltda., Minas Gerais, Brazil) using a modified Hummers method.[24,25] Promethazine hydrochloride standard was acquired from Sigma-Aldrich (St. Louis, Mo, USA). All reagents were of analytical grade and used as received.

Sulfuric acid, acetic acid, sodium acetate, sodium hydroxide, boric acid, hydrochloric acid, acetonitrile and potassium mono-hydrogen phosphate were purchased from Merck (Darmstadt, Germany). All solutions were prepared with ultrapure water from a Millipore Milli-Q system with resistivity ≥ 18.2 MΩ cm (Barnstead, Dubuque, IA, USA). A promethazine hydrochloride stock solution (0.10 mol L^{-1}) was prepared by dissolving the solid salt in ultrapure water, which was later stored in a dark flask and under refrigeration. Analyte standard solutions were properly diluted with supporting electrolyte just before measurements. Commercial pharmaceutical products (*Fenergan®* - tablets, from Sanofi-Aventis Farmacêutica Ltda) from different fabrication lots (09/2013 and 01/2014) were purchased from a local drugstore.

The influence of two different supporting electrolytes (Britton-Robinson (B-R) buffer (0.10 mol L^{-1}, pH 2-7) and sulfuric acid solution (0.10 mol L^{-1})) for promethazine hydrochloride oxidation was evaluated by cyclic voltammetry. Sulfuric acid produced better results and was adopted along the main measurements.

A 0.10 mol L^{-1} acetic acid/sodium acetate (pH = 5.0) solution was used for the electrochemical reduction of GO film deposited over the glassy carbon surface. This solution was prepared by mixing 0.18 mol L^{-1} sodium acetate and 0.10 mol L^{-1} acetic acid.

Instrumentation

Cyclic voltammetry and amperometry: All measurements were performed using an EcoChemie PGSTAT-20 potentiostat (EcoChemie, The Netherlands) connected to a conventional 5 mL electrochemical cell. The rGO modified glassy carbon was used as the working, a platinum wire as the auxiliary and $Ag/AgCl_{(KClsat)}$ as the reference electrode.

Characterization techniques: Scanning electron microscopy (SEM) images were obtained with a JSM-7401F field emission scanning electron microscope (FESEM, JEOL Ltd., Japan). Transmission electron microscope (TEM, Tecnai G2-Spirit-2006 from FEI Co., OR, USA) was used to observe the surface morphology. Raman spectra were recorded using an iHR 550 Raman spectrometer (Horiba Jobin Yvon Ltd., France) with excitation made by an argon-ion laser beam (λ = 514 nm and 1.0 mW) and with a BX-41 confocal microscope (Olympus, 50 x objective). X-ray diffraction (XRD) experiments were carried out with an Ultima IV diffractometer (Rigaku, Japan) using Cu Kα (λ = 1.5406 Å) radiation.

Comparison techniques: Two methodologies (spectrophometry and high-performance liquid chromatography) were used to compare the results obtained by the proposed method. Absorbance measurements were carried out at 249 nm, using a model 8453 spectrophotometer (HP Co., CA, USA) equipped with a D_2 lamp and using a 1.00 cm optical path quartz cuvette. The chromatograms were recorded on a LC-10 AS chromatograph (Shimadzu Co., Japan) equipped with an UV-Vis wavelength spectrophotometer operated at the previously described wavelength (249 nm) and equipped with a Phenomenex C18 chromatographic column (4.6x250 mm, 4µm particle size).

Preparation of GCE modified with rOG

Glassy carbon electrode (GCE, geometric area = 0.071 cm^2) was polished with 0.3 and 0.05 µm alumina slurry, followed by through rinsing with ultrapure water and sonication for 3 min in ethanol medium. The graphite oxide (from Nacional Grafite Ltda.) was dispersed in ultrapure water (0.5 mg mL^{-1}) and exfoliated to GO by

ultrasonic bath for 2 h. Next, it was centrifuged at 7000 rpm for 20 min and the excess (mainly the unexfoliated GO and unoxidized graphite) was removed. A 6 µL GO solution (volume used to ensure coverage of the GCE area) was transferred to the GCE surface with a micropipette, and later allowed to dry at room temperature.[26] Reduction of the GO film was performed in a 0.10 mol L^{-1} acetic acid/sodium acetate solution (pH = 5.0) by successive potentials scanning (30 voltammetric cycles, from 0 V to -1,5 V , *vs* Ag/AgCl) at a scan rate of 50 mVs^{-1}.[27] The so modified GCE (rGO-GCE) was used for all of the following voltammetric experiments involving the determination of promethazine hydrochloride.

Sample preparation

Amperometry: For the quantification of promethazine hydrochloride in pharmaceutical products, 3 tablets of each sample were weighed and an average mass per tablet was determined. Tablets were finely powdered and a portion of the powder, which was equivalent to 28.2 mg of promethazine hydrochloride, was transferred to a 100 mL volumetric flask, which was then filled to its mark with electrolyte (0.10 mol L^{-1} H_2SO_4 solution). The resulting solution was exposed to ultrasonic bath for 2 min and filtered through paper. An 500 µL aliquot of this solution was transferred to an electrochemical cell containing 4.50 mL of electrolyte. The analyte content at each tablet was determined using the standard addition method.

Spectrophotometry: Spectrophotometric experiments were performed following the procedure described in the British Pharmacopoeia.[28] About 50 mg macerated tablets were weighed and dissolved in 10.0 mL of 2.0 mol L^{-1} hydrochloric acid solution. These samples were stirred for 15 minutes and diluted with ultrapure water up to 100 mL.

From each solution, aliquots of 50 mL were centrifuged (5000 rpm for 10 minutes) and in sequence, 5 mL of the supernatant was mixed with 10 mL of 0.2 mol L^{-1} hydrochloric acid. This final solution was then diluted to 100 mL with ultrapure water.

HPLC analysis: High performance liquid chromatography analyses of the commercial samples were performed following a methodology described in the literature.[29] About 15 mg of macerated tablets were weighed and dissolved in 10.0 mL of methanol. The samples were centrifuged at 5000 rpm over 15 minutes. An 200 µL aliquot of supernatant was transferred to a 10.0 mL volumetric flask and dissolved in the mobile phase (0.025 mol L^{-1} K_2HPO_4 solution adjusted to pH 7 and acetonitrile, in a proportion of 50:50). Then, 50 µL of the resulting solution was injected into the chromatograph.

Results and discussion

Production of rGO-GCE

Graphite powder was oxidized in acidic medium according to the modified Hummers method and the formed product (graphite oxide) was then exfoliated in ultrapure water to obtain GO (methodology). Graphite oxide has a layered structure, similar to graphite but it presents oxygen based functional groups on both basal planes and edges which expands the interlayer distance, as well as the atomic-thick layers hydrophilicity. As consequence, these oxidized layers can be exfoliated in water or polar organic media by ultrasonic bath producing only one or few layers of carbon atoms similar to graphene. Figure 1 presents a scanning electron microscopy (SEM) and a transmission electron microscope (TEM) of the obtained GO surface.

INSERT FIGURE 1

Figure 1 displays the SEM images of GCE (A) with its typical glazed surface and GCE modified with GO (B). It is possible to observe a typical wrinkled structure (which arises from the π-π interaction between graphene sheets) with plenty of corrugations in Figure 1B. Moreover, this figure is in accordance with previous studies reported in the literature.[30] As shown at TEM image (Figure 1C), GO surface has a silk veil-like waved structure with thin and wrinkled sheets, revealing the random overlay of the individual sheets.

Raman spectroscopy is a technique widely used for the characterization of sp^2 and sp^3 hybridized carbon atoms at carbon-based materials, in manner to distinguish the order and disorder/defect structures. X-ray diffraction (XRD) is other nondestructive characterization technique used to obtain typical d-spacing (interlayer distance of the (002) peak) measurements. In this study, both techniques were used to confirm the material oxidation. Raman spectra (A) and XRD patterns (B) of both graphite (a) and GO (b) are shown in Figure 2.

INSERT FIGURE 2

Figure 2A shows a prominent peak at 1586 cm^{-1} and a weak peak at 1356 cm^{-1} which corresponds to the G and D bands of raw graphite, respectively (a). The presence of D band for graphite indicates that there are a considerable number of defective sites within the structure (sp^3-hybridized carbons). However, a significant increase in the intensity of D band and a shift of G band to 1598 cm^{-1} were observed for GO (b), indicating that a large number of sp^2- hybridized carbons have been converted to sp^3-

hybridization through the chemical oxidation. The relative intensity ratio of D and G bands (I_D/I_G) is usually considered proportional to the number of defective sites in the material.[30] The I_D/I_G value for GO was of 1.06, it is higher than the one found for raw graphite (~0.09), which indicates that there are significant edge-plane-like defective sites existing on the material surface due to the oxidation step.[25]

XRD patterns of raw graphite (a) and GO (b) are shown in Figure 2B. The diffraction (002) peak of graphite was centered at $2\theta = 26.5°$ (a) with interlayer d-spacing of 0.336 nm. After chemical oxidation of graphite the (002) peak disappeared, but a new peak at $2\theta = 11.6°$ (b) with d-spacing of 0.756 nm is observed. This indicates the generation of oxygen based functional groups at the graphite interlayer spaces.[30] From these results, GO film was deposited on GCE surface and then, electrochemically reduced in 0.10 mol L^{-1} acetic acid/sodium acetate solution (pH = 5.0).

Figure 3 shows successive scanning for the electrochemical reduction of GO, in a potential range from 0 to -1.5 V (*vs.* Ag/AgCl), recorded over a scan rate of 50 mV s^{-1}.

INSERT FIGURE 3

The cyclic voltammograms of GCE modified with GO (Figure 3) show a large cathodic peak at -0.71 V on the first cycle likely due to the reduction of the oxygen based functional groups present at the extremities of GO structures, since reduction of water to hydrogen occurs at more negative potential (about -1.5 V).[10] On the 2nd cycle, the reduction peak decreased considerably and disappeared in the following cycles. No difference could be observed between the 20th and 30th cycles. This voltammetric result demonstrated that the reduction step occurred quickly and irreversibly and GO

could be reduced electrochemically on the GCE surface without the use of toxic reagents that would have been required in the chemical reduction of GO.

Voltammetric studies of promethazine at rGO-GCE

The electrochemical behavior of promethazine hydrochloride was investigated at bare and modified GCE electrode. Initial studies were carried out with 1.0 x 10^{-3} mol L^{-1} of analyte in 0.10 mol L^{-1} H_2SO_4 solution. This electrolyte was chosen based on the fact that many phenothiazine compounds, including promethazine, can easily oxidize in acidic medium[15]. The cyclic voltammograms recorded at bare GCE (a) and rGO-GCE (b) are compared in Figure 4. For both sensors, it was possible to observe two oxidation waves corresponding to the formation of a cation (at +0.72 V (a) and +0.70 V (b)) and a dication radical (at +0.95 V (a) and +0.90 V (b)) during the forward sweep.

INSERT FIGURE 4

The inset of Figure 4 shows the proposed mechanism for the electrochemical reaction of promethazine in acid medium. The first process corresponds to the reversible electrooxidation (reduction at + 0.64 V) of the analyte nitrogen atom (situated at the ring) and the second anodic peak is related to the irreversible electrooxidation of the sulfur atom associated with a coupled hydrolysis reaction.

Comparing the oxidation and reduction peaks, the small magnitude of current at the reduction process can be attributed to the fact that promethazine radical is fairly stable on a voltammetric time scale and at low pH value. For higher scan rates the difference between the cathodic and anodic signals decreases (see Figure 5). These results are in agreement with those reported by Sackett *et.al.*[31] However, rGO-GCE

appears to be a more favorable surface for the analyte oxidation and reduction processes, considering the small displacement observed between the anodic and cathodic peaks. The anodic current magnitude at the rGO-GCE was greater than the one at the bare GCE, probably due to the surface area increase by the presence of graphene film.

Other voltammetric studies, for a 1.0 x 10^{-3} mol L^{-1} promethazine solution (not shown here), involving a 0.1 mol L^{-1} B-R buffer (in a pH range over 2.0 and 7.0) were also carried out. The results showed that increasing the pH value, makes the second peak shift to less positive potential, until a critical point and above (pH 3) where higher values made the two oxidation peaks coalesced. Therefore, it was observed a single oxidation peak at the first scan and two new couples at the second scan, implying in the formation of new oxidation products, different from the ones formed during first scan. Moreover, slight peak current decrease (around 2 %) was observed after 11 cycles at pH 2.0. At pH 7.0 this decrease was far more significant (42 %) when compared with the initial current value. The reason for this current signal decrease is not obvious but probably at this pH the products generated during the oxidation process remains on the rGO-GCE surface, partially blocking it. Based on these results and considering the good repeatability obtained in 0.10 mol L^{-1} H_2SO_4 solution (RSD = 1.82 % for 11 scans), this electrolyte was selected for all the experiments performed in the following sections.

Figure 5A presents cyclic voltammograms corresponding to successive additions of promethazine in 0.10 mol L^{-1} H_2SO_4 at rGO-GCE, with scan rate of 100 mV s^{-1}. The current signals obtained for each concentration of analyte were very stable and reproducible. A very linear relationship between cathodic and anodic currents and promethazine concentration (from 2.49 to 14.8 x 10^{-4} mol L^{-1}) was observed (inset of Figure 5A). Figure 5B shows the variation of cathodic and anodic currents with scan

rate from 0.01 to 0.8 V s^{-1}, using a 1.0 x 10^{-3} mol L^{-1} promethazine solution in 0.10 mol L^{-1} H_2SO_4 medium. An increase in the height of the oxidation and reduction peaks occurred and anodic peak potential shifted towards more positive values. The same was not so pronounced for cathodic peak potential. Moreover, the cathodic and anodic current values varied linearly with the square root of the scan rate (inset of Figure 5B).

INSERT FIGURE 5

Determination of promethazine hydrochloride

The effect of anodic working potential (from +0.70 to +0.80 V, *vs.* Ag/AgCl, not shown here) was investigated in order to find the most suitable condition for amperometric quantification of promethazine in pharmaceutical products. During the amperometric experiments in 0.10 mol L^{-1} H_2SO_4, +0.78 V was selected as the working potential based on the best compromise between sensitivity and reproducibility.

Under the optimized conditions (electrolyte: 0.10 mol L^{-1} H_2SO_4 and working potential: +0.78 V), a series of experiments were performed using promethazine standard solutions at different concentrations, in order to build the analytical curve. Linearity between oxidation current and analyte concentration was observed over a wide range, from 1.99 x 10^{-6} to 10.34 x 10^{-4} mol L^{-1}. Figure 6A shows a series of amperometric responses obtained in one of these experiments. The dynamic range presented a slope of 6.28 ± 3.67 x 10^{-2} mA mol^{-1} L and an intercept of 1.68 x 10^{-1} ± 1.62 x 10^{-2} μA, with a correlation coefficient of 0.999. The estimated detection limit was 1.99 x 10^{-7} mol L^{-1} (S/N=3) and the quantification limit was calculated as 6.63 x 10^{-7} mol L^{-1}.

The so prepared rGO-GCE sensor was used for the determination of promethazine hydrochloride in tablet samples which contains also amide, lactose, sugar,

hydrated silica, talcum, magnesium stearate, methacrylate copolymer, polyethylene glycol, titanium dioxide and riboflavin. Parallel experiments involving 1 mM of promethazine and 5 mM amide, lactose, glucose or riboflavin showed that these compounds do not cause any interference under the established conditions. Figure 6B presents the results of successive additions of promethazine standard solution into the electrochemical cell containing a commercial pharmaceutical sample dissolved in 0.10 mol L^{-1} H_2SO_4. The inset of this figure depicts a good linear relationship between successive additions of analyte standard solution (from 1.99 to 9.80 x 10^{-4} mol L^{-1}) and amperometric signals. Values of promethazine hydrochloride concentration in tablet samples were obtained by extrapolating the data of the standard addition plots which corresponds to the current variation of the added promethazine.

INSERT FIGURE 6

The results obtained using the proposed method were compared with those from the spectrophotometric and high-performance liquid chromatography procedures.[29] Spectrophotometry is the technique recommended by the British Pharmacopoeia for promethazine determination.[28] Table 1 presents these results together with the corresponding standard deviations calculated from three independent measurements for each sample.

INSERT TABLE 1

As presented in Table 1, results found by the proposed method are very close to the labeled value (28.2 mg) with a standard deviation of ±1.4 %. Moreover, these results were compared favorably with the values obtained for spectrophotometry[28] and

HPLC.[29] In the case of sample 1, the average value obtained by amperometry showed to be the same of that found by spectrophotometry and it was very close to the result obtained by HPLC. A significance test (null hypothesis) was applied to results showed in Table 1, resulting in experimental *t-values* of 1.00 (for amperometry and spectrophotometry) and 0.33 (for amperometry and HPLC). These values suggest there is no evidence of systematic errors for both situations (amperometry in comparison with spectrophotometric analysis and amperometry in comparison with HPLC analysis), considering 95 % of confidence interval and a critical *t-value* of 12.71.[32] The rGO-GCE sensor presented good analytical performance when compared with other carbon sensors modified with different agents for promethazine determination.[33,34] Recently, Primo *et al.* have reported the use of GCE modified with carbon nanotubes dispersed in DNA and, after 5 min of accumulation at an open circuit potential, they obtained a linear range from 1.0×10^{-7} to 6.0×10^{-6} mol L^{-1} and a detection limit of 2.3×10^{-8} mol L^{-1}. [34]

Conclusion

The results obtained in this work demonstrate the potentiality of a GCE modified with rGO sensor for the amperometric quantification of promethazine hydrochloride in pharmaceutical products. Low detection limit (1.99×10^{-7} mol L^{-1}) and a wide linear range (from 1.99×10^{-6} to 1.03×10^{-3} mol L^{-1}) with good repeatability were achieved along all amperometric experiments. Moreover, the fabrication process of the proposed sensor includes a simple electrochemical reduction of GO, avoiding the use of an excess of reducing agents that could contaminate the resulting material, as occurs when chemical reduction is chosen. Finally, this amperometric method allows rapid, simple, accurate and precise analysis without any sample pretreatment such as extraction or derivatization, making this methodology very suitable for quality control applications.

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Captions

Figure 1 – SEM images of (A) GO modified GCE and (B) bare GCE and (C) TEM image of GO.

Figure 2 – (A) Raman spectra obtained for (a) graphite and (b) GO. (B) XRD patterns of (a) graphite and (b) GO.

Figure 3 – 30 sucessive cyclic voltammograms recorded for GO reduction process in 0.10 mol L^{-1} acetic acid/sodium acetate solution (pH = 5.0) using GCE modified with GO. Scan rate, 50 mVs^{-1}. (Dotted line) 1st scan and (solid line) 2nd, 10th, 20th and 30th scans.

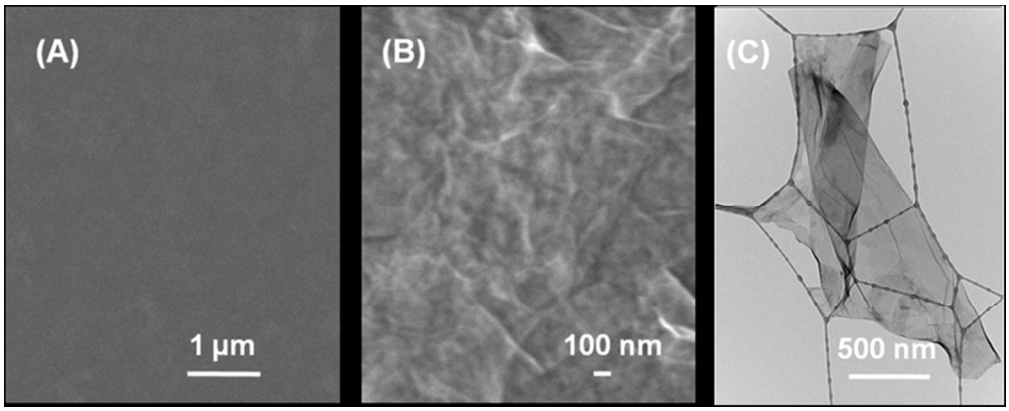
Figure 4 – Cyclic voltammograms recorded for bare (a) GCE and rGO-GCE (b) in 0.10 mol L^{-1} H_2SO_4 medium (dashed line) and in 1.0 x 10^{-3} mol L^{-1} promethazine solution (solid line). Scan rate, 100 mV s^{-1}. Inset shows the probable mechanism of promethazine oxidation.

Figure 5 – Cyclic voltammograms obtained with rGO-GCE in 0.10 mol L^{-1} H_2SO_4 for different: promethazine concentrations (A) (a-f, 2.49 to 14.8 x 10^{-4} mol L^{-1}) and (B) scan rates (0.01 to 0.8 V s^{-1}). For insets: (■) anodic and (●) cathodic currents.

Figure 6 – Amperometric response for: (A) successive additions of promethazine standard solution in 0.10 mol L^{-1} H_2SO_4 medium (1.99 x 10^{-6}-10.34 x 10^{-4} mol L^{-1}) and (B) promethazine analysis in commercial tablet sample using successive additions of standard solution (1.99-9.80 x 10^{-4} mol L^{-1}). E = +0.78 V.

Table

Table 1 – Results obtained after analysis of commercial tablet samples of promethazine hydrochloride by proposed amperometric method and the values found by spectrophotometry[28] and HPLC.[29] Labeled value: promethazine hydrochloride = 28.2 mg.



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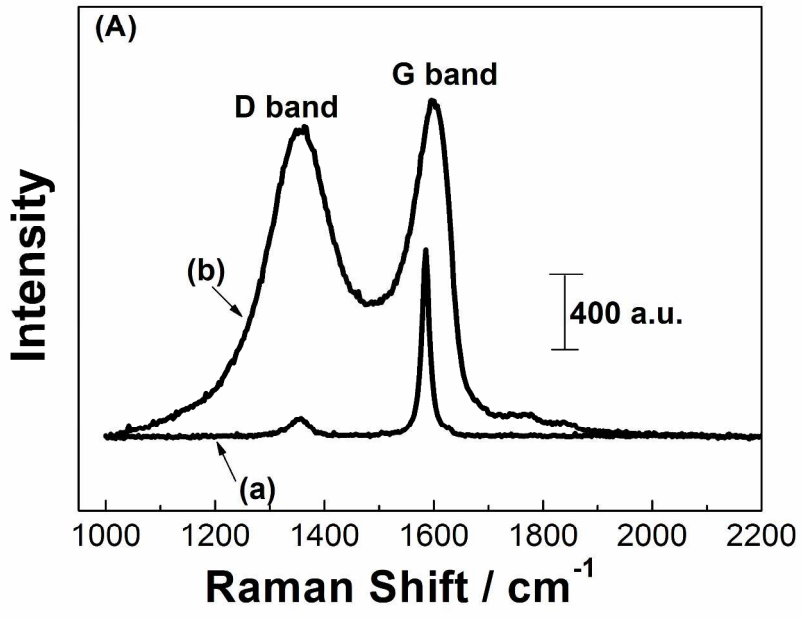

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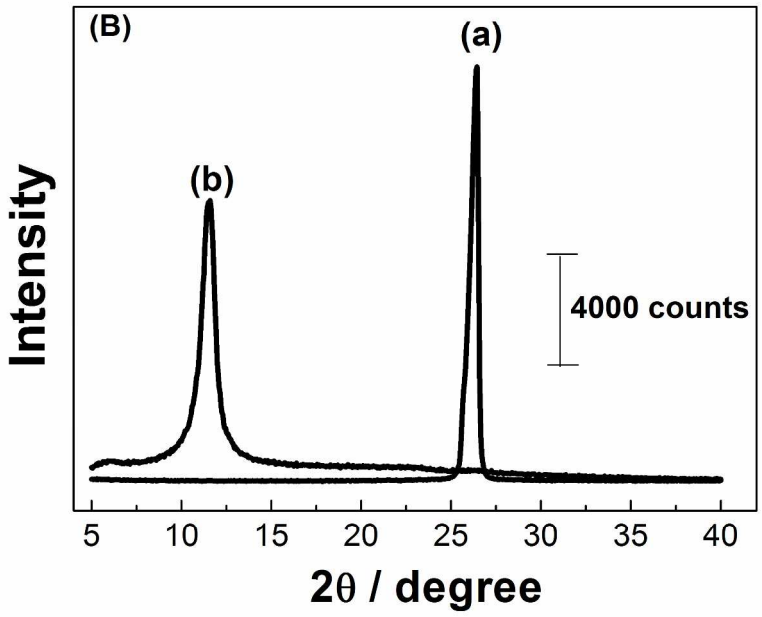

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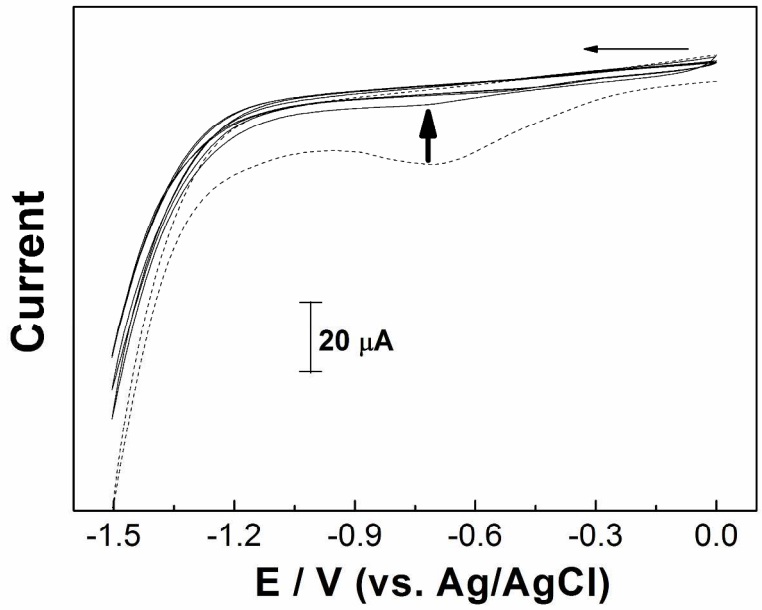

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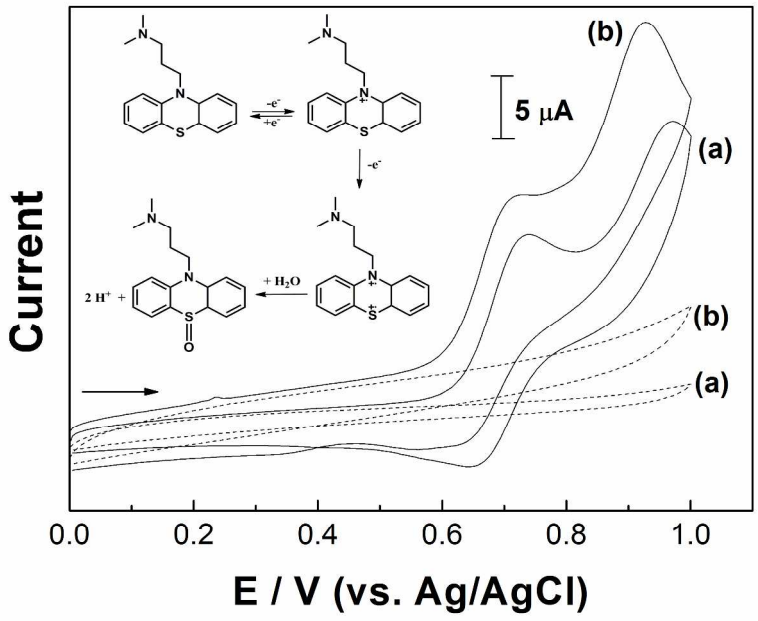

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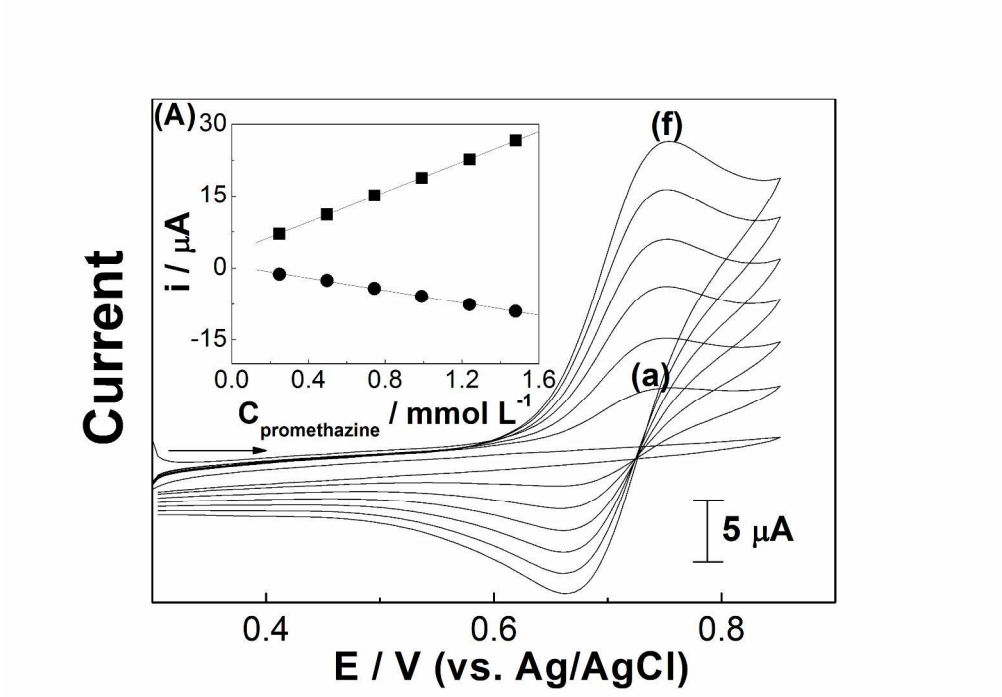

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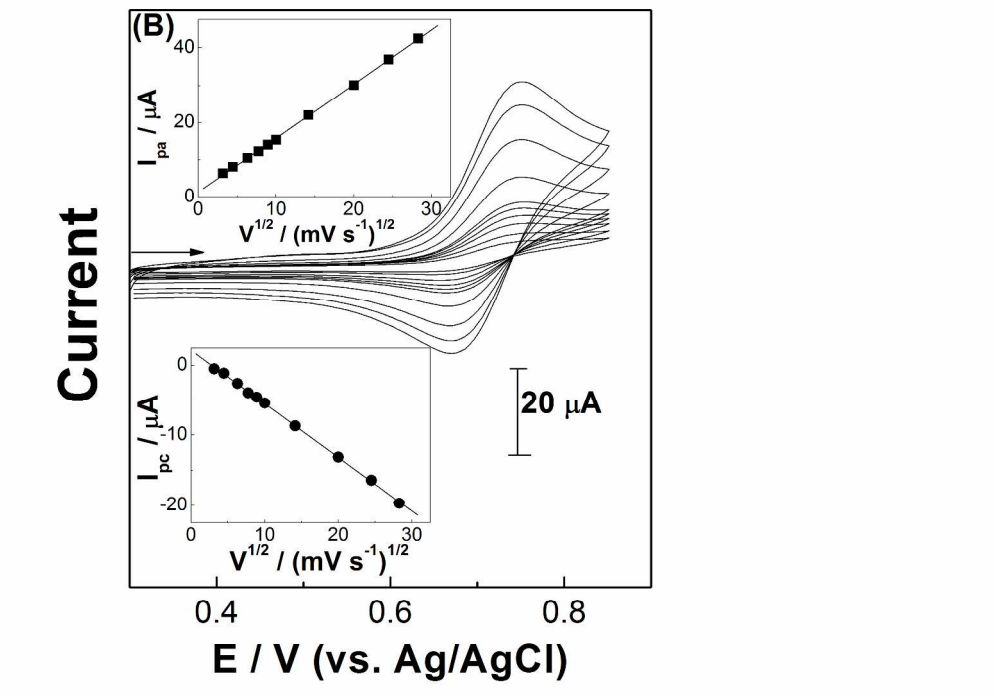

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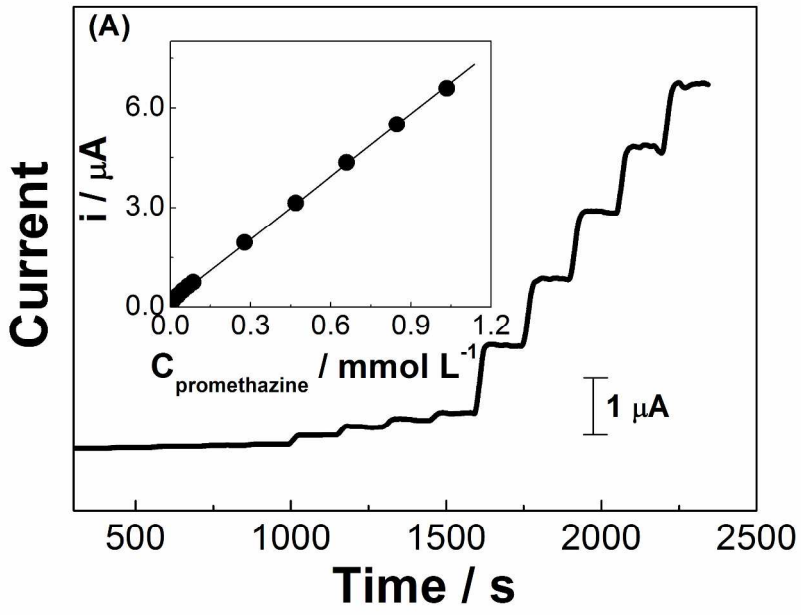

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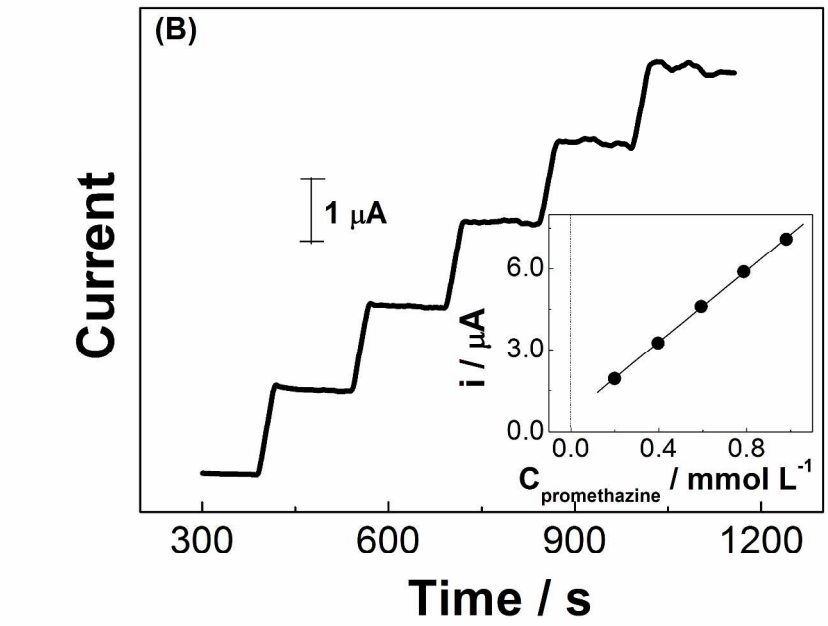
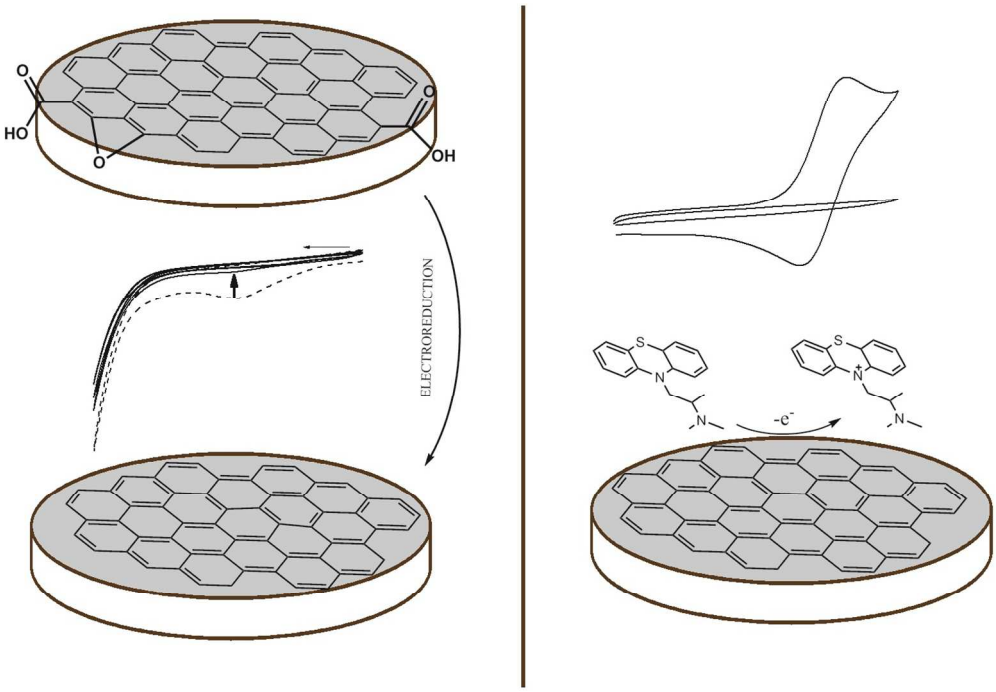


573x403mm (150 x 150 DPI)

Sample	Amperometry ± S.D.[a] (mg)	Spectrophotometry ± S.D.[a] (mg)	HPLC ± S.D.[a] (mg)
1	28.0 ± 0.4	28.0 ± 0.2	28.1 ± 0.6
2	28.6 ± 0.5	28.3 ± 0.2	28.4 ± 0.3

[a] Average ± standard deviation for three determinations.



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