Original Article

Electrochemical Sensor Based on Multiwall Carbon Nanotube-Paste Electrode for Determination of Promethazine in Pharmaceutical Formulations and Blood Plasma

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Abstract

A multi-walled carbon nanotube modified paste electrode (MWCN-PE) was used for determination of promethazine (PMZ) in drug formulations and blood plasma by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. Results revealed that the MWCN-PE shows an electrocatalytic activity toward the anodic oxidation of PMZ by a marked enhancement in the current response in buffered solution at pH 5.0. At the MWCN/CPE, the anodic peak potential of PMZ was shifted by 28.0 mV to more negative potential in comparison with bare carbon paste electrode. The detection limit for this method was 0.025 µmol L-1. Results indicated that the modified electrode could be employed for the determination of promethazine hydrochloride in pharmaceutical formulations and plasma samples.

Abbreviations: BHT-Butylated hydroxytoluene; TEAC-trolox-equivalent antioxidant capacity; AUC-area under the curve.

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Introduction

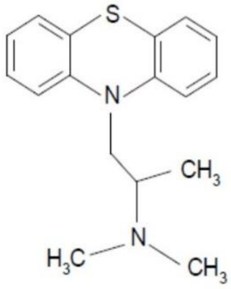
Promethazine hydrochloride, PMZ, (10-N, N-dimethyl-1-(10-H-phenothazine-10-yl) propan-2-amine hydrochloride or 10-H-phenothiazine-10-ethanamine, N, N, a-tri methyl-mono hydrochloride) (Scheme 1), which belongs to the phenothiazine group, is a pharmaceutical compound widely used for its antihistaminic, sedative, antipsychotic, analgesic and anti-cholinergic properties. However, promethazine hydrochloride can cause adverse effects in humans, such as endocrinal, cardiac and reproductive alterations. Therefore, its determination in commercial formulations and biological samples is extremely important (1, 2). Several methods have been reported for the determination of promethazine, including spectrophotometry (3), chemiluminescence (4) and turbidimetry (5). HPLC methods are now widely used for promethazine determination (6). However these methods require time-consuming sample extractions, expensive instrumentation and high-running costs (7). Electrochemical techniques have proved to be excellent alternatives to determine this and other pharmaceutical compounds, since they are simple, cost little and require relatively short analysis times, without the need for derivatization or time-consuming extraction steps (8).

As for the evaluation of the electrochemical behavior and quantification of promethazine by electro analytical techniques, several electrode materials such as gold electrode (2), glassy carbon electrode (9), wax impregnated graphite electrode,10 and modified carbon paste electrode,8 have already been used as working electrodes.

Carbon-pastes, prepared from carbon powder and a suitable water-immiscible binder, provide a suitable medium for incorporating modifiers into electrodes (11). CPEs have been applied in electrochemical studies and as electrochemical sensors for determination of various species (12-14). These electrodes offer advantages such as low background current (compared to solid graphite or noble metal electrodes such as gold electrode, born doped diamond electrode, etc.), easy preparation and use (against gold and glassy carbon electrodes that multi-stage cleaning process are needed), low cost, large potential window and simple surface renewal (15, 16). Moreover, the feasibility of incorporating different substances during the paste preparation, allows fabrication of electrodes with desired composition and therefore, with pre-determined properties (17, 18).

Carbon nanotubes (CNT) are one of the most promising nano-materials for a wide range of technological innovations. Applications of CNT in biosensors have been investigated widely in the past, due to its special properties (low resistance, large specific surface area, nano-size effect and high stability). Furthermore, the abilities of CNT to promote electron-transfer reactions have been attributed to their electronic structure and high electrical conductivity.

In this work we report construction and application of a MWCNT modified carbon paste electrode (MWCNT/CPE) as an amplified electrochemical sensor for the determination of PMZ in syrup pharmaceutical formulation and human blood serum samples. The performance of this sensor was compared with bare carbon paste electrode (bare CPE). The electrochemical behavior of PMZ at the modified electrode was investigated using voltammetry such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. According to the results, the MWCNT/CPE displayed a low detection limit and a high sensitivity to PMZ determination in real samples according to diffusion process (against other papers that are according to adsorbtion process with vary accumulation times) (19-21).



Scheme 1. The molecular structure of promethazine

Experimental particulars

Apparatus for electrochemical measurement

Voltammetric measurements were carried out using an AutolabPotentiostat/ Galvanostat PGSTAT30 (Eco Chemie, Utrecht, Netherlands) controlled by General Purpose Electrochemical Systems (GPES) software, which a conventional three-electrode system was adopted. The working electrode was multi-walled carbon nanotube incorporated to carbon paste electrode (MWCN/CPE) and the auxiliary and reference electrodes were platinum wire and Ag/AgCl (3.0 mol/L of KCl) electrode, respectively. A digital pH meter (Metrohm model 691) was used when preparing phosphate buffer solution (PBS) that served as the supporting electrolyte in the voltammetric experiments. The cyclic voltammograms (CV) were recorded from 0.0 to 1.2 V and differential pulse voltammetric (DPV) analysis was used for determination of promethazine in samples. All electrochemical measurements were done in an unstirred electrochemical cell at 25 ± 0.5 ºC.

Materials

All solutions were freshly prepared using double distilled water. PMZ, H3PO4, NaOH and other reagents were analytical grade, purchased from Sigma-Aldrich and used without any further purification. Pure fine graphite powder (Merck) and paraffin oil (DC 350, Merck, ρ=0.88 g/cm3) were used as binding agents for the graphite pastes. The MWCNTs were obtained from China and had an outside diameter of 10–20 nm, a length <1–2 µm, and purity over 95% while for more purification an acid treatment was used. PMZ solutions were prepared immediately prior to use and all experiments were carried out at ambient laboratory temperature (25 °C). Phosphate buffer solutions (PBS, 0.1 mol/L) of different pH values were prepared from stock solutions of 0.1 mol/L H3PO4 and a saturated solution of NaOH.

Fabrication of carbon paste electrodes (CPEs)

The bare carbon paste electrode (CPE) was prepared by thoroughly hand-mixing 0.50 g graphite powder with approximately 0.2 ml of paraffin oil. The multi walled carbon nanotube modified paste electrode (MWCN/CPE) was prepared by thoroughly mixing 0.50 g graphite powder and 0.006g of MWCNT prior to adding paraffin oil. Finally, a portion of the pastes (bare CPE or MWCNT/CPE) was put into plastic syringe tubes with the inner diameter of 2.0 mm to form bare CPE or MWCN/CPE. Electrical contact to the paste was established by inserting a copper wire down the syringe, making contact to the back of mixture. The surface was mechanically polished with a piece of weighing paper.

Results and discussion

Voltammetric behavior of PMZ at MWCNT/CPE

Figure 1 shows the voltammograms of PMZ on the surface of bare CPE (blue curve) and MWCN/CPE (red curve) in PBS of pH 5.0. At the bare electrode, PMZ shows a relatively weak oxidation peak at 723.0 mV, which at the MWCN/CPE, the oxidation peak becomes sharp, with a negative potential shift of 28.0 mV. Similarly comparison of the oxidation of PMZ at bare CPE and that at MWCN/CPE reveals a dramatic enhancement in the anodic peak current observed at MWCN/CPE relative to value obtained at bare CPE. Results are summarized in Table 1. The lower overpotential and the increase in current response are clear evidence of the catalytic effect of the modified electrode for the oxidation of PMZ (22).

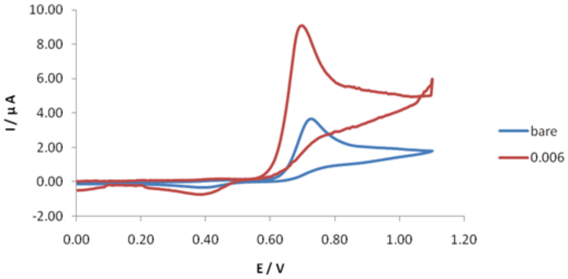


Figure 1. Cyclic voltammograms observed for 2.0×10-4 M PMZ in PBS of pH 5.0 at a bare CPE and MWCN/CPE (0.006 g) at the scan rate of 30 mV/s.

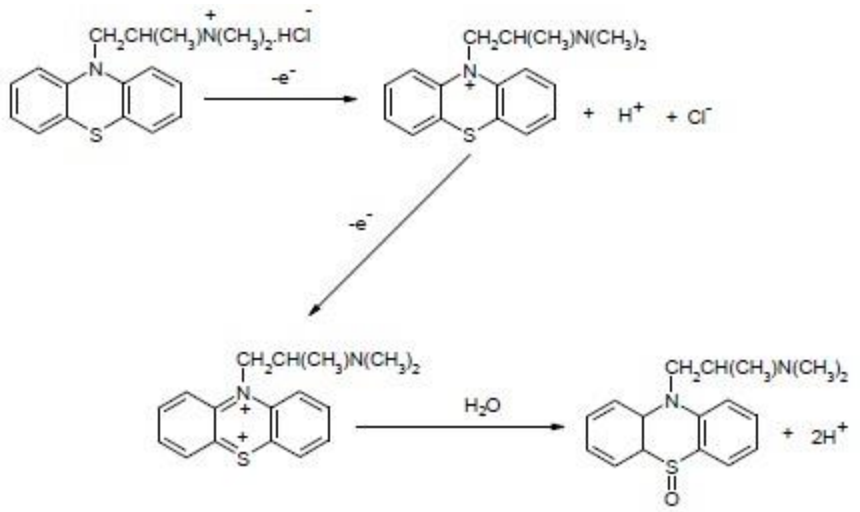
Electrode	Ep / V	Ip / µA
Bare CPE	0.723	061/3
MWCN/CPE	0.695	563/7

Table 1. Potential and current peaks of bare CPE and MWCN/CPE

The effect of pH on electro-oxidation of PMZ

PMZ can be oxidized at positive potential depending on the electrode type and solution pH. In order to ascertain this, the CVs response of 200.0 μmol/L of PMZ at a surface of MWCN/CPE was obtained in PB solutions with pH values ranging from 2.0 – 7.0. The PMZ oxidation response increased with pH, reaching a maximum at pH 5.0, and then decreased (Figure 2). The peak potential (Ep) was shifted to more negative values with increasing pH. The linear regression equation was obtained as: Ep (V) = -0.039 pH +0.905 (R2= 0.991) (pH=2.0-7.0), which showed that the overall process was proton dependent and the oxidation of PMZ involved two electrons - one proton (see Scheme. 2) according to slope of Nernst equation (Eq.1). The probable catalytic processes of PMZ may be expressed as follows (23):

$$Ep=E^{o}+0.0591V(1/n)\log[(Ox)a/(R)b]-0.0591V(m/n)pH$$



Scheme 2. The probable oxidation processes of PMZ at the surface of MWCNT/CPE.

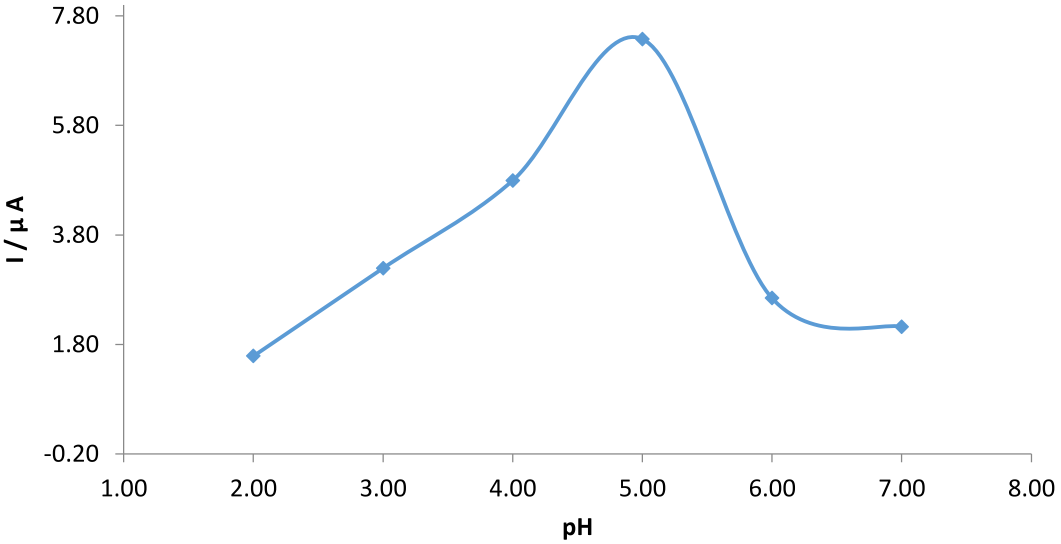


Figure 2. Peak currents of PMZ vs. pH in the range of 2.0 -7.0 in PBS by cyclic voltammetry

Calibration plot

Since differential pulse voltammetry (DPV) has a much higher current sensitivity and better resolution than cyclic voltammetry, it was used to estimate the lower limit of detection of PMZ. In addition, the charging current contribution to the background current, which is a limiting factor in the analytical determination, is negligible in DPV mode. So, the DPVs of PMZ at the surface of MWCNT/CPE was recorded in the range of 0.02 to 250.0 µmol L-1 (Figure 3). The DPVs shown two linear relation between peak current and concentration of PMZ in the ranges of 0.02 to 6.0 µmol L-1 (Ip = -0.485 +5.013C, R2= 0.997) and 6.0 - 250.0 µmol L-1 (Ip = -18.39 +0.539C, R2= 0.990).

According to slope of equation in low concentrations of PMZ, the detection limit (3Sb/m) was found to be 1.2 × 10-9 mol/L.

The relative standard deviation (RSD) of 4.15% in oxidation peak current and 0.72% in peak potential for five repeated detections of 2.5×10-5 mol/L PMZ suggests excellent repeatability of results using the modified electrode. When the electrode was stored in our laboratory at room temperature, the modified electrode retained 94.2% of its initial response after 15 days. These results indicate that Au-NPs/CPE has both good stability and satisfactory reproducibility. When using three different electrodes, the RSD% for five measurements was 5.8%.

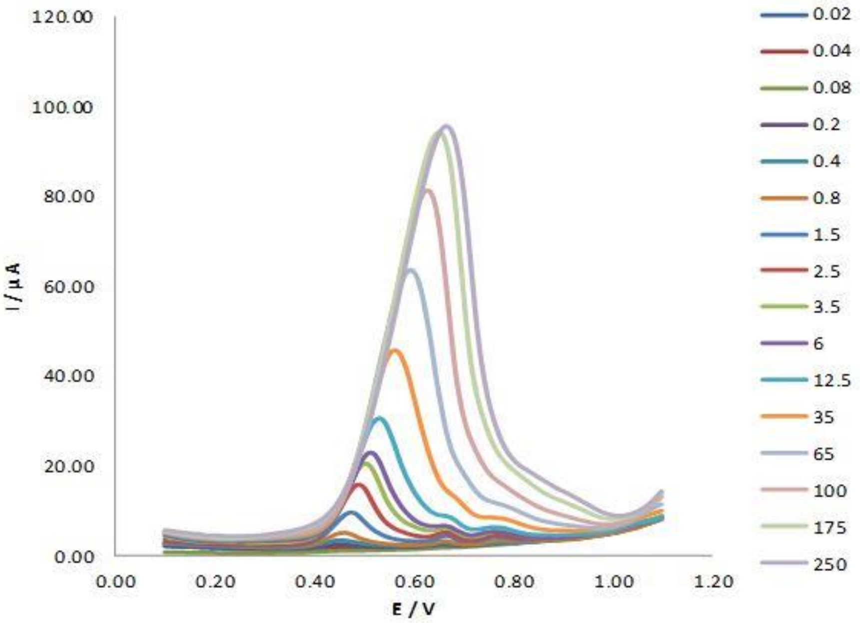


Figure 3. DPV of PMZ at the MWCN/CPE in the concentration range of 0.02-250.0 µM in PBS, pH 5.0

Interference study

In order to evaluate the selectivity of the proposed method in the determination of PMZ, the influence of various foreign species such as K+, Cu2+, NO3-, Cl-, alanine, glycine, ascorbic acid and uric acid on the determination of 10.0 µmol/L PMZ was investigated. The tolerance limit was taken as the maximum concentration of foreign substances, which caused no more than ±5% relative error in the determination. The results showed that no conventional cations, anions, or organic substances affected the peak current of PMZ. The tolerated concentrations were 50.0 µmol/L for uric acid and 30.0 µmol/L for ascorbic acid.

Determination of PMZ in human blood serum

In order to evaluate the analytic applicability of the proposed electrode, it was applied for the measurement of PMZ concentration in a pharmaceutical formulation and a human blood serum sample. Firstly, the diluted blood plasma sample was spiked with various concentrations of

PMZ and its DPV was obtained by the modified electrode. The results are shown in Table 2.

The MWCN/CPE responds well for the recovery of spiked PMZ with high selectivity and sensitivity. This implies a promising feature for the applicability of the MWCN/CPE for the selective and sensitive determination of PMZ in the real samples.

Added (μmol/L)	Found (μmol/L)	Recovery (%)
0.0	Not detected	-
70.80	73.60	103.095

Table 2. Application of the proposed method to the determination of PMZ in spiked human blood plasma

Measurement of PMZ concentration in pharmaceutical formulations

In order to evaluate the analytic applicability of the proposed electrode, it was applied for the measurement of PMZ concentration in pharmaceutical formulation. So, 1.0 mL PMZ syrup was taken and added into 5.0 mL PBS (0.1 mol/L). 0.4 mL of this sample was added to a 25.0 mL buffer cell and a standard addition method was applied to measure accuracy. The results are given in Table 3. Satisfactory agreement of the experimental results was found for the PMZ concentration.

Sample	Stated content (μmol/L)	Detected content (μmol/L)	Recovery %	RSD % (n=3)
PMZ syrup.1	12.00	10.74	89.5	1.45
PMZ syrup.2	45.00	44.60	99.11	0.98
PMZ syrup.3	150.0	147.75	98.50	1.05

Table 3. Determination of PMZ in syrup pharmaceutical formulations

Conclusion

This work describes a simple and sensitive method for the determination of PMZ. High sensitivity, low detection limit, easy regeneration of the electrode surface, low cost, simple methods of preparation and excellent stability and reproducibility make the MWCN-PE useful for the determination of PMZ in pharmaceutical dosage forms and blood plasma samples.

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