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Voltammetric determination of promethazine hydrochloride at a multi-wall carbon nanotube modified glassy carbon electrode

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A simple and sensitive method is described for voltammetric determination of promethazine hydrochloride (PMZ), a widely used phenothiazine drug, based on its electrochemical oxidation at a multi-wall carbon nanotube (MWCNT) modified glassy carbon electrode (GCE). Compared with bare GCE, the MWCNT-modified GCE exhibited excellent enhancement effect on the electrochemical oxidation of PMZ. PMZ yielded two anodic peaks at about 0.61 V and 0.78 V, and the peak at 0.61 V was applied to the determination. Under optimized conditions, the anodic peak current was linear to the concentration of PMZ in the range from 5.0×10^{-8} to 4.0×10^{-4} M with the detection limit of 1.0×10^{-8} M. The relative standard deviation (RSD) was 2.28% for 8.0×10^{-6} M PMZ ($n = 10$). To further validate its possible application, the proposed method was successfully used for the quantification of PMZ in pharmaceutical formulations and biological fluids with satisfactory results.

Keywords: promethazine hydrochloride; voltammetric determination; multi-wall carbon nanotube; modified electrode

Introduction

Promethazine hydrochloride, 10-N,N-dimethyl-1-(10H-phenothiazin-10-yl)propan-2-amine hydrochloride (Figure 1), is a prominent compound in the large group of phenothiazine derivatives. It is widely used as an antihistaminic for the symptomatic relief of hypersensitivity reactions or for enhancing the analgesic, anesthetic, and sedative effect of other drugs.[1] However, massive overdose of PMZ may cause side effects such as endocrinal, cardiac and reproductive alterations, coma, respiratory depression, or other undesirable reactions, even death.[2] Therefore, the determination of PMZ is of great importance in biological samples and pharmaceutical formulations analysis. Numerous analytical techniques have been reported for the determination of PMZ, which include titrimetric,[3,4] spectrophotometry,[5,6] potentiometric,[7] electrophoresis,[8] high performance thin-layer chromatography (HPTLC),[9] high performance liquid chromatography (HPLC),[10] liquid chromatography-mass spectrometry (LC-MS),[11] resonance Rayleigh scattering,[12] etc. Like other phenothiazine derivatives, PMZ is a good electron donor; it can be oxidized electrochemically and produce voltammetric response. Based on its electroactivity, PMZ has also been investigated and determined conveniently by electrochemical methods.[13–17]

Since the discovery of carbon nanotubes (CNTs) by Iijima,[18] they have attracted tremendous research interest in chemical, physical, and material areas due to their unique structural, mechanical, electronic, and chemical properties.[19] According to the number of carbon atom layers within the sidewall, CNTs can be divided into multi-wall carbon nanotubes (MWCNTs) and single-wall carbon nanotubes (SWCNTs). The subtle electronic properties of CNTs reveal that they have the ability to promote electron-transfer reactions when used as an electrode material in electrochemical reactions.[20,21] To our knowledge, voltammetric determinations of PMZ at a MWCNT-modified GCE have not been reported yet. In continuation of our previous studies on electrochemical determination,[22,23] the objective of the present work is to develop a convenient and sensitive method for the determination of PMZ based on the unusual properties of MWCNT-modified electrode. In this study, the electrochemical behaviour of PMZ at the MWCNT-modified GCE was investigated. The results showed that MWCNT-modified GCE enhanced the oxidation peak current of PMZ as compared to bare GCE. Consequently, a voltammetric method based on MWCNT-modified electrode was developed for the determination of PMZ in pharmaceutical formulations and biological fluids. This newly proposed method possesses several advantages such as high sensitivity, rapid response, good reproducibility, low cost, and simplicity.

Experimental

Reagents and materials

MWCNTs were kindly provided by the Laboratory of Life Analytical Chemistry, Nanjing University, China. They were synthesized with a catalytic pyrolysis method and then purified with concentrated HNO_3.[24] The chemical treatment causes segmentation and carboxylation of MWCNTs at their terminus. Dihexadecyl hydrogen phosphate (DHP), a hydrophobic surfactant, was purchased from Fluka and used as a solublizing agent to prepare MWCNT-DHP suspension. Standard PMZ was purchased from Material Medical

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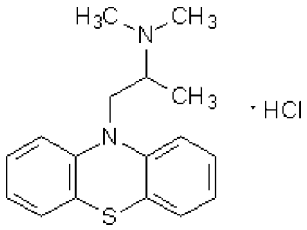

Figure 1. Chemical structure of PMZ.

and Biologic Product Verification Institution of China, and its stock solution of 1.0×10^{-3} M was prepared in water. The working standard solutions were freshly prepared by serial dilution of the stock solution with 0.1 M phosphate buffer solution (PBS, pH 7.4). PMZ injection (Shanghai Harvest Pharmaceutical Co. Ltd, Shanghai, China) was obtained from a local hospital. All other reagents were of analytical grade and used as received without further purification. Double-distilled water was used throughout. A 0.1 M PBS solution of pH 7.4 served as the supporting electrolyte unless otherwise stated.

Apparatus

All the electrochemical measurements were performed with a CHI 660A Workstation (CH Instruments, Austin, Texas, USA). A conventional three-electrode system, consisting of a MWCNT-modified GCE (3-mm diameter) as the working electrode, an Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode, was employed.

Fabrication of MWCNT-modified GCE

Five milligrams each of MWCNT and DHP were dispersed into 5 mL of water by ultrasonication for about 20 min to give a stable and homogeneous MWCNT-DHP suspension of 1 mg/mL. Prior to modification, a GCE was mechanically polished with alumina slurry of different grades to a mirror finish, and rinsed in water under sonication for 3 min. A MWCNT-modified GCE was prepared by first dropping 15 µL of 1 mg/mL MWCNT-DHP suspension onto the GCE surface and then allowing water to evaporate at room temperature in the air.

Analytical procedure

A desired volume of PMZ standard or sample solution was pipetted to a 10 mL electrolytic cell containing 0.1 M PBS (pH 7.4), followed by deaeration by pumping oxygen-free nitrogen for 10 min. An accumulation step was then conducted with stirring of the solution for 3 min at open-circuit. After a quiescent interval of 30 s, linear sweep voltammograms from 0.00 to 1.00 V were recorded. Prior to each measurement, the MWCNT-modified GCE was activated by successive cyclic voltammetric sweeps from 0.00 to 1.00 V at 100 mV/s in the blank electrolyte solution until the voltammograms remained unchanged, indicating that a fresh electrode surface had been reproduced.

Results and Discussion

Electrochemical characterization of the MWCNT-modified GCE

The microscopic areas of the MWCNT-modified GCE and bare GCE were obtained by cyclic voltammetry (CV) using 1.0 mM $K_3Fe(CN)_6$ as a probe at different scan rates. For a reversible process, the following Randles-Sevčik equation can be used:[25]

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

where i_{pa} refers to the peak current, n is the number of electrons transferred, A is the surface area of the electrode, D_R is diffusion coefficient, C_0 is the concentration of $K_3Fe(CN)_6$ and v is the scan rate. For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, $n = 1$, $D_R = 7.6 \times 10^{-6}$ cm^2/s, the microscopic areas can be calculated from the slope of the i_{pa} versus $v^{1/2}$ relation. The experiment results showed that the electro-active surface areas of bare GCE and MWCNT-modified GCE were measured to be 0.046 cm^2 and 0.078 cm^2, respectively.

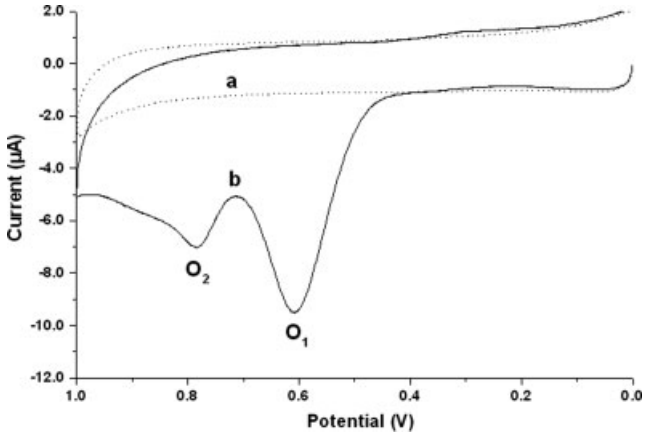

Figure 2. Cyclic voltammograms of the MWCNT-modified GCE in 0.1 M PBS (pH 7.4) without (a) and with (b) 8.0×10^{-6} M PMZ. Scan rate: 100 mV/s.

Electrochemical behaviour of PMZ

The electrochemical behaviour of PMZ at the MWCNT-modified GCE was examined by CV within a certain potential window. Figure 2 compares cyclic voltammograms of the MWCNT-modified GCE in 0.1 M PBS (pH 7.4) in the absence (a) or presence (b) of 8.0×10^{-6} M PMZ. No observable redox peaks appeared in the blank PBS. Upon addition of 8.0×10^{-6} M PMZ, two sensitive, well-defined oxidation peaks at about 0.61 V (O_1) and 0.78 V (O_2) were observed during the first anodic sweep from 0.00 to 1.00 V (versus Ag/AgCl), but no corresponding reduction peak was observed on the reverse scan, implying that the electrode reaction of PMZ was totally irreversible. The electrode process is attributed to the oxidation of the phenothiazine ring in PMZ in two sequential steps involving two electrons and two protons to the corresponding sulfoxide.[16,17] As it can be seen, peak O_1 is much higher and sensitive than peak O_2, hence, peak O_1 was employed to examine PMZ in subsequent studies. The oxidation peak currents of PMZ dramatically decreased during the successive cyclic voltammetric sweeps. After four potential sweeps with a scan rate of 100 mV/s, the peak currents remained nearly unchanged. This phenomenon might be ascribed to the adsorption of PMZ or its oxidation product onto the surface of the MWCNT-modified GCE and the resultant inactivation of the electrode surface. In addition, the effect of scan rate on the peak current of PMZ was investigated by CV. It was found that the peak current was proportional to the scan rate over the range from 10 to 200 mV/s, providing further evidence that the electrode reaction of PMZ at the MWCNT-modified GCE is adsorption-controlled.

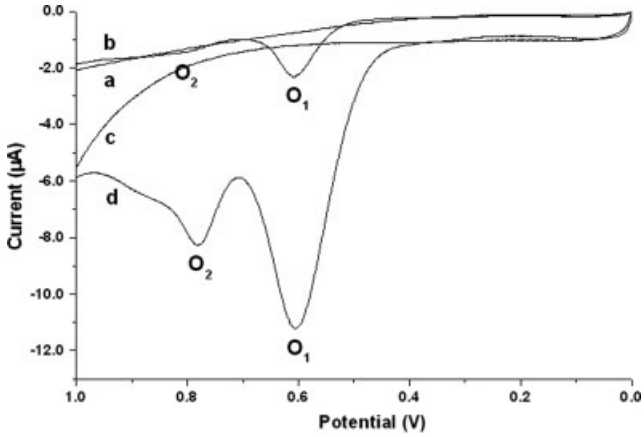

Figure 3. Linear sweep voltammograms of the bare GCE (a, b) or the MWCNT-modified GCE (c, d) when placed in 0.1 M PBS (pH 7.4) in the presence (b, d) and absence (a, c) of 8.0×10^{-6} M PMZ. Scan rate: 100 mV/s.

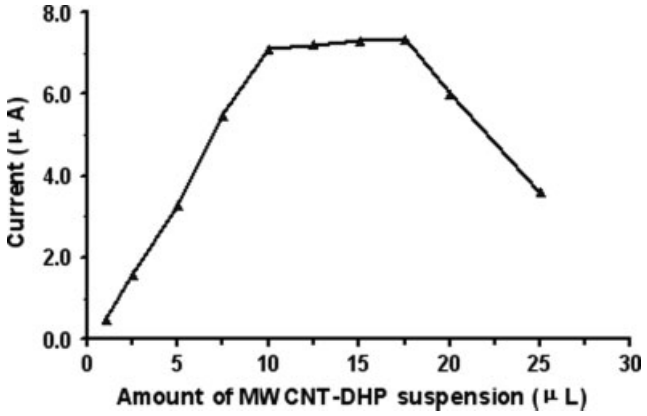

Figure 4. Effect of amount of MWCNT-DHP suspension on the oxidation peak current of 8.0×10^{-6} M PMZ.

To have more insight into the significance of MWCNT for the analysis of PMZ, the oxidation behaviours of PMZ at bare GCE and MWCNT-modified GCE were compared by linear sweep voltammetry (LSV). As shown in Figure 3, 8.0×10^{-6} M PMZ also exhibited two anodic peaks at the bare GCE, one relatively sensitive anodic peak was at about 0.63 V (O_1) and another very ill-defined oxidation peak with very low current occurred at about 0.82 V (O_2). However, under the same conditions, the oxidation peaks shifted negatively to 0.61 V (O_1) and 0.78 V (O_2) with considerable enhancement in the peak current at the MWCNT-modified GCE. The remarkable peak current enhancement and the negative shift of oxidation peak potential are undoubtedly attributed to the unusual structure and properties of MWCNT such as high aspect ratio and strong adsorptive ability.

Effect of supporting electrolyte

The electrochemical oxidation of 8.0×10^{-6} M PMZ in different supporting electrolyte solutions, including pH 5.0–8.0 PBS, pH 2.0–10.0 Britton-Robinson buffer, pH 1.0–5.0 sodium citrate-HCl buffer, pH 4.0–6.0 HAc-NaAc buffer (0.1 M of each buffer), were examined by CV. The best oxidation response was obtained in pH 7.4 PBS in that the electrochemical response was well defined with the highest peak current as compared to that in other buffer systems.

It was also demonstrated that in 0.1 M PBS the oxidation peak current of PMZ gradually increased as pH values increased from 5.0 to 7.4 and then levelled off within the pH range from 7.4 to 8.0. Hence, 0.1 M PBS (pH 7.4) was chosen as the supporting electrolyte for the determination of PMZ. Moreover, the dependence of the oxidation peak potential (E_{pa}) on the pH value was examined. The pH value strongly affected the E_{pa} of PMZ. It showed that the E_{pa} shifted negatively with increasing pH from 5.0 to 8.0, and a good linear relationship was observed between pH value and E_{pa} with a slope of −61 mV/pH. This slope value indicates that identical numbers of protons and electrons are involved in the electrode process. Obviously, the result is in accordance with the reaction mechanism mentioned above.

Effect of amount of MWCNT-DHP suspension

The thickness of the MWCNT-DHP film on the GCE surface is determined by the amount of MWCNT-DHP suspension dropped on the GCE surface. Figure 4 depicts the relationship between amount of MWCNT-DHP suspension and the oxidation peak current of PMZ. The peak current significantly increased with increasing the amount from 0 to 10 μL. As the amount of suspension further increased, the peak current changed very slightly, and when the amount of suspension exceeded 17.5 μL, the peak current conversely decreased. This is probably attributed to the compromising effects of DHP on the electrochemical performance of the composite film due to the hydrophobic and insulating actions of DHP. As a result, an appropriate amount for the fabrication of MWCNT-modified GCE was determined as 15 μL of 1 mg/mL MWCNT-DHP suspension.

Effect of accumulation potential or accumulation time

Accumulation prior to voltammetric measurements could influence the electro-oxidation of PMZ at the MWCNT-modified GCE. It was found that the peak current was practically independent on the accumulation potential within the potential window of 0.40 to −0.40 V. Thus, an open circuit accumulation was performed.

As to the effect of the accumulation time on the oxidation peak current, the current increased with the accumulation time in the range of 3 min; when the accumulation time exceeded 3 min the current reached a plateau, suggesting that the accumulation process of PMZ had achieved its saturation adsorption on MWCNT-DHP film. Therefore, 3-min accumulation was used to improve sensitivity of the method and shorten the time consumption.

Calibration plot and stability

Under the optimized experimental conditions, the linear sweep voltammograms of PMZ with different concentrations at the MWCNT-modified GCE were recorded (Figure 5). The peak current increased linearly with incremental concentration of PMZ in the range from 5.0×10^{-8} to 4.0×10^{-4} M, giving a regression equation of i_p (μA) $= 3.32 + 4.03 \times 10^5 C$ (M) ($r = 0.9986$). The detection limit was found to be 1.0×10^{-8} M (according to $S/N = 3$). The relative standard deviation (RSD) of 2.28% for 8.0×10^{-6} M PMZ ($n = 10$) indicated a good reproducibility.

For evaluating the long-term stability of the MWCNT-modified GCE, it was stored in the air and used for monitoring 8.0×10^{-6} M PMZ daily over a period of 4 weeks. The deviation of current responses was only 4.68%.

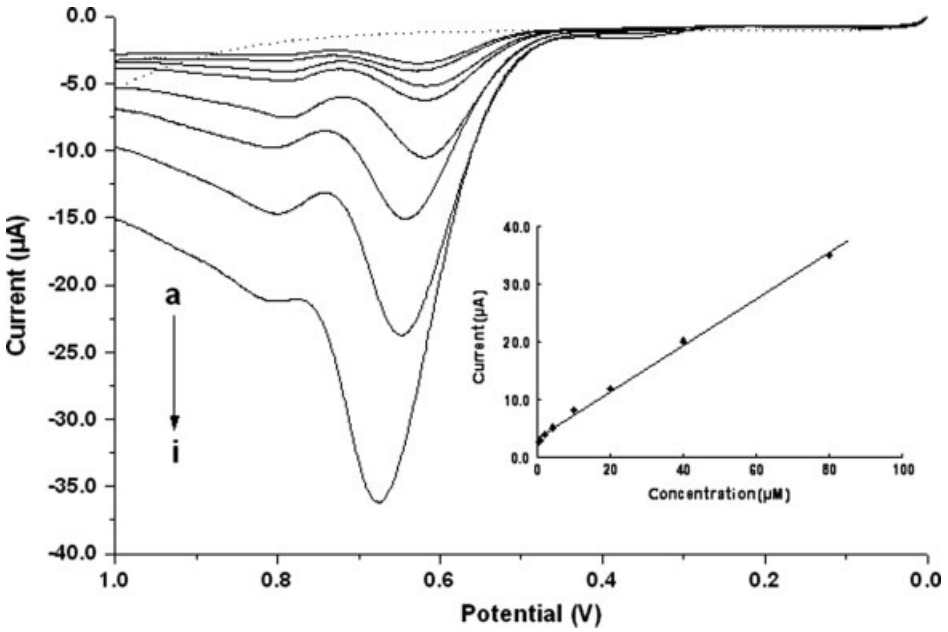

Figure 5. Linear sweep voltammograms of PMZ at the MWCNT-modified GCE in 0.1 M PBS (pH 7.4) containing 0 (a), 4.0×10^{-7} (b), 8.0×10^{-7} (c), 2.0×10^{-6} (d), 4.0×10^{-6} (e), 1.0×10^{-5} (f), 2.0×10^{-5} (g), 4.0×10^{-5} (h), 8.0×10^{-5} (i) M PMZ, respectively. Accumulation time: 3 min, scan rate: 100 mV/s. Also shown is the resulting calibration plot (inset).

Interference study

A systematic study was carried out to evaluate the interferences of foreign species on the determination of PMZ at the level of 1.0×10^{-6} M. We found that 200-fold concentration of Na^+, K^+, Mg^{2+}, Pb^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Cl^-, Br^-, I^-, NO_3^-, or SO_4^{2-}, 50-fold concentration of uric acid, oxalic acid, citric acid, lactic acid, tartaric acid, glucose, lactose, saccharose, starch, or carboxymethylcellulose, and 20-fold concentration of vitamin B_1, vitamin B_2, vitamin B_{12}, or vitamin C in the solution had almost no influences on the determination of PMZ (signal change below 5%). However, some phenothiazines such as chlorpromazine hydrochloride, fluphenazine, trifluoperazine hydrochloride could potentially interfere with the determination of PMZ.

Applications

Drug analysis

The developed method was used for the determination of PMZ in injections. Before determination, no pretreatment for the PMZ injection was done except for dilution with 0.1 M PBS (pH 7.4). After that, a known volume of the diluted PMZ was added to an electrochemical cell containing 10 mL of 0.1 M PBS (pH 7.4), and then the linear sweep voltammograms were recorded as in standard PMZ described in analytical procedure. The content of PMZ was calculated from the calibration equation (Table 1). Furthermore, the results obtained by our proposed method were compared with those determined by the pharmacopeia method.[3] Both method results were in good agreement with each other, suggesting that the MWCNT-modified GCE had great promise for practical application in pharmaceutical formulation analysis. In order to establish the suitability of the proposed method, the known amounts of the standard PMZ were added into the analyte solution, and the same procedure was applied. Recoveries were found to be in the range of 98.8 and 103.2%, indicating that the proposed method had good accuracy and satisfactory repeatability.

Determination of PMZ in human serum and urine samples

The proposed method was also applied to the determination of PMZ in human serum and urine samples which were obtained from volunteers. For sample pretreatment, the serum and urine samples were diluted 50 and 20 times with 0.1 M PBS (pH 7.4) respectively, and the urine samples were further centrifuged for 5 min at 4000 rpm to remove the suspended particles. The determination of PMZ in biological fluid samples was performed by a standard

Table 1. Determination of PMZ in injections

Sample	Declared content (mg/mL)	Detected by this method (mg/mL)	Detected by the pharmacopeia method[3] (mg/mL)	Recovery of this method (%)
1	25.0	25.2	25.4	103.2
2	25.0	24.6	24.8	99.4
3	25.0	26.0	24.6	101.6
4	25.0	25.8	25.2	98.8
5	25.0	24.2	24.8	102.0

Table 2. Determination of PMZ in human serum and urine samples

Sample	added (μM)	Found (μM)*	Recovery (%)	RSD (%)
serum 1	0.400	0.390	97.5	2.87
2	1.00	1.02	102.0	2.42
3	5.00	5.06	101.2	1.36
urine 1	0.400	0.412	103.0	3.14
2	1.00	0.992	99.2	1.58
3	5.00	4.94	98.8	2.65

* Average of five assays

addition method. The recoveries were found to be in the range from 97.5 to 103.0% (Table 2).

Conclusion

In this study, a MWCNT-modified GCE was fabricated for the voltammetric determination of PMZ. The enhancement in the oxidation current and the negative shift of peak potential of PMZ at the MWCNT-modified GCE might be attributed to the surface properties of MWCNTs. This newly developed method is sensitive, convenient, rapid, and suitable for the determination of PMZ in pharmaceutical formulations and biological fluids.

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