Sensitive Detection of Haloperidol and Hydroxyzine at Multi-Walled Carbon Nanotubes-Modified Glassy Carbon Electrodes

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Abstract: Haloperidol (i.e. HPD) and hydroxyzine (i.e. HXY), two effective and important tranquilizers with low redox activity, were found to generate an irreversible anodic peak at about +0.86 V (vs. SCE) or two anodic peaks at about +0.83 and +0.91 V in 0.05 M NaH2PO4-Na2HPO4 (pH=7.0) buffer solution with a multi-walled carbon nanotubes-modified glassy carbon electrode (i.e. MWNTs/GC), respectively. Their sensitive and quantitative measurement based on the first two anodic peaks was established under the optimum conditions. The anodic peak current was linear to HPD and HXY concentration from 1×10⁻⁷ to 2.5×10⁻⁵ M and 5×10⁻⁸ to 2.5×10⁻⁵ M, the detection limits obtained were 8×10⁻⁹ and 5×10⁻⁹ M, separately. The modified electrode exhibited some excellent characteristics including easy regeneration, high stability, good reproducibility and selectivity. The method proposed was successfully applied to the detection of HPD and HXY in drug tablets and proved to be reliable compared with ultraviolet spectrophotometry. The modified electrode was characterized by electrochemical methods.

Keywords: Haloperidol, Hydroxyzine, Multi-walled carbon nanotubes, Detection, Excellent characteristics, Characterization.
1. Introduction

As two effective and important tranquilizers, haloperidol and hydroxyzine (Scheme 1) are widely used in the treatment of schizophrenia [1, 2]. Based on their importance, some analytical methods have been applied to investigate their characteristics, such as spectrophotometry [3], high-performance liquid chromatography [4, 5], capillary electrophoresis [6], mass spectrometry, etc. [7]. Nevertheless, owing to their low redox activity under normal conditions, there are few papers about them studied by means of electrochemical methods. Among the few examples, ion-selective electrodes [8, 9], hanging mercury electrodes [10, 11], glassy carbon electrodes [1, 2] were used to study and determine them. However, their practical applications might be restricted due to some disadvantages including complicated electrode preparation, unsatisfactory detection limit, toxicity of electrodes and time-consuming preconcentration. Therefore, it is necessary to develop a new analytical method for them.

Scheme 1. Molecular structure of haloperidol (HPD) or hydroxyzine (HXY).

Since carbon nanotubes (CNTs) were discovered in 1991, they have attracted much attention of researchers [12, 13]. Owing to their high stability and mechanical strength, good conductivity and catalytic activity, they are usually used as electrode materials and as such are widely applied in electroanalytical chemistry [14]. It has been reported that CNT-modified electrodes have been successfully applied to study and determine some biological, organic, inorganic molecules or ions [15-17]. However, there is no report about electrochemical sensors based on CNTs-modified electrodes for these applications.

In this paper, a MWNTs/GC modified electrode was fabricated and characterized by electrochemical methods. Meanwhile, the electrochemical behaviors of HPD and HXY at the modified
electrode were investigated. It was found that the modified electrode showed some excellent characteristics. Therefore, a more sensitive and simpler determination method was proposed.

2. Experimental

2.1 Reagents

Haloperidol (C₂₁H₂₃ClFNO₂) and hydroxyzine hydrochloride (C₂₁H₂₇ClN₂O₂·2HCl) were purchased from Sigma. Their stock solutions (0.002 M or 0.01 M) were prepared with ethanol or twice distilled water and stored at +4 ºC in darkness. Buffer solution was a 0.05 M NaH₂PO₄-Na₂HPO₄ (pH=7.0) aqueous solution. Multi-walled carbon nanotubes came from Shenzhen Nanotech Port Co. Ltd. (diameter 10-30 nm, length 0.5-40 µm, Shenzhen, China). The drug tablets were obtained from Sine JiuFu Pharmaceutical Company (Shanghai, China). Prior to determination, the tablets were ground into powder, dissolved in ethanol or water, filtered into a container and diluted to certain volume. Other reagents (Sigma or Aldrich) used were of analytical grade or chemical grade, and their solutions were prepared with twice distilled water or ethanol.

2.2 Apparatus

Electrochemical measurements were carried out on a CHI 440 electrochemical analytical instrument (ChenHua Instrument Company, Shanghai, China). A three-electrode system was used, including a bare GC electrode (Φ=3.0 mm) or MWNTs/GC modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire electrode as counter electrode. Electrochemical impedance spectroscopy (EIS) measurements were performed on an EG&G PAR Model 273 A bipotentiostat (Princeton, USA) in conjunction with a lock-in amplifier. A 5-mV amplitude sine wave was applied to the electrode under potentiostatic control and the frequency range was from 0.1 Hz to 100 KHz. Ultraviolet spectrophotometry (UV) measurements were performed on HP8453 ultraviolet-visible spectrophotometer (Agilent, USA). Solution pH was measured with a Mettler-Toledo 320-s pH meter (Mettler-Toledo Instruments Limited Company, Shanghai, China). All experiments were carried out at about 25 ºC.

2.3 Fabrication of MWNTs/GC modified electrode

According to the literature [14], MWNTs were refluxed in the mixture of concentrated H₂SO₄ and HNO₃ for about 6 h, then washed with twice distilled water and dried under vacuum at room temperature. The suspension of MWNTs was prepared by dispersing the MWNTs (2 mg) in N,N-dimethylformamide (DMF, 2 mL) with the aid of ultrasonic agitation.

The bare GC electrode was first polished to a mirror-like surface with 0.3 and 0.05 µm α-alumina slurry, then rinsed and ultrasonicated sequentially in water, acetone, diluted HNO₃ and water, then dried in air. The effective area of GC electrode could be calculated to be 0.0674 cm² [14]. The MWNTs/GC modified electrode was fabricated by dropping MWNTs suspension (5 µL) on the GC electrode surface and evaporating the solvent in air.
2.4 Procedure

The three-electrode system was immersed in a 10 mL cell containing a suitable amount of HPD or HXY and 0.05 M NaH$_2$PO$_4$-Na$_2$HPO$_4$ (pH=7.0) buffer solution. After accumulating for 180 s at open circuit under stirring and following quiet for 10 s, potential scan was initiated and cyclic voltammograms were recorded between +0.2 and +1.2 V at a scan rate of 100 mVs$^{-1}$. Before every test, the modified electrode needed easy regeneration in blank buffer solution. With the potential cycle repeating, the anodic peak current decreased rapidly. Generally, voltammograms corresponding to the first cycle were recorded.

3. Results and Discussion

3.1 Electrochemical characterization of the MWNTs/GC modified electrode

The MWNTs/GC modified electrode was characterized by electrochemical methods. As shown in Figure 1, K$_3$Fe(CN)$_6$ exhibited a pair of quite reversible redox peaks at bare GC electrode. The separation of peak potential ($\Delta E_p = E_{pa} - E_{pc}$) was about 68 mV and the formal potential ($E_F = (E_{pa} + E_{pc})/2$) was 256 mV. At the modified electrode, a pair of higher and reversible redox peaks could still be observed. On one hand, under the same conditions, both the anodic peak of K$_3$Fe(CN)$_6$ on GC and MWNTs/GC grew with scan rate increasing, it was found that both the electrode process was controlled by diffusion, the regression equations were $I_p = 1.271 + 3.160v^{1/2}$ ($I_p$: µA, $v$: mV/s, $r=0.999$) and $I_p = -59.946 + 15.933v^{1/2}$ ($r=0.997$), respectively.

Figure 1. CVs of 2 mM K$_3$Fe(CN)$_6$ at bare GC electrode (A) and MWNTs/GC modified electrode (B). Scan rate: 100 mV s$^{-1}$; supporting electrolyte: 0.05 M KNO$_3$. 

![Figure 1](image-url)
A reversible system should satisfy the Randles-Sevcik equation \[ I_p = 2.69 \times 10^5 n^{3/2} A C_0 D_R^{1/2} v^{1/2} \]. According to the ratio of slopes of the two lines, the apparent area of the MWNTs/GC modified electrode was about 5 times as large as that of the GC electrode. Meanwhile, the peak current was about 3.5 times as high as that of the bare GC electrode (in this experiment, the anodic peak current was only linear (not proportional) to the square root of scan rate or apparent area), which was attributed to the significant enhancement of apparent area. Furthermore, the increase in background current also suggested this. On the other hand, the \( \Delta E_p \) and \( E_F \) reduced to 56 and 248 mV, indicating the electrode process on MWNTs/GC became more reversible and easier. This should be attributed to the better conductivity of modified electrode. Figure 2 shows the EIS spectra of the two electrodes in the given solution. Apparently, the resistor of modified electrode was much smaller than that of bare GC electrode, also indicating MWNTs/GC had better conductivity.

Figure 2. EIS spectra of bare GC electrode (A) and MWNTs/GC modified electrode (B). Frequency used: 0.1 Hz to 100 KHz; solution: 2 mM K₃Fe(CN)₆/K₄Fe(CN)₆ containing 0.1 M KNO₃. The insert is the amplification of (B).

Cyclic voltammograms of HPD and HXY at MWNTs/GC modified electrode

In blank buffer solution, no obvious HPD or HXY peaks were observed at both the bare GC electrode or the MWNTs/GC modified electrode. As shown in Figure 3, HPD generated a small anodic peak at about +0.86 V at the bare GC electrode. However, at the modified electrode, this anodic peak current increased about 40 times and became very sensitive, which suggested MWNTs/GC had better conductivity and electrocatalytic activity. HXY also produced a small anodic peak at about +0.83 V at bare GC electrode, while two anodic peaks occurred at the modified electrode, one at about +0.83 V and the other at about +0.91 V. The former peak was more sensitive than the latter, and its current was also about 40 times as high as that of the bare GC electrode. This also suggested that the modified electrode had better conductivity and electrocatalytic activity. Apparently, the MWNTs/GC modified electrode could promote the electrochemical oxidation of HPD and HXY. In addition, no obvious
cathodic peaks appeared for these substances at both the bare GC electrode or the modified electrode, which indicated the electrochemical oxidation of HPD or HXY was irreversible.

**Figure 3.** CVs of 5×10⁻⁶ M HPD or HXY at the bare GC electrode (A) and the MWNTs/GC modified electrode (C). CVs of MWNTs/GC modified electrode in blank solution (B). Scan rate: 100 mV s⁻¹; supporting electrolyte: 0.05 M NaH₂PO₄-Na₂HPO₄ (pH=7.0); accumulation time: 180 s (at open circuit).

3.2 Effect of scan rate

For both HPD and HXY, when the scan rate was increased from 10 to 300 mV s⁻¹, the anodic peak grew. It was found that anodic peak current was linear to scan rate, the regression equations were: \( I_p = 13.141 + 0.749v \) (\( I_p \) (µA), \( v \) (mVs⁻¹), \( r = 0.996 \)) and \( I_p = 9.838 + 0.820v \) (\( r = 0.997 \)), respectively. This suggested their electrode processes were controlled by adsorption. Furthermore, the anodic peak potential and the logarithm of scan rate showed a linear relationship, following the equation: \( E_p = 0.380 + 0.104 \ln v \) (\( E_p \) (V), \( r = 0.995 \)) and \( E_p = 0.407 + 0.0916 \ln v \) (\( r = 0.999 \)), separately. According to the equation [18]: \( I_p = n^2F^2v\Gamma_0^*/4RT = nFQv/4RT \), the factor \( n \) was calculated to be 1.38 (HPD) and 1.84 (HXY), respectively. This indicated one or two electrons were lost during the first step of electrochemical oxidation, separately. In addition, according to the equation [19]: \( E_p = E^0+[RT/(1-\alpha)nF]\ln[RTK/(1-\alpha)nF] +[RT/(1-\alpha)nF]\ln v \), the charge-transfer coefficient \( \alpha \) could be calculated to be 0.753 (HPD) and 0.786 (HXY), respectively.
3.3 Optimization of the analytical conditions. Influence of pH and buffer solution

For both HPD and HXY (Figure 4), within the range of pH 2.3~10.0, the peak potential shifted negatively as pH increased, following the linear equations: $E_p=1.395-0.0716pH$ ($r=0.994$) and $E_p=1.202-0.0509pH$ ($r=0.994$), respectively. This meant $H^+$ was involved in the oxidation.

**Figure 4.** Influence of pH on the anodic peak current (A) and peak potential (B) of HPD or HXY. Supporting electrolyte: 0.05 M phosphate. Other conditions as in Figure 3.

The slopes of 71.6 and 50.9 mV/pH suggested the number of protons was equal to that of electrons taking part in the oxidation, indicating one or two protons participated in the oxidation, which was in accordance with the literature [1, 2]. Meanwhile, the peak current increased until it attained the maximum at pH 7.0, then it decreased. According to the literature [20, 9], the pK$_a$ values of HPD and HXY are 8.1 and 7.2, separately. Meanwhile, neutral HPD and HXY were hydrophobic, meaning they were poorly soluble in water [3]. In general, when the pH of the solution was much lower than the pK$_a$, owing to the strong protonation, the oxidation might become difficult and responses were lower. As the pH increases, the responses are enhanced due to the deprotonation. However, if the pH exceeded the pK$_a$ and increased further, their solubility decreased gradually and drug precipitation occurred, which could affect the accumulation and resulted in the decrease of the responses. Therefore, pH 7.0 was chosen as the optimum pH value. When the pH was set at 7.0, several supporting electrolytes including NaH$_2$PO$_4$-Na$_2$HPO$_4$, Tris-HCl, Na$_2$B$_4$O$_7$-HCl, NH$_3$-NH$_4$Cl were tested. It was found that the response obtained in NaH$_2$PO$_4$-Na$_2$HPO$_4$ buffer solution was a little better than that in other media. It was thought that the response probably had some relation with the kind of medium, which is under investigation. Additional, the variation in buffer concentration from 0.02 to 0.4 M had no obvious influence on the responses. Furthermore, pH 7.0 was near to the pK$_a$ of NaH$_2$PO$_4$-Na$_2$HPO$_4$ (pK$_a$=7.20 [21]) buffer solution. Therefore, its buffer capacity was good and adequate. Hence, 0.05 M NaH$_2$PO$_4$-Na$_2$HPO$_4$ (pH=7.0) was chosen as the buffer solution in the experiment.
3.4 Influence of accumulation time and potential

For both HPD and HXY, as the accumulation time increased, the anodic peak currents also increased. After 180 s, the responses reached the maximum, which indicated the accumulation reached saturation. As too long accumulation time might reduce the stability of MWNTs film, 180 s was generally chosen as accumulation time. When the accumulation potential varied from –0.8 to +0.6 V, the peak current increased until it attained the maximum at –0.4 or –0.2 V, then it decreased. However, compared with at open circuit, the effect of accumulation potential was negligible. Hence, open circuit accumulation was adopted.

3.5 Influence of amount of MWNTs suspension

Figure 5 showed the amount of MWNTs suspension had influence on the peak current. When the amount varied from 4 to 8 µL, the peak current remained constant. Otherwise it decreased. This related to the thickness of the film. If the film was too thin, the amount of adsorbed HPD and HXY was fewer, thus the response was smaller; while it was too thick, its conductivity decreased. At the same time, the film became a little instable because MWNTs could leave off the electrode surface. Therefore, the peak current decreased. Generally, 5 µL MWNTs suspension was chosen in this case.

Figure 5. Influence of amount of MWNTs suspension on the anodic peak current of HPD or HXY. Other conditions as in Figure 3.

3.6 Calibration curve

Under the optimum analytical conditions, the determination of HPD and HXY with different concentrations was performed. It was found that the anodic peak current was linear to concentration within the range from $1\times10^{-7}$ to $2.5\times10^{-5}$ M and $5\times10^{-8}$ to $2.5\times10^{-5}$ M, the regression equations were:
I_p=14.546+11.998c (I_p (µA), c (µM), r=0.997) and I_p=7.369+14.475c (r=0.997), respectively. The detection limits obtained were 8×10^{-9} and 5×10^{-9} M (S/N=3), separately.

3.7 Regeneration, reproducibility, stability and selectivity of MWNTs/GC modified electrode

The MWNTs/GC modified electrode could be easily regenerated by repetitive cycling in a blank solution for several times. For eight parallel determinations of a 5×10^{-5} M HPD or HXY solution with the same electrode, the RSD of peak current was calculated to be 2.12% and 2.72%, respectively. This indicated the modified electrode had good reproducibility. After continuous use for 7 days, the modified electrode could retain 95% and 91% of its initial responses, separately, which suggested the modified electrode had high stability.

The influence of foreign compounds was tested. For HPD and HXY, it was found that several kinds of surfactants such as sodium dodecyl sulfate (SDS), cetylpyridinium bromide (CPB), cetyltrimethylammonium bromide (CTAB), Tween-20, Triton X-100 made the peak decrease. 50-fold Cu^{2+}, Ag^{+}, propranolol, glucose, phenylalanine, glycine, allopurinol, captopril, 100-fold Fe^{3+}, Pb^{2+}, Zn^{2+}, thiamine, riboflavin, folic acid, antipyrine, norfloxacin did not interfere the detection, while ascorbic acid and epinephrine interfered severely. This suggested the MWNTs/GC modified electrode had certain resistance to some interference.

3.8 Applications

This method was applied to the detection of HPD or HXY in drug tablets. The pretreatment and determination procedure were the same as described in Experimental. The analytical results are shown in Table 1, the recovery was 95.5-102.0% and 96.4-101.3%, respectively.

| No. | Add (µM) | Found (µM) | Recovery % |
|-----|----------|------------|------------|
| HPD (2.66 µM) | | | |
| 1 | 2.66 | 5.20 | 95.5 |
| 2 | 5.32 | 7.82 | 97.0 |
| 3 | 7.98 | 10.80 | 102.0 |
| 4 | 10.64 | 13.50 | 101.9 |
| HXY (2.79 µM) | | | |
| 1 | 2.79 | 5.48 | 96.4 |
| 2 | 5.58 | 8.27 | 98.2 |
| 3 | 8.37 | 11.30 | 101.7 |
| 4 | 11.16 | 14.10 | 101.3 |

According to Table 1, the HPD or HXY contents could be calculated, which was 1.98 and 24.9 mg/tablet (their declared contents were 2 and 25 mg/tablet), respectively. In order to examine the accuracy of this method, the drug samples were also measured by UV method proposed by Chinese
Pharmacopoeia [3]. By means of $F$-test and $t$-test [22], the calculated $F$-value (2.01 and 1.70) and $t$-value (0.279 and 0.375) of the results obtained did not exceed the theoretical values (5.05 and 2.23, respectively, 95% confidence level). It suggested that the determination method proposed was reliable.

4. Conclusions

A MWNTs/GC-modified electrode was fabricated and characterized by electrochemical methods. When the potential was scanned, HPD and HXY could generate one or two anodic peaks at the modified electrode, respectively. Sensitive and quantitative measurements of these substances based on the first two anodic peaks was established under the optimum conditions. The anodic peak current was linear to HPD or HXY concentration over a certain range. The modified electrode exhibited some excellent characteristics, including easy regeneration, high stability, good reproducibility and selectivity. This method proposed could be successfully applied to the determination of HPD or HXY in practical drug samples and proved to be reliable.

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