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Electrochemical behavior and determination of fluphenazine at multi-walled carbon nanotubes/(3-mercaptopropyl)trimethoxysilane bilayer modified gold electrodes

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Abstract

A novel multi-walled carbon nanotubes/(3-mercaptopropyl)trimethoxysilane (MPS) bilayer modified gold electrode was prepared and used to study the electrochemcial behavior of fluphenazine and determine it. Fluphenazine could effectively accumulate at this electrode and produce two anodic peaks at about 0.78 V and 0.93 V (versus SCE). The peak at about 0.78 V was much higher and sensitive, thus it could be applied to the determination. Various conditions were optimized for practical application. Under the selected conditions (i.e. 0.05 M pH 3.5 HCOOH–HCOONa buffer solution, 5 μl 1 mg ml^{-1} multi-walled carbon nanotubes for $\Phi = 2.0$ mm electrode, accumulation at open circuit for 180 s), the anodic peak current was linear to fluphenazine concentration in the range from 5×10^{-8} to 1.5×10^{-5} M with correlation coefficient of 0.9984, the detection limit was 1×10^{-8} M. For a 1×10^{-5} M fluphenazine solution, the relative standard deviation of peak current was 2.51% ($n = 8$). This method was successfully applied to the determination of fluphenazine in drug samples and the recovery was 96.4–104.4%. The electrode could be easily regenerated and exhibited some selectivity, but some surfactants reduced the peak current greatly. The modified electrode was characterized by alternating current impedance and electrochemical probe.

Keywords: Electrochemical behavior; Fluphenazine; Multi-walled carbon nanotubes; (3-Mercaptopropyl)trimethoxysilane; Gold electrodes

1. Introduction

Fluphenazine is a derivative of phenothiazine. Its molecular structure is as shown in Scheme 1. It has neuroleptic and antidepressive actions, hence has been widely used in the treatment of psychotic patients [1]. The characteristics and detection of fluphenazine were studied by means of spectrophotometry [2], chemiluminescence [3], colorimetry [4], high-performance liquid chromatography [5], gas-chromatography [6], titrimetry [7], flow-injection analysis, etc. [8]. Because of the electroactivity of fluphenazine, it was studied and determined electrochemically. Bishop and Hussein [9], Senturk et al. [10] and Bouklouze et al. [11] used different electrodes including Au, Pt, ion-selective electrodes to study its electrochemical mechanisms and got some kinetic parameters. Peng et al. [12], and Jarbawi and Heineman [13], respectively, used carbon paste electrodes and wax-impregnated graphite electrodes to determine it, the detection limits were 1×10^{-8} and 5×10^{-8} M.

Since carbon nanotubes (CNTs) were discovered in 1991, they have attracted much attention of researchers [14]. Owing to their unique advantages such as high conductivity, high chemical stability, extremely high mechanical strength and modulus, CNTs are expected to be widely used in many fields including nanoelectronic devices, electron field emission sources and potential hydrogen storage material [15]. In electroanalytical field, as they can promote electron-transfer reactions, they are used as electrode materials and to prepare sensors [16]. It has been reported that CNTs modified electrodes were successfully applied to study and determine many biological and organic molecules such as xanthine [17], daunomycin [16], dopamine [18], cysteine [19], uric acid [20], amino acids [21], 6-mercaptopurine [22], 3,4-dihydroxy-phenylacetic acid [23], hydrazine [24] and nitrite [25]. Nevertheless, there is no report about phenothiazine and its derivatives studied by using CNTs modified

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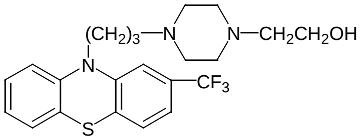

Scheme 1. Molecular structure of fluphenazine.

electrodes. Among the previous documents, the substrate electrodes used were mainly glassy carbon electrodes and powder microelectrodes. Metal substrate electrodes were seldom utilized probably due to the inconvenience to immobilize CNTs on metal surfaces. Among the few examples, Wang et al. directly dropped minor amount of single-walled carbon nanotubes (i.e. SWNTs) on gold electrodes to prepare modified electrodes. Using this resulting electrode, they studied the electrochemical behavior of uric acid and got a satisfactory result [20]. However, the CNTs modified gold electrodes prepared by such way is not stable enough. In order to fabricate stable CNTs modified metal electrodes, chemical covalent method was adopted to immobilize CNTs, but the preparation procedure was relative complicated and time-consuming.

(3-Mercaptopropyl)trimethoxysilane (MPS) is a good conjunction reagent with two functional groups. It was once used to immobilize gold nanoparticles on gold electrode surface so as to fabricate a horseradish peroxidase biosensor [26]. In this paper, MPS was let to assemble on gold electrode to immobilize MWNTs. Thus, a novel multi-walled carbon nanotubes/(3-mercaptopropyl)trimethoxysilane bilayer modified gold electrode (i.e. MWNTs/MPS/Au) was fabricated. The resulting electrode could be regenerated easily and exhibited good reproducibility and stability. Using such modified electrodes, the electrochemical behavior of fluphenazine was investigated and a procedure for its voltammetric determination was proposed.

2. Experimental

2.1. Reagents

Fluphenazine hydrochloride ($C_{22}H_{26}F_3N_3OS\cdot 2HCl$) was purchased from Sigma and its stocking solution (5×10^{-3} M) was prepared with *N*,*N*-dimethyl formamide (DMF). 5×10^{-2} M (3-mercaptopropyl)trimethoxysilane ($HS(CH_2)_3Si(OCH_3)_3$, Aldrich, 95%) stocking solution was also prepared with DMF. Multi-walled carbon nanotubes came from Shenzhen Nanotech Port Co. Ltd. (diameter 10–30 nm, length 0.5–40 μm, Shenzhen, China). Buffer solution was a 0.05 M HCOOH–HCOONa ($pH = 3.5$) aqueous solution. The drug sample came from JIUFU Pharmaceutical Company of Shanghai (Shanghai, China). Prior to determination, it was ground into powder, dissolved in DMF, filtered into a container and then diluted to certain volume. Other reagents used were of analytical grade or chemical grade, and their solutions were prepared with twice distilled water or DMF.

2.2. Apparatus

Electrochemical measurements were carried out on a CHI 617A electrochemical analytical instrument (CH instrument company, Shanghai, China). A three-electrode system was used, including a bare gold electrode ($\Phi = 2.0$ mm) or modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. Electrochemical impedance spectroscopy (EIS) was performed with a Model 273A bipotentiostat in conjunction with a lock-in amplifier (EG&G PAR Co., USA). The frequency range was from 0.1 to 100 kHz. Solution pH was measured with a Mettler-Toledo 320-s pH meter (Shanghai, China).

2.3. Preparation of MWNTs and modified electrode

Multi-walled carbon nanotubes (i.e. MWNTs) was refluxed in the mixture of concentrated H_2SO_4 and HNO_3 for 4–5 h, then washed with twice distilled water and dried in vacuum at room temperature. The MWNTs suspension was prepared by dispersing 2 mg MWNTs in 2 ml DMF in the aid of ultrasonic agitation.

The bare gold electrode was first polished to a mirror-like surface with 0.3 and 0.05 μm α-alumina slurry, then washed with water and rinsed ultrasonically for 3 min in twice distilled water, followed by absolute ethanol, diluted sulfuric acid, twice distilled water, and dried in air. If necessary, the electrode was immersed in a Piranha solution (a mixed solution of 30% H_2O_2 and concentrated H_2SO_4, $V_1/V_2 = 1/3$) for 3 min before washing with water. The electrode was then cycled between 0 and $+1.5$ V (versus SCE) in 0.5 M H_2SO_4 until a stable cyclic voltammogram (CV) was obtained. According to the cathodic peak area of the gold electrode, the effective area of the electrode was calculated to be 0.0522 cm^2. Then the clean electrode was immersed in 0.05 M (3-mercaptopropyl)trimethoxysilane (i.e. MPS) solution for 12 h, then taken out and rinsed thoroughly with twice distilled water to remove physically adsorbed MPS. Thus MPS self-assembled monolayer modified (SAM) gold electrode was obtained (i.e. MPS/Au). The MWNTs/MPS/Au bilayer modified electrode was prepared by means of dropping 5 μl of MWNTs suspensions on the SAM modified electrode surface and evaporating the solvent in air.

2.4. Procedure

The three-electrode system was immersed in a 10 ml cell containing proper amount of fluphenazine and 0.05 M HCOOH–HCOONa ($pH = 3.5$) 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 $+0.3$ and $+1.0$ V, with a scan rate of 100 $mV\,s^{-1}$. All measurements were carried out at room temperature.

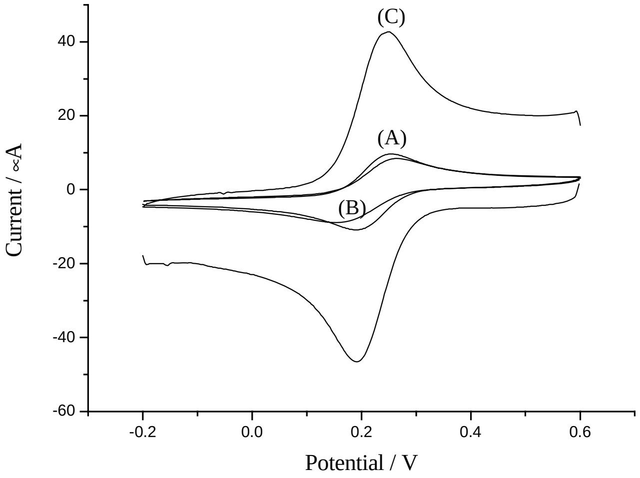

Fig. 1. Cyclic voltammograms (CV) of 2 mM $K_3Fe(CN)_6$ at Au electrode (A), MPS/Au (B) and MWNTs/MPS/Au (C). Supporting electrolyte: 0.5 M KNO_3.

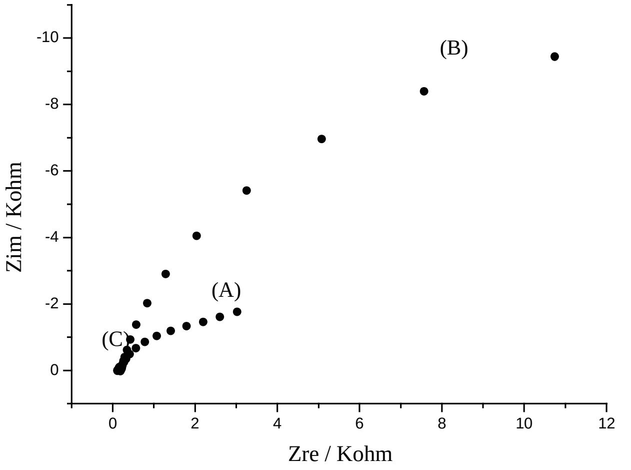

Fig. 2. Electrochemical impedance spectroscopy of Au electrode (A), MPS/Au (B) and MWNTs/MPS/Au (C). Frequency range used: 0.1–100 kHz. Solution: 2 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ containing 0.1 M KNO_3.

3. Results and discussion

3.1. Electrochemical characterization of modified electrode

As shown in Fig. 1, $K_3Fe(CN)_6$ exhibits a pair of reversible redox peaks at a bare gold electrode. The separation of peak potential ΔE_p is about 66 mV and the ratio of peak current i_{Pa}/i_{Pc} is about 1. When it is modified with a MPS SAM, the ΔE_p becomes about 112 mV and the peak current decreases by 20%, indicating the monolayer can restrain $K_3Fe(CN)_6$ from arriving at the electrode surface so that the electrode process becomes not so reversible. On the contrary, when it is further modified by MWNTs, the ΔE_p reduces to about 56 mV and the i_{Pa}/i_{Pc} also returns to about 1, suggesting the electrode process is more reversible. However, the peak current is much higher than that of bare gold electrode. This should be attributed to the significant increment in electrode surface area. Under the same conditions, when scan rate increased from 50 to 500 $\mathrm{mV\,s^{-1}}$, the peak of $K_3Fe(CN)_6$ grew for both bare gold and bilayer modified electrode. It was found the electrode process was controlled by diffusion, the regression equations were $i_p = 0.531 + 1.005v^{1/2}$ (i_p (μA), v ($\mathrm{mV\,s^{-1}}$), $r = 0.9995$) and $i_p = -11.503 + 5.289v^{1/2}$ ($r = 0.9994$), respectively. For a reversible system, it should satisfy the Randles–Sevcik equation: $i_{pa} = 2.69 \times 10^5 n^{3/2} A C_0 D_R^{1/2} v^{1/2}$ [27]. According to the ratio of slopes of the two lines, the area of the bilayer modified electrode was calculated to be about five times as large as that of gold electrode.

Fig. 2 shows the ac impedance spectroscopy of the above three electrodes in the given solution. The resistor of the MPS SAM modified electrode is larger than that of the bare gold electrode, which means it has stronger hindrance to the transfer of $Fe(CN)_6{}^{4-/3-}$. According to the impedance of the two electrodes in high frequency range, the coverage (θ) of MPS monolayer was estimated to be 85.6% ($R_{ct} = 3.448\,\mathrm{k\Omega}$, $R'_{ct} = 23.89\,\mathrm{k\Omega}$, $\theta = 1 - R_{ct}/R'_{ct}$ [28]). However, the resistor of MWNTs/MPS/Au bilayer modified electrode is relative smaller, indicating it has much less hindrance than the other two electrodes. The results of EIS are in accordance with the CV characterization by $K_3Fe(CN)_6$.

3.2. Cyclic voltammograms of fluphenazine

There were no observable peaks for fluphenazine at bare gold and MPS SAM modified electrode, suggesting it did not accumulate at the two-electrode surface and the electrochemical reaction was very slow. At MWNTs/MPS/Au bilayer modified electrode, however, two anodic peaks occurred. One was at about 0.78 V and another at about 0.93 V (versus SCE). As in a blank solution no peaks appeared, it could be inferred that fluphenazine effectively accumulated at bilayer modified electrode surface and its electrochemical oxidation was promoted (Fig. 3). At the same time, the background current became greater due to the increased electrode surface. As it can be seen, the peak at about 0.78 V was much higher and sensitive, so it was favorable for fluphenazine determination. With the potential cyclic repeating, the anodic peak current decreased rapidly. Therefore, the voltammograms corresponding to the first cycle were generally recorded.

3.3. Influence of scan rate

With scan rate increasing, the peak current grows and peak potential shifts positively. There is a good linear relationship between peak current and scan rate from 50 to 300 $\mathrm{mV\,s^{-1}}$. The regression equation is $i_p = 30.05 + 0.2175v$ ($r = 0.9987$), indicating the electrode process is controlled by adsorption. In addition, the peak potential and the logarithm of scan rate shows a linear relationship, following

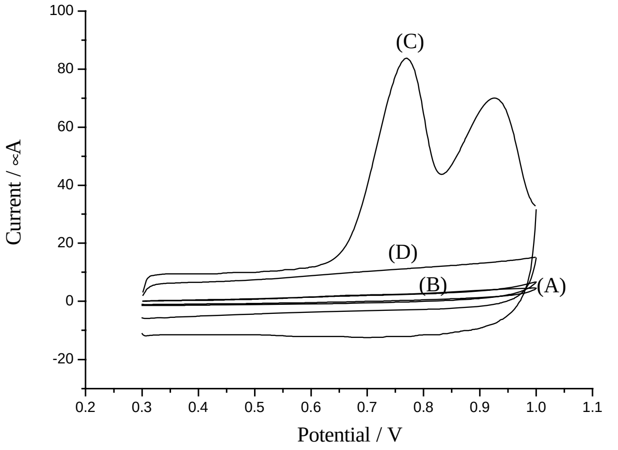

Fig. 3. CVs of Au electrode (A), MPS/Au (B) and MWNTs/MPS/Au (C) in a 1×10^{-5} M fluphenazine solution. MWNTs/MPS/Au in a blank solution (D). Scan rate: 100 mV s^{-1}; supporting electrolyte: 0.05 M HCOOH–HCOONa (pH 3.5); accumulation time: 180 s (at open circuit); volume of MWNTs suspension: 5 µl.

the equation: $E_{p,a} = 0.4092 + 0.091 \ln v$ ($E_{p,a}$ (V), $r = 0.993$), which suggests the oxidation process is irreversible. The invisible or smaller cathodic peak at CV also can confirm this point. For a irreversible adsorbed system, it should satisfy the equation $i_p = n^2F^2vA\Gamma_0^*/4RT = nFQv/4RT$ [29], thus n was calculated to be 1.2, meaning one electron was lost during the first step of oxidation. According to the equation $E_{p,a} = E^{\Phi} + [RT/(1-\alpha)nF]\ln(1-\alpha) - [RT/(1-\alpha)nF]\ln(RTk_s/nF) + [RT/(1-\alpha)nF]\ln v$ [30], the charge-transfer coefficient α was estimated to be 0.73.

3.4. *Influence of pH*

Within the range of pH 2–6, the peak potential shifted negatively with pH increasing, following the linear equation $E_p(V) = 0.8759 - 0.02785$ pH ($r = 0.993$), suggesting H^+ took part in the oxidation. Meanwhile, the peak current increased gradually until it attained the maximum at pH 3.5, then it decreased (Fig. 4). When pH was lower than 2 or higher than 6, both peaks became poor-defined. Especially in basic solutions, more peaks appeared, meaning the oxidation mechanism changed to some extent (Fig. 5). Thus, pH 3.5 was chosen as the optimum pH value.

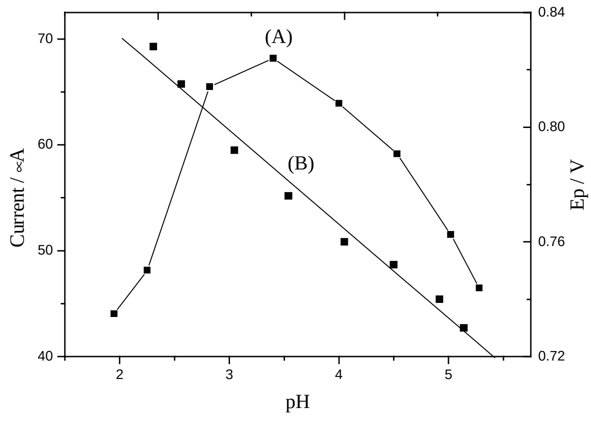

Fig. 4. Influence of pH on the anodic peak current (A) and peak potential (B). Blank solution: 0.05 M phosphate salt; other conditions as in Fig. 3.

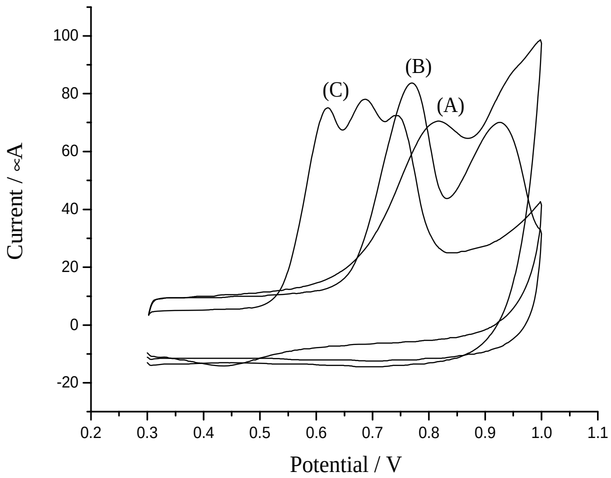

Fig. 5. Influence of pH on the shape of anodic peak. pH: 2.25 (A), 3.50 (B) and 7.40 (C). Other conditions as in Fig. 3.

3.5. *Influence of buffer solution*

When the solution pH was set at pH 3.5, several different supporting electrolytes such as $KHC_8H_4O_4$–HCl, HCOOH–HCOONa, HAc–NaAc, H_3PO_4–NaH_2PO_4 were tested. The result shows that the peak was more stable and higher in HCOOH–HCOONa buffer solution. In addition, there was no obvious change observed in peak current and peak potential when the concentration of buffer solution changed from 0.05 to 0.2 M. In order to maintain sufficient buffer capacity and avoid the increment of background current because of the concentration of buffer solution increasing, a 0.05 M HCOOH–HCOONa buffer was chosen as the supporting solution.

3.6. *Influence of accumulation time and accumulation potential*

With accumulation time increasing, the peak current increased. After 180 s, it got the maximum value for a 1×10^{-5} M fluphenazine solution (Fig. 6). This indicates the saturation accumulation was reached. As too long accumulation time might reduce the stability of MWNTs film, 180 s was generally chosen as accumulation time. When accumulation potential varied from +0.6 to −0.6 V, the peak current increased and attained the maximum at about −0.2 V, then it decreased (Fig. 7). However, compared with at open circuit, peak current for accumulation at −0.2 V was a little higher. Hence accumulation at open circuit was adopted.

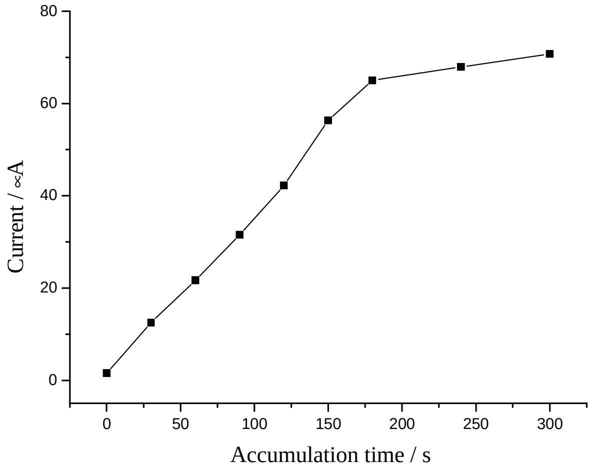

Fig. 6. Variation of the anodic peak current with accumulation time. Other conditions as in Fig. 3.

3.7. *Influence of amount of MWNTs*

Fig. 8 shows that the amount of MWNTs has influence on the peak current. When the amount is from 5 to 8 μl, the peak current is more stable and higher. Otherwise, it decreases. This is related to the thickness of the film. If the film was too thin, the fluphenazine amount adsorbed was small, thus the peak current was small. When it was too thick, the film conductivity reduced and the film became not so stable as MWNTs could leave off the electrode surface. Meanwhile, the peak current also decreased. Generally, 5 μl MWNTs suspension solution was used in this case.

3.8. *Calibration curve*

Under the selected analytical conditions, the determination of fluphenazine solutions of different concentrations was carried out. There was a good linear relationship observed between the anodic peak current and the concentration from 5×10^{-8} to 1.5×10^{-5} M. The regression equation was $i_p = 1.591 + 5.255c$ (i_p (μA), c (μM), $r = 0.9984$), the detection limit was 1×10^{-8} M.

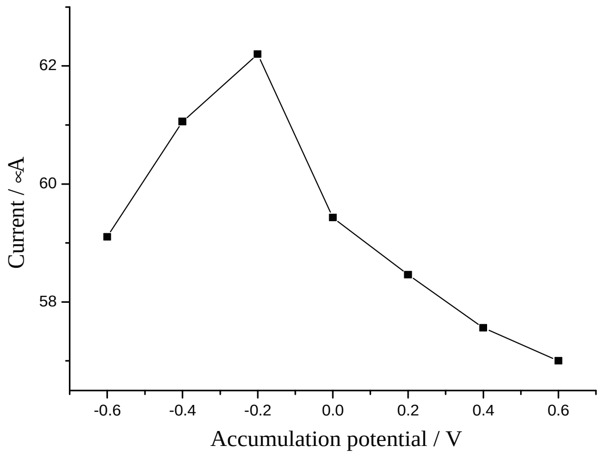

Fig. 7. Variation of the anodic peak current with accumulation potential. Other conditions as in Fig. 3.

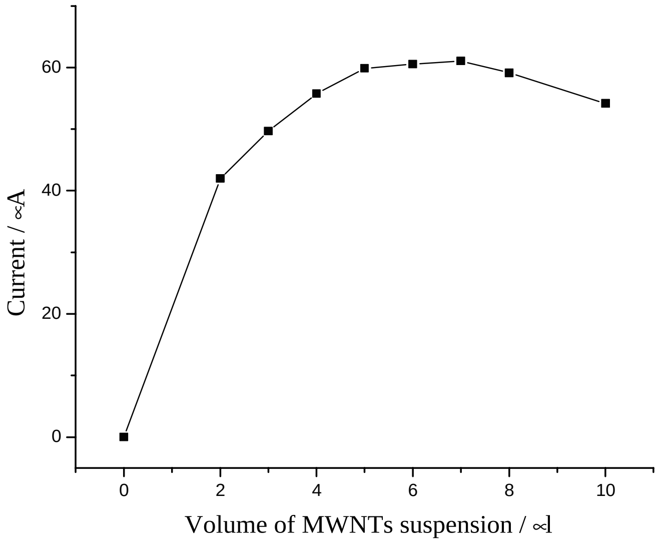

Fig. 8. Influence of MWNTs suspension (1 mg ml^{-1}) volume used on the anodic peak current. Other conditions as in Fig. 3.

3.9. *Regeneration and reproducibility of MWNTs/MPS/Au bilayer modified electrode*

The bilayer modified electrode could be easily regenerated by repetitive cycling in a blank solution for several times. For eight successive determination of a 1×10^{-5} M fluphenazine with the same electrode regenerated after every determination, the R.S.D. was calculated to be 2.51%. A 1×10^{-5} M fluphenazine solution was measured with the same electrode every several hours within-day, the R.S.D. of the peak current was 2.64% ($n = 5$). As to the between-day reproducibility, it was similar to that of within-day if the temperature was kept almost unchanged. Owing to the loss of the bilayer, the current response of the modified electrode would decrease after successive use for some time. In this case, the electrode should be modified again.

3.10. *Interference*

The influence of some heavy metal ions and organic compounds were tested. The results show 100-fold of Cu^{2+}, Zn^{2+}, Fe^{3+}, Pb^{2+}, thiamine, glycine, glucose, 50-fold of ascorbic acid, epinephrine, folic acid, riboflavine, chloramphenicol did not interfere the determination, while Ag^{+} and L-cysteine interfered severely. This suggests the bilayer modified electrode had certain resistance to some interference. Meanwhile, several kinds of surfactants such as Tween-20, Triton X-100, sodium dodecyl sulfate (SDS), cetylpyridinumbromide (CPB),

Table 1
Measurement results of fluphenazine in drug

Sample solution	Add (μM)	Expected (μM)	Found (μM)	Recovery (%)	Average recovery (%)
1[a]	0	–	2.51	–	
	2.51	5.02	5.24	104.4	
	5.02	7.53	7.69	102.1	101
	7.53	10.04	9.68	96.4	

[a] Drug tablet came from JIUFU Pharmaceutical Company of Shanghai (China).

Table 2
Replicated measurement results of fluphenazine in drug sample

	Solution no.[a]							
	1	2	3	4	5	6	7	8
Found (μM) (spectrophotometry)	2.53	2.57	2.55	2.53	2.57	2.58	2.57	2.53
Found (μM) (this method)	2.56	2.56	2.54	2.48	2.50	2.49	2.52	2.48

[a] The sample solutions were prepared as in Table 1. Statistical analysis results: $F_{\mathrm{exp}} = 2.45$, smaller than the F_{crit} value (3.79, at the 5% probability level); $t_{\mathrm{exp}} = 2.1$, smaller than the corresponding t_{crit} (2.2, at the 95% confidence level) [31].

cetyltrimethylammonium bromide (CTAB) were tested and it was found that they all depressed the peak current. This might have a relation with the interaction between surfactants and fluphenazine.

3.11. Applications

This method was applied to the determination of fluphenazine in drug sample (i.e. Fufennaijing). The pretreatment and determination procedure were the same as described in Section 2. The analytical results were shown in Table 1, the recovery was 96.4–104.4%. According to Table 1, the fluphenazine contents could be calculated, which was 2.1 mg per tablet (its declared content was 2 mg per tablet). In order to examine the accuracy of this method, the drug sample was also measured by absorption spectrophotometry, the analytical results are shown in Table 2. By means of F-test and t-test, it can be concluded this method is reliable [31].

4. Conclusions

Fluphenazine could effectively accumulate at the MWNTs/MPS/Au bilayer modified electrode. When potential was made to move, it produced two anodic peaks at about 0.78 and 0.93 V (versus SCE) in 0.05 M pH 3.5 HCOOH–HCOONa buffer solution. The peak at about 0.78 V was suitable for analysis and the peak current was linear to fluphenazine concentrations over a certain range under the selected conditions. This procedure could be applied to the determination of fluphenazine in drug tablets. The electrode could be easily regenerated and had some selectivity, but some surfactants reduced the peak current greatly.

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