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Voltammetric Determination of Tetracycline by Using Multi-Wall Carbon Nanotube – Ionic Liquid Film Coated Glassy Carbon Electrode

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A voltammetric method was developed for the determination of tetracycline (TC) by using an ionic liquid (IL, 1–octyl–3–methylimidazolium–hexafluorophosphate) – multi-wall carbon nanotube (MWNT) film coated glassy carbon electrode (GCE). Experiment showed that both IL and MWNT could facilitate the TC oxidation. Thus on the electrode TC exhibited a sensitive anode peak at 0.54 V (vs. SCE) in pH 7.0 phosphate buffer solutions. Under the optimized experimental conditions, the peak current was linear to TC concentration in the range of 1.1×10^{-7}–2.2×10^{-5} M for 150 s accumulation. The electrode had good reproducibility. It was successfully applied to the detection of TC in egg and pharmaceutical samples.

Keywords: Tetracycline, ionic liquid, carbon nanotube, voltammetry

1. INTRODUCTION

Tetracycline (TC) is a widely used antibiotic and it has high activity against nearly all Gram–positive and Gram–negative bacteria [1]. However, owing to the extensive use of this antibiotic sometimes TC residue is found in food products such as milk, egg and shrimp [2]. TC can adsorb strongly onto environmental materials, where it still keeps its activity [3]. Various studies indicate that low-level dose of antibiotics for long period could show bacteria resistance [4]. In order to ensure food safety, maximum residue limits (MRLs) have been set for TC, chlortetracycline and oxytetracycline in a number of food products, for examples, 0.3 mg kg^{-1} in liver, 0.6 mg kg^{-1} in kidney, 0.2 mg kg^{-1} in egg and 0.1 mg kg^{-1} in milk and muscle tissue [5,6]. And several methods for TCs determination have been developed, such as microbiological method [7], high–performance liquid chromatographic method (HPLC) with UV [8-10], fluorescence [11], chemiluminescence [12] and mass spectrometry [13-15] detection. Although these methods have advantages of sensitivity and accuracy, their high cost and complicated operation procedure limit their extensive application. Electrochemical methods are simple, speedy, sensitive and inexpensive, thus they are alternative for TC determination [1,16].

Carbon nanotubes (CNTs) are widely used in many fields [17]. CNTs based modified electrodes can promote the electron transfer of analytes and enhance their response sensitivity. Ionic liquid is composed entirely of ions. It possesses lots of merits such as wide electrochemical window, excellent chemical stability, thermal stability and high conductivity. As electrode modifying material it can immobilize other material on electrode surface and promote the electron transfer [18]. Taking into account the advantages of IL and CNT, they were combined to fabricate modified electrodes. Zhao et al. prepared a CNT–IL gel modified electrode, it could be used for the selective detection of dopamine in the presence of ascorbic acid and uric acid [19]. Choi et al. immobilized organophosphorus hydrolase on CNT–ionic liquid electrode for organophosphate chemical detection [20]. Liu et al. immobilized glucose oxidase (GOD) on CNT–IL gel coated GCE and the GOD showed more reversible electron transfer process and higher electron transfer rate [21]. Liu et al. used single-wall carbon nanotube–ionic liquid gel modified electrode to realize the sensitive detection of p–nitroaniline [22]. Fan et al. fabricated an ionic liquid – single-walled carbon nanotube paste coated glassy carbon electrode; the electrode was applied for the determination of methylparathion in real samples [23].

In the present paper, ionic liquid–carbon nanotube paste modified electrode is fabricated and used for the detection of TC, and its performance with respect to sensitivity, linear range and selectivity is evaluated and discussed. The applicability of the nanocomposite electrode is demonstrated through determining TC in samples.

2. EXPERIMENTAL PART

2.1. Reagents

Tetracycline (TC) was obtained from Medication Inspection Bureau of Hubei (Wuhan, China), and the TC stock solution (2.25×10^{-3} M) was prepared with absolute ethanol. The multi-walled carbon nanotubes used (MWNTs, diameter: 10–30 nm, length: 0.5–40 µm, purity: ≥95%) came from Shenzhen Nanotech. Port Co. Ltd. (Shenzhen, China). The ionic liquids 1–octyl–3-methylimidazolium hexafluorophosphate (OMIMPF$_6$) was purchased from Acros Organics and used as received. Paraffin liquid was obtained from Sinopharm Group Chemical Reagent Co. (Shanghai, China). All other chemicals used were of analytical reagent grade. The water used was redistilled.

2.2. Apparatus

Voltammetric experiments were carried out on a CHI660A electrochemical workstation (CH Instrument Company, USA). A conventional three-electrode system was employed, including a modified glassy carbon electrode (2 mm in diameter) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. The scanning electron microscope (SEM) image was obtained using a HITACHI X–650 SEM (Hitachi, Japan). The pH value of solution was measured with a Mettler Toledo 320–S pH meter (Shanghai, China).

2.3. Preparation of modified electrode

Prior to modification, the glassy carbon electrode was carefully polished to mirror smooth with 0.05 μm alumina slurry on a polishing pad, ultrasonicated in ethanol and distilled water for several minutes respectively. The MWNTs were pretreated by reflowing in HNO_3–H_2SO_4 (volume ratio 1:1) mixture for 2 h at 55 ℃ and then for 3 h at 80 ℃, washed with redistilled water and dried in vacuum. Six milligram treated MWNTs and 20 μL IL were mixed and ground in an agate mortar to make a uniform paste. Then proper amount of IL–MWNT mixture was transferred to the cleaned GCE, and smoothed with a spatula. Thus, an IL–MWNT paste modified glassy carbon electrode (denominated as IL–MWNT/GCE) was obtained. The thickness and area of the paste film were controlled by using a sticking film with a hole. The paraffin liquid–MWNT/GCE was fabricated in similar way.

2.4. Experimental procedure

A 10 mL 0.1M phosphate buffer solution (pH = 7.0) with certain amount of TC was transferred to the electrochemical cell. Then the electrode system was immersed into it. After an accumulation on open circuit for 150 s under stirring, potential scan was initiated. The linear sweep voltammogram (LSV) was recorded from 0.2 to 0.8 V at scan rate of 0.1 Vs^{-1}. For the study on the effect of solution pH, the voltammogram was recorded between 0.1 and 1.0 V. After each measurement, the modified electrode was regenerated by successive potential scan between 0.2 V and 0.8 V in a 0.10 M phosphate buffer solution (pH=7.0) for four times. All electrochemical experiments were performed at room temperature.

2.5. Sample preparation

The pharmaceutical sample was treated as follows: some commercial tablets containing TC were pulverized and dissolved with anhydrous ethanol. The obtained solution was filtered, and the filtrate was diluted to certain volume for detection.

Egg sample and redistilled water was transferred into a flask, stirred until a homogeneous solution was formed, then proper amount of the solution was introduced into a 50 mL volumetric flask, absolute ethanol was added to the mark. The mixture was shaken vigorously for about 15 min. Then 10 mL of the sample was centrifuged for 30 min at 3500 rad/s. The supernatant was determined.

3. RESULTS AND DISCUSSION

3.1. Characterization of different films

Figure 1 shows the SEM images of paraffin oil–MWNT film and $OMIMPF_6$–MWNT film on GCE. The surface of both films is quite even and the MWNT is indiscernible, which can be attributed to the binding and masking effect of viscous paraffin oil and $OMIMPF_6$. But the $OMIMPF_6$–MWNT film is more uniform than the paraffin oil–MWNT film. Furthermore, owing to the interaction between

MWNT and OMIM$^+$, MWNT bundles tend to becoming fine bundles and crosslink, and the OMIMPF$_6$–MWNT composite behaves like gel [24].

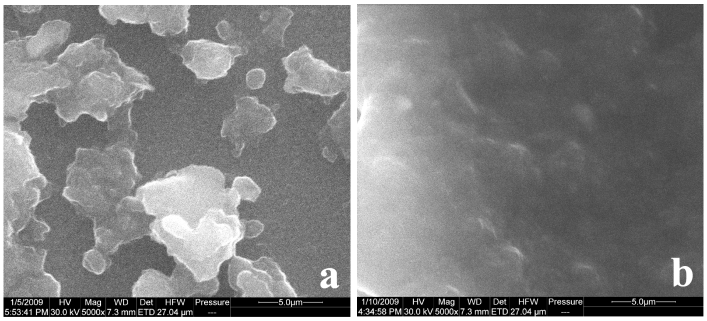

Figure 1. SEM images of paraffin oil–MWNT film (a) and OMIMPF$_6$–MWNT film (b).

3.2. Electrochemical performance of TC on different electrodes

The LSVs of TC on paraffin oil–MWNT/GCE and OMIMPF$_6$–MWNT/GCE are displayed in Fig. 2. Two bigger anodic peaks are observed for the latter, while on the former the peaks are small and broad. This reveals that IL can facilitate TC oxidation. In addition, the interaction of OMIMPF$_6$ and MWNT makes the effective area of MWNTs increase to some extent due to the improved dispersion of MWNTs. Hence, the OMIMPF$_6$–MWNT/GCE is chosen for the following experiments.

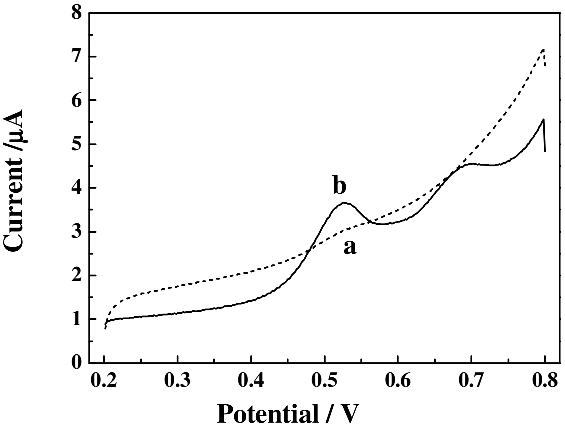

Figure 2. LSVs of paraffin oil–MWNT/GCE (a) and OMIMPF$_6$–MWNT/GCE (b) in solutions containing 9.0 µM TC. Scan rate: 0.1 V s^{-1}; supporting electrolyte: 0.10 M phosphate buffer solution (pH 7.0); accumulation time: 150 s (on open-circuit).

3.3. Optimization of some parameters

3.3.1. Solution pH

The influence of pH on the peak current (i_{pa}) and peak potential (E_{pa}) is investigated from pH 2.0 to pH 8.0 (Fig. 3). When the pH is lower than 5.0, the shape of the curve is worse and the peak current of TC decreases with pH rising, it reaches a minimum value at about pH 5.0. When solution pH exceeds 5.0 the peak current begin to increase, and the curve shape is improved. The best response of TC is observed at pH 7.0.

The peak potential moves in negative direction with pH rising and they show such relationship as: E_{pa} (V) = 1.00 – 0.064 pH (r = 0.996). The slope of –64 mV/pH demonstrates that the numbers of electron and proton transferred in the electrochemical reaction of TC are equal.

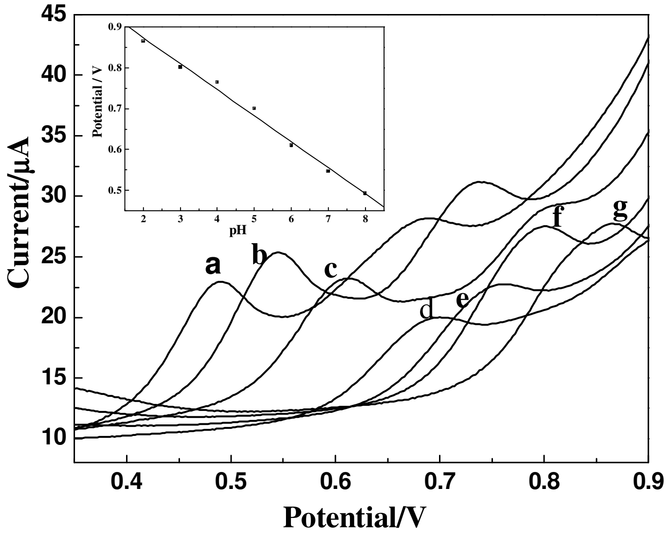

Figure 3. LSVs of 9.0 μM TC solutions with different pH. Solution pH: 8, 7, 6, 5, 4, 3 and 2 (from a to g); inset: E_{pa} vs. pH plot. Other conditions as in Fig. 2.

3.3.2. Accumulation time and accumulation potential

The peak current of TC grows rapidly with accumulation time prolonging, but when the accumulation time is above 150 s, the peak current keeps almost unchanged for a 9.0 μM TC solution. This indicates that the adsorption of TC on the film reaches equilibrium. In general, the accumulation time is fixed at 150 s. The influence of accumulation potential is tested from –0.5 to 0.3 V. The peak current of TC is almost independent of it. Thus, the accumulation is performed on open circuit.

3.3.3. Influence of scan rate

The influence of scan rate (v) on the voltammetric peak has been investigated in the range from 0.003 to 0.6 V s^{-1}. It is found that the anodic peak current of TC is linear to the scan rate (Fig. 4). The linear regression equation is: i_{pa} (μA) = 0.388 + 58.0 v (Vs^{-1}, r=0.998), indicating that the electrochemical process is adsorption-controlled. The peak potential moves in positive direction when scan rate increases, the relationship between peak potential and the logarithm of v follows such linear regression equation: E_{pa} (V) = 0.647 + 0.0374 log v (r=0.992), implying that the electron transfer is not very fast.

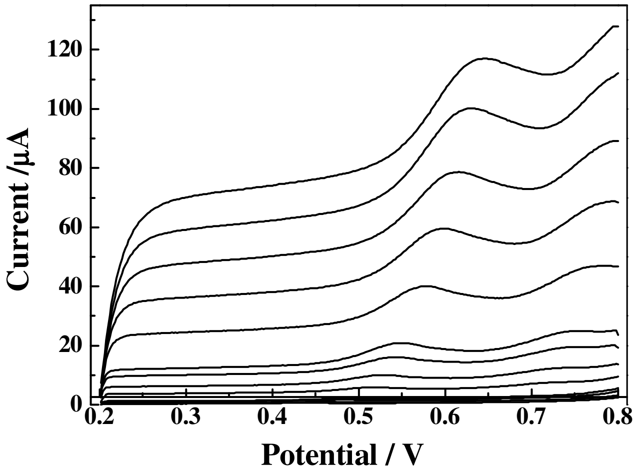

Figure 4. Influence of scan rate on LSV of 9.0 μM TC. Scan rate: 0.003, 0.005, 0.008, 0.01, 0.03, 0.05, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 V s^{-1} (from inner to outer). Other conditions as in Fig. 2.

3.4. Analytical performance of the OMIMPF$_6$–MWNT/GCE

3.4.1. Variation of peak current with TC concentration

Under the optimized conditions, a series of standard solutions of TC are prepared and determined to explore the relationship between peak current and TC concentration. It is found that the peak current is linear to TC concentration over the range of 1.1×10^{-7} – 2.2×10^{-5} M (Fig. 5). The regression equation is i_p (μA) = 0.94 + 0.76 c (μM, r=0.996). The detection limit is estimated to be 3×10^{-8} M.

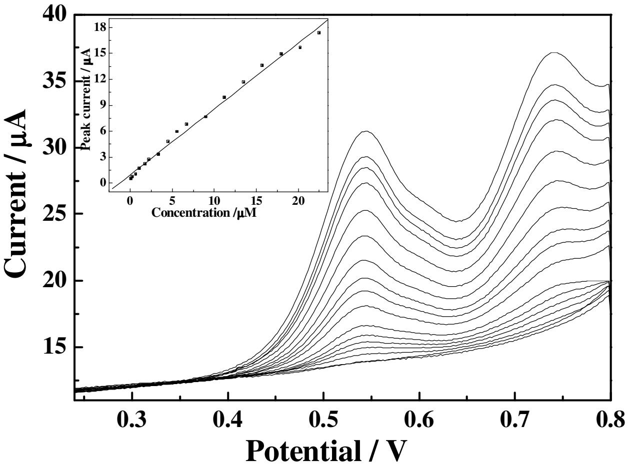

Figure 5. Variation of peak current with TC concentration. TC concentration: 0.11, 0.23, 0.68, 1.13, 1.80, 2.25, 3.38, 4.5, 5.63, 6.75, 9, 11.25, 13.50, 15.75, 18.00, 20.25 and 22.50 μM (from inner to outer). Inset: the plot of peak current vs. TC concentration. Other conditions as Fig. 2.

3.4.2. Repeatability and reproducibility

To test the repeatability and reproducibility of IL–MWNT/GCE, a 9 μM TC solution is measured for 10 times with a modified electrode regenerated after every determination. As a result, the relative standard derivation (RSD) for the peak current is about 2 %. Additionally, 9 μM TC solution is determined with 10 modified electrodes prepared by the same method, and the relative standard derivation (RSD) for the peak current is less than 7 %.

3.5. Application

This method was applied to the determination of TC in pharmaceutical and egg samples. A standard addition method was adopted to estimate the accuracy. The measurement results are shown in Table.1. The recoveries for the standards added are 91 % –105 %, which are acceptable. This indicates that the method has some reliability.

Table 1. Determination results of TC in tetracycline tablets and egg sample.

	Added (μM)	Expected (μM)	Found (μM)	Recovery(%)
Tetracycline tablet	0	3.38	3.11	-
	3.38	6.76	6.56	97.0
	6.75	10.1	10.3	102
	10.1	13.5	12.5	92.6
Egg	0	-	Nd	-
	2.25	2.25	2.36	105
	4.50	4.50	4.22	93.8
	6.75	6.75	6.14	91.0

ND: not detected.

4. CONCLUSIONS

IL–MWNT paste film can enhance the voltammetric response of TC and thus it can produce a sensitive anodic peak on IL–MWNT/GCE. The electrochemical process is irreversible and controlled by adsorption. The peak current and TC concentration show a linear relationship in a certain range. The electrode can be regenerated and it exhibits good reproducibility. This IL–MWNT/GCE could be applied to the determination of TC in some samples.

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