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Voltammetric determination of terbinafine in biological fluid at glassy carbon electrode modified by cysteic acid/carbon nanotubes composite film

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Abstract

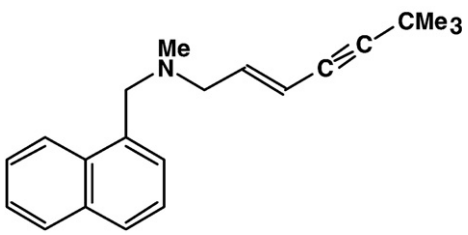
The electrochemical oxidation of L–cysteine (CySH) in presence of carbon nanotubes (CNTs) formed a composite film at a glassy carbon electrode (GCE) as a novel modifier for directly electroanalytical determination of terbinafine without sample pretreatment in biological fluid. The determination of terbinafine at the modified electrode with strongly accumulation was studied by differential pulse voltammetry (DPV). The peak current obtained at +1.156 V (vs. SCE) from DPV was linearly dependent on the terbinafine concentration in the range of 8.0×10^{-8}–5.0×10^{-5} M in a B–R buffer solution (0.04 M, pH 1.81) with a correlation coefficient of 0.998. The detection limit (S/N = 3) was 2.5×10^{-8} M. The low-cost modified electrode showed good sensitivity, selectivity, and stability. This developed method had been applied to the direct determination of terbinafine in human serum samples with satisfactory results. It is hopeful that the modified electrode will be applied for the medically clinical test and the pharmacokinetics in future.

Keywords: Terbinafine; Cysteic acid; *L*−cysteine; Carbon nanotubes; Voltammetry; Human serum

1. Introduction

Terbinafine (Scheme 1) is an allylamine derivative. Chemically it is (*E*)–*N*–(6, 6–dimethyl–2–hepten–4–ynyl)–*N*–methyl–1–naphthalenemethanamine ($C_{21}H_{25}N$). Like other allylamines, it inhibits ergosterol synthesis by inhibiting squalene epoxidase — an enzyme that is part of the fungal cell wall synthesis pathway [1]. In layman's terms, it inhibits fungal and bacterial cell wall growth, causing the contents of the cell to be unprotected and eventually die. So it is applied to the skin in the occurrence of dermatophytoses, pityriasis versicolor, and cutaneous candidiasis occurrence or superficial fungal infections like seborrheic dermatitis, tinea capitis, and onychomycosis especially for its short duration therapy [2]. Generally, terbinafine hydrochloride is the main chemical form of terbinafine for pharmaceutical purposes. Terbinafine comes as a tablet to take by mouth. It is usually taken once a day for 6 weeks for fingernail fungus and once a day for 12 weeks for toenail fungus. As a 1% cream or powder, it is used for superficial skin infections such as jock itch (*Tinea cruris*), athlete's foot (*Tinea pedis*) and Ringworm. It is highly lipophilic in nature and tends to accumulate in skin, nails, and fatty tissues. Excessive terbinafine may cause some side effects as follows: an allergic reaction (difficulty breathing; closing of your throat; swelling of lips, tongue, or face; or hives), a rash, changes in vision or blood problems and so on [3].

Quantitative determination of terbinafine in pharmaceutical formulations and human physiological fluids is of considerable



Scheme 1. Structure of terbinafine.

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significance in both quality control of preparations and clinical diagnoses. There are only a limited number of techniques described for the determination of terbinafine in its pharmaceutical formulations including capillary zone electrophoresis [4], HPLC [5,6], UV−spectrophotometric method [7–9], agar diffusion method [10] and polarography [11]. Pharmaceutical analysis in biological fluids plays an important role in studies of pharmacokinetics and pharmacodynamics both in experimental animals and human. Some studies on pharmacological actions of terbinafine have been done, however, there have been a very few methods reported about the determination of terbinafine in biological media. The determination of active contents of terbinafine in biological fluids is often achieved by chromatography but there are certain problems that still exist which require more sophisticated models to be solved. Denouel et al. developed a reliable reversed-phase high-performance liquid chromatographic method for the determination of terbinafine in human plasma with the detection limit was 2.0 ng/mL [12]. Zehender et al. detected terbinafine in human plasma and urine by high-performance liquid chromatography using on line solid-phase extraction, and the linear range was $0–2.5\times10^3$ ng/mL [13]. Though these methods showed good selectivity and sensitivity, they required several time-consuming manipulation steps, sophisticated instruments and special training.

Electrochemical detection of analysis is a very elegant method in analytical chemistry. The interest in developing electrochemical-sensing devices for use in environmental monitoring, clinical assays or process control is growing rapidly. Electrochemical sensors satisfy many of the requirements for such tasks particularly owing to their inherent specificity, rapid response, sensitivity and simplicity of preparation. Up to date, there is only an available polarography with the hanging mercury drop electrode for the determination of terbinafine in the literatures [11]. Arranz et al. determined terbinafine at −1.47 V vs. Ag/AgCl by square wave voltammetry and differential pulse polarography with a hanging mercury drop electrode [11]. This technique showed good reproducibility and sensitivity. However, the utilization of the hanging mercury drop electrode would contaminate the environment because of their environmental toxicity if the mercury is handled with no special care. Furthermore, their test solution must be degassed with oxygen-free nitrogen for 10 min, and they determined of terbinafine in spiked human urine samples based on a preseparation step at a solid phase C-18 cartridge. For these reasons, the simple, rapid, and sensitive method for the determination of terbinafine in biological media with high sensitivity is expected to be established.

Since carbon nanotubes (CNTs) were discovered in 1991, they have attracted much attention of researchers [14]. Some progress in application of CNTs as biosensors has been made, which greatly benefits from the ability of carbon nanotubes to promote the electron−transfer reactions of important biomolecules, including enzymes, DNA and proteins et al. [15]. CNTs modified electrodes have been proved to have excellent electroanalytical properties, such as wide potential windows, low background current and good biocompatibility. Different types of CNTs modified electrodes have been reported, including carbon nanotube paste electrodes [16], carbon nanotube-intercalated graphite electrodes [17] and CNTs film coated electrodes [18,19], etc. Recently, conducting polymer/CNTs composites have received significant interest, because the incorporation of CNTs into conducting polymers can lead to new composite materials possessing the properties of each component with a synergistic effect that would be useful in particular applications [20].

In the present paper, we described the use of CNTs and cysteic acid based on electrochemical oxidation of *L*−cysteine (CySH) to form a novel composite film material at a glassy carbon electrode (GCE) for directly electroanalytical determination of terbinafine in human serum samples without sample pretreatment. The negatively-charged functional groups of the cysteic acid, the sulfonated group, can strongly attract protonized amine groups of terbinafine molecules with positive charges onto the modifier film in an acid solution, and improve favorable accumulation of terbinafine to result in higher sensitive current response. Compared with the bare glassy carbon electrode, the modified electrode showed significantly enhanced accumulation of terbinafine, and the detection limit of this method decreased one order of magnitude. This method has the advantages of rapid and simple operation, very low interference and high accuracy in the serum for the determination of terbinafine. This cysteic acid/CNTs composite film is considered to be a promising, low-cost, steady and biocompatible material for the modification of electrodes.

2. Experimental

2.1. Apparatus

Cyclic voltammetric (CV) and differential pulse voltammetric experiments were carried out at a CHI 660B electrochemical workstation (Chenhua Instruments, China). All electrochemical experiments employed a conventional three-electrode system with a glassy carbon electrode or a cysteic acid/CNTs modified glassy carbon electrode (3.0 mm in diameter) as a working electrode, a platinum wire as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. SEM images were obtained from XL-30E scanning electron microscopy (Philips, Netherlands). All potentials reported in this paper were referenced to the SCE. All of the electrochemical experiments were carried out at 25 °C.

2.2. Chemicals and solutions

L−cysteine (CySH) was obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Terbinafine hydrochloride was purchased from the National Institute for the Control Pharmaceutical and Biological Products (Beijing, China). The multi-wall carbon nanotubes (30–50 nm) were obtained from the Chinese Academy of Sciences Organic Chemistry Company (Chendu, China). All the other reagents used were of analytical grade. Doubly distilled water was obtained by purification through a Millipore water system and was used throughout. A stock standard solution of terbinafine (1.0×10^{-3} M) was prepared, and kept it in the dark under refrigeration (below 4 °C). The B−R buffer solutions (0.04 M) at various pH values were used as base solutions for the electrochemical determination of terbinafine.

2.3. Fabrication of the cysteic acid/CNTs modified glassy carbon electrodes

Before modification, the glassy carbon electrode was polished respectively with 1, 0.3 and 0.05 μm α-alumina powder, rinsed thoroughly with doubly distilled water within each polishing step, and then sonicated in 1:1 nitric acid, acetone and doubly distilled water successively. The CNTs was purified according to the previous report [14]: the CNTs was refluxed in the mixture of concentrated H_2SO_4 and HNO_3 for 24 h, then washed with twice distilled water and dried in vacuum at room temperature. The CNTs suspension was prepared by dispersing 2.5 mg the CNTs in 25 ml dimethyl formamide (DMF) under sonication for 10 min. A 10 μL aliquot of black suspension was dropped directly on the glassy carbon electrode surface, and left it dried under an infrared lamp. The cysteic acid/CNTs/GCE was prepared by cycling scanning the CNTs/GCE between −1.2 and +2.6 V (vs. SCE) at the scan rate of 200 $mV \bullet s^{-1}$ in 0.04 M HCl solution containing 2.5×10^{-3} M CySH with 20 consecutive cycles (Fig. 1). The modified electrode was then electroactivated by cyclic scanning from +0.6 to +1.5 V in the 0.5 M H_2SO_4 solution until a steady cyclic voltammogram was obtained. Finally, the electrode was dried with a stream of high purity nitrogen.

2.4. Determination of terbinafine

The cysteic acid/CNTs modified-glassy carbon electrode, the platinum wire counter electrode, and the saturated calomel reference electrode (SCE) were immersed in 20.00 mL B–R buffer solution (0.04 M, pH 1.81). A certain amount of terbinafine was added in the solution, with stirring by a magnetic stirrer. The stirring was stopped after the electrochemical accumulation for 60 s was at −0.30 V. Then the differential pulse voltammetry (DPV) was immediately performed to scan from +0.5 to +1.4 V after quiet time of 20 s. To establish the optimum conditions for the determination of terbinafine by means of the DPV technique, various instrumental parameter variables were studied. The optimum conditions were as follows: scan rate, 5 $mV \bullet s^{-1}$; sampling width, 0.05 s; pulse amplitude, 50 mV; and pulse period, 0.2 s. An anodic peak current of terbinafine at +1.156 V was recorded (Fig. 2). The standard addition method was applied to quantitative determination of terbinafine. After the determination, the renewal of the electrode was accomplished by soaking the modified electrode in the 0.5 M H_2SO_4 solution to cyclically scan between +0.6 and +1.5 V about 10 cycles.

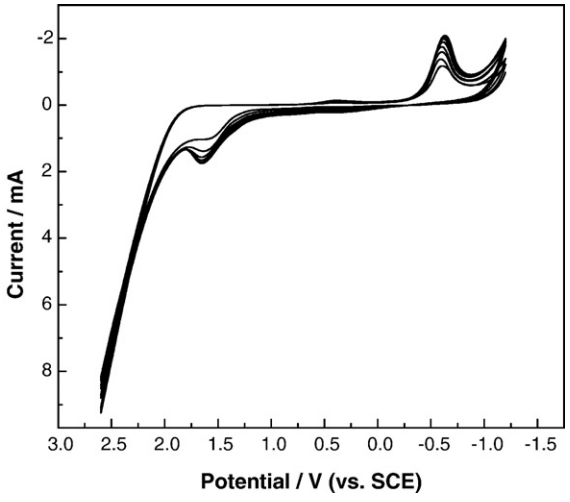

Fig. 1. Cyclic voltammograms of the CNTs/GCE in 0.04 M HCl solution containing 2.5×10^{-3} M *L*–cysteine (CySH). Scan rate: 100 $mV \bullet s^{-1}$; Scan potential: −1.4 to 2.6 V; Consecutive cycle: 20.

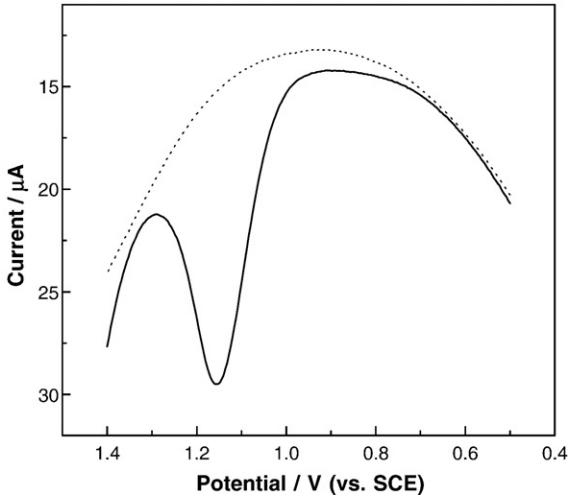

Fig. 2. Differential pulse voltammograms of 1.0×10^{-6} M terbinafine (solid line) and blank (dot line) at the cysteic acid/CNTs/GCE in the 0.04 M B–R buffer solution (pH, 1.81). Accumulation potential under stirring: −0.3 V; Accumulation time: 60 s; Quiet time: 20 s; Scan rate: 0.010 $V \bullet s^{-1}$; Pulse height: 0.050 V; Sampling width: 0.05 s; Pulse period: 0.2 s; Sensitivity: 1.0×10^{-5} $A \bullet V^{-1}$.

3. Results and discussions

3.1. The role of materials modified on the GCE

The high electroactive surface area and excellent electronic conductivity of CNTs make this material an attractive candidate for use as an active electrode modifier [21]. Fig. 3A-a and A-c shows the differential pulse voltammetric behavior of 1.0×10^{-6} M terbinafine at bare GCE and the CNTs/GCE in the 0.04 M B–R buffer solution (pH 1.81). The current response of terbinafine at the modified electrode is about five times higher than that at the bare GCE, at same oxidation potential with identical experimental conditions. This is attributed to further beneficial effects in terms of branched electrical conductivity coupled to increased electrode surface area of CNTs. On the other hand, after the CNTs were treated with concentrated H_2SO_4 or HNO_3 during the purification process, the carboxylic acid groups were introduced on the CNTs surface [22]. In B−R buffer solution of pH 1.81, the surface of the CNTs/GCE should be negatively charged because carboxylic acid groups bring negatively charged ones [19], while terbinafine exits as cation (*pKa* 7.1 [23]). Terbinafine with positive charges could be attracted to the surface of the modified electrode. So the CNTs/GCE would enhance the current response of terbinafine.

Fei et al. described that the oxidation product of CySH can be oxidized further to chemisorbing molecules (cysteic acid) under high positive potential [24]. Ralph et al. demonstrated that CySH was adsorbed on the electrode by using AC voltammetry on GCE,

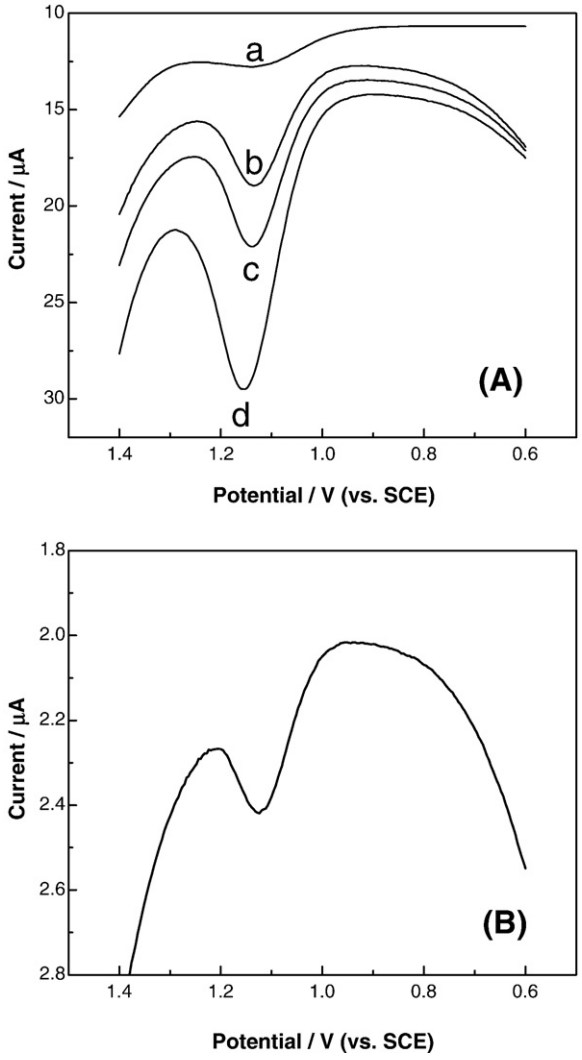

Fig. 3. Differential pulse voltammograms of 1.0×10^{-6} M terbinafine in the 0.04 M B–R buffer solution (pH, 1.81). (A): a — the bare GCE; b — the cysteic acid modified GCE; c — the CNTs modified GCE; d — the cysteic acid/CNTs/GCE. (B) The Nafion/CNTs/GCE. The experimental parameters are similar to Fig. 3.

and its further oxidation to cysteic acid was proposed [25]. Spataru et al. confirmed that the functional group SO_3H of cysteic acid was strongly adsorbed at GCE by using cyclic voltammetric and polarization measurements [26].

Up to now, it is not very clear about the electrochemical oxidation mechanism of CySH. According to Jin and coworker's report [27], they used a primary amine, aminobenzoic acid to fabricate a modified GCE by electrochemical oxidation. A certain yellow substance was produced at the electrode surface. They thought the aminobenzoic acid was oxidized to free radical at the surface of the electrode, the radicals then combined together rapidly to form to a polymer. Previously, however, and his coworker described a method utilized with amine-containing compounds and thought the electrooxidation of amines to their analogous cation radicals to form a chemically stable covalent linkage between the nitrogen atom of the amine and edge plane sites at the GCE surface [28]. By use of X-ray photoelectron spectroscopy (XPS) for coverage assessment, the capability of this route is demonstrated by the immobilization of a simple primary amine at the GCE surface. Their investigation of the influence of substituents on the nitrogen atom (e.g., primary, secondary, tertiary amines) revealed that the surface coverage of primary amines was ~ 3 times higher than that of secondary amines, whereas tertiary amines were not immobilized at a detectable level. This behavior is attributed to a strong steric effect whereby bulky substituents on the nitrogen atom hinder accessibility of the reactive amine cation radical to surface binding sites. Amine salts and amides also showed no detectable coverage by XPS. Subsequently, β-alanine [29] and glutamic acid [30] were reported to fabricate modified GCEs based on electrochemical oxidations and the mechanisms discussed for the immobilization process between amines and carbon was coincident with Deinhammer. As proposed, the process proceeded initially via the one-electron oxidation of the amine functionality to its corresponding cation radical, which subsequently formed a carbon-nitrogen linkage at the carbon surface. To verify that the electroxidation can immobilize *L*–cysteine on the surface of the electrode, XPS, SEM and ATR spectrum were used for the analysis of the electrode surface. As shown in Fig. 4, XPS spectra shows that the position of the peak maximum was at 399.46 eV, which was consistent with the formation of a carbon-nitrogen bond between the amine cation radical and the GCE surface. It verified the immobilization of *L*–cysteine on the GCE. The ATR spectrum was shown in Fig. 5. The stretching frequency of C=O in the COOH group shows up at about 1731 cm^{-1}. 1159 cm^{-1} is the C–NH bond between the carbon of the glassy carbon electrode and the N of the *L*–cysteine, while 1232 cm^{-1} is the HN–CH bond between the NH of *L*–cysteine and CH of tertiary carbon. The stretching frequency of S=O in the $-SO_3H$ group shows up at about 765 cm^{-1} and the wide absorption peak and strong stretching frequency of $-SO_3H$ is at about 1000 cm^{-1} [31,32].

In recent years, the incorporation of highly conductive CNTs into Nafion can promote electrochemical responses, and Nafion/CNTs based electrodes have been increasingly used to solve demanding electrochemical problems, further they offer advantages over other types of electrode materials [33–35]. We think that cysteic acid based on the electrochemical oxidation of CySH is

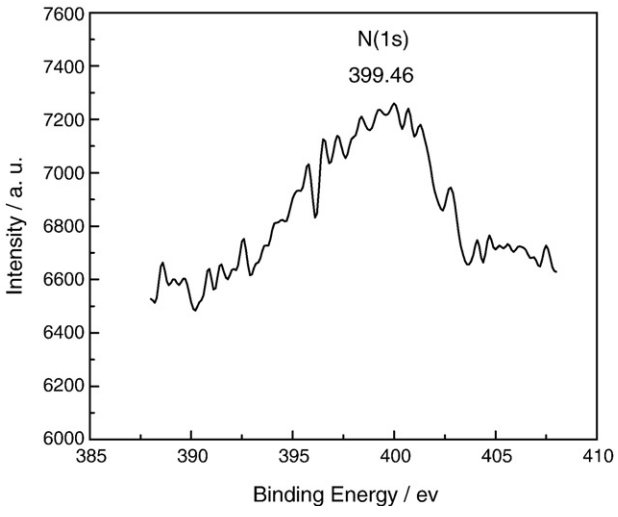

Fig. 4. X-ray photoelectron spectra in the N (1s) region for the cysteic acid modified glassy carbon electrode.

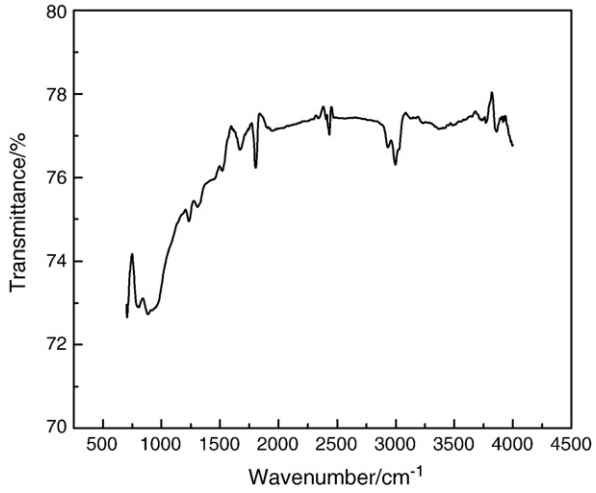

Fig. 5. ATR spectrum of the cysteic acid/CNTs/GCE surface.

similar to Nafion with a sulfonated group, and it can be used as an electrode modifier due to its attractive ion-exchange characteristics. Fig. 3(A–b) shows the peak current response of terbinafine at cysteic acid/GCE is enhanced compared with the bare GCE under above identical experimental conditions. It was attributed to the electrostatic attraction of modifier layer for the protonized amine groups of terbinafine molecules carrying positive charges in the acid solution. The negatively-charged functional groups of the cysteic acid, the sulfonated group, can attract terbinafine onto the modifier film and improve favorable accumulation of terbinafine to result in higher sensitive current response. Fig. 3(A-d) shows the current response of terbinafine is the most remarkable at cysteic acid/CNTs/GCE compared with cysteic acid/GCE and CNTs/GCE, which the current response of terbinafine at the modified electrode is about ten times higher than that at the bare GCE. Obviously, the CNTs (which are now commercially available in high quality and uniformity) can provide high electrical conductivity and high surface area in the cysteic acid film. The higher accumulation effect of the cysteic acid/CNTs modified electrode reflects the synergistic effect of cysteic acid with CNTs. In addition, according to the previous literature [32], a Nafion/CNTs/GCE was prepared for comparison with the proposed electrode. A 0.1 wt.% Nafion solution used in this work was prepared by diluting the 5 wt.% Nafion solution in ethanol. CNTs (2.5 mg) was added into 25 mL 0.1% Nafion solution and sonicated for 30 min until a homogeneous dispersion was achieved. A same GCE was coated with 10 μL of the CNTs–Nafion dispersion above mentioned. The solvent was left to evaporate at room temperature in air. Fig. 3(A-d) and Fig. 3(B) show that the oxidation peak potential at the Nafion/CNTs/GCE is similar to that at the cysteic acid/CNTs/GCE, implying that both cysteic acid and Nafion play the same role at the two modified electrodes. Nafion/CNTs/GCE also enhanced the peak current response of terbinafine compared with GCE under above identical experimental conditions, but its current response is lower than that at cysteic acid /CNTs/GCE, and decreases one order of magnitude because of poor conductivity of Nafion. On the other hand, Nafion is more expensive than CySH. So cysteic acid/CNTs film has more significant advantages and electroanalytical application than Nafion/CNTs film.

The peak current response of the cysteic acid/CNTs/GCE to terbinafine is expected to be affected by the amount of the CNTs on the electrode surface, which can be controlled by using same volume (10 μL) of the suspensions with different content of the CNTs to prepare the CNTs films. After the cysteic acid film is formed on the CNTs/GCE by electroxidation of CySH, the anodic peak current of 1.0×10^{-6} M terbinafine at these composite modified electrodes was recorded, and the relationship between the anodic peak current and the CNTs content on the electrode is shown in Fig. 6 (A). With the increment of the CNTs concentration, the peak current increased accordingly, implying that higher the CNTs content was, higher the sensitivity of the electrode was. However, it was observed that the background current and noise level also increased with excessive CNTs content at the electrode in the experiment. Additionally, the cysteic acid/CNTs composite film would not be stable because excessive CNTs could leave off the electrode surface to decrease the peak current. Therefore, a moderate CNTs concentration of 0.10 mg•ml^{-1} was selected for the fabrication of the cysteic acid/CNTs composite modified electrodes in this work.

The anodic peak current of terbinafine was considerably related to the amount of cysteic acid at the modified electrode, too. When the CNTs/GCE was carried out by the cyclic voltammetry, the more cycles of sweep were, the more cysteic acid based on electrochemical oxidation of CySH was adsorbed onto the surface

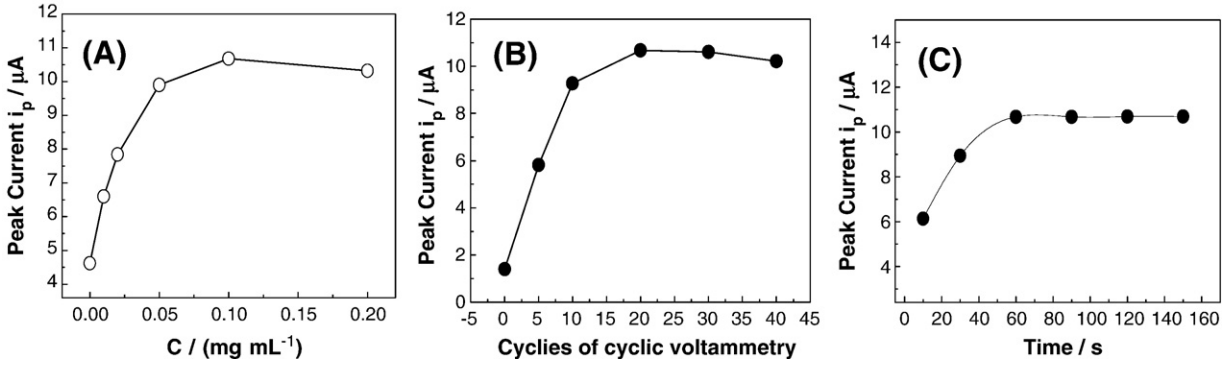

Fig. 6. The DPV current responses of 1.0×10^{-6} M terbinafine in the 0.04 M B–R buffer solution (pH, 1.81) at the cysteic acid/CNTs/GCE. (A) Effect of the CNTs concentration on the anodic peak currents. (B) Effect of scan circles for electrochemical oxidation of *L*–cysteine on the anodic peak current. (C) Effect of accumulation time on the anodic peak currents. The experimental parameters are similar to Fig. 3 except for the variable objects investigated.

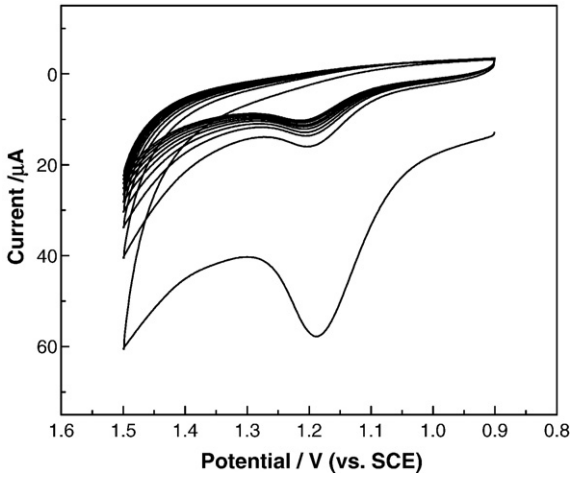

Fig. 7. Cyclic voltammogram of 5.0×10^{-6} M terbinafine at the cysteic acid/CNTs/GCE in the B–R buffer solution (0.04 M, pH 1.81). Scan rate: 100 mV•s^{-1}.

of the CNTs/GCE. Fig. 6 (B) shows the remarkable effect of the cysteic acid amount to the peak current. The anodic peak current gradually increased with the amount of cysteic acid on the CNTs/GCE surface. It might be ascribed to the quick accumulation of terbinafine. However, the anodic peak current decreased when excessive cysteic acid was adsorbed onto the CNTs/GCE. The reason is that the cysteic acid could leave from the electrode surface to decrease the peak current. So a 20-sweep-circle under the cyclic voltammetry was selected in present experiment.

3.2. Optimization of experimental conditions

To establish the optimum conditions for the determination of terbinafine by means of DPV technique, some conventional supporting electrolytes were tested, and various instrumental variables were studied. Higher sensitive and well-defined peaks of terbinafine in voltammograms were obtained in the B–R buffer solution (0.04 M) compared with other supporting electrolytes, such as HCl, H_2SO_4, H_3PO_4, NaOH, and phosphate buffer solution (PBS). The pH value of the base solution has a significant influence on the oxidation of terbinafine at the cysteic acid/CNTs/GCE. Experimental results about the effect of solution pH on the anodic peak currents obtained with recording differential pulse voltammograms of terbinafine, in a series of B–R buffers (0.04 M) with varying pH, in the range of 1.81 to 7.0. The anodic peak current of terbinafine would increase monotonically and peak potential would simultaneously shift toward positive direction with decreasing the pH value. This was attributed to the existence of terbinafine (pK_a 7.1) in the cationic form in the acid solution and the cysteic acid/CNTs/GCE undertook negative charges, and the modified film can attract and accumulate the cationic terbinafine onto the electrode surface. But the background current was too large to facilitate the determination of terbinafine when the acidity was too high. So the B–R buffers (pH 1.81) solution was selected for the determination.

The effects of accumulation potential on the DPV current response of terbinafine were studied. The accumulation step proceeded in a constantly stirred solution and the voltage scanning step was performed after 20 s of quiet time. We found the peak current of terbinafine was the highest at −0.3 V as the accumulation potential. This is attributed to the fact that terbinafine exists in the cationic form at pH 1.81 and the negative potential is more favorable to the accumulation. Therefore an accumulation potential of −0.3 V was chosen in all of the subsequent work.

The effect of accumulation time at −0.3 V on the peak current was also investigated. Fig. 6(C) shows that the peak current of terbinafine increased with increasing accumulation time within 60 s, which indicates that terbinafine on the modified electrode surface was gradually adsorbed. Further postponement of the accumulation time of exceeding 60 s did not increase the response of terbinafine on the electrode, and the peak current remained almost constant, owing to the surface adsorption saturation. For practical purposes, a 60 s accumulation time was sufficient for the determination of terbinafine.

3.3. Electrochemical behavior of terbinafine on the cysteic acid/CNTs/GCE

In order to study some aspects of electrochemical behaviors of terbinafine, cyclic voltammograms were then recorded at different potential scan rates from the range of 5–100 mV•s^{-1} in the B–R buffer solution (Fig. 7). Only an irreversible oxidation wave at +1.2 V was observed and a positive shift in the peak potential (E_p) was observed, which confirms the irreversibility of the process [36], with the simultaneous increase in peak current (i_p) when the scan rate (v) was increased.

The experimental results indicate a good linear dependence of the oxidation peak potential upon the log of the scan rate, log v, with a slope of 0.053 V. The peak potential also shows a linear dependence upon pH over the pH range from 1.8 to 7.0, with a slope of −0.069 V. This behavior can be interpreted on the basis of the treatment of an irreversible electrode process by cyclic voltammetry [37], which relies on a general approach of electrode kinetics [38].

According to this treatment, the dependence of the oxidation peak potential upon the scan rate v is expressed by the equation (see Eq. (6) of Ref. [37]):

$$\left(\frac{\partial E_p}{\partial \log v}\right)_{\mathrm{pH}} = \frac{2.3\mathrm{RT}}{F\left(\overrightarrow{\mathrm{n}}+\delta\alpha\right)} \tag{1}$$

while its dependence upon pH is expressed by the equation (see Eq. (7) of Ref. [37]):

$$\left(\frac{\partial E_p}{\partial pH}\right) = \frac{-2.3RTh}{F\left(\overrightarrow{\mathrm{n}}+\delta\alpha\right)} \tag{2}$$

Here, R, T and F have their usual significance, $\overrightarrow{\mathrm{n}}$ is the number of elementary electron-transfer steps preceding the rate-determining step, δ is a parameter equal to 1 for a rate-determining electron-transfer step or to 0 for a rate-determining chemical step, $\alpha \cong 0.5$ is the symmetry factor for an electron-transfer step (if rate-determining), and h is the number of elementary deprotonation steps the precede the rate-determining step. Considering that 2.3RT/F equals 0.059 mV at room temperature, the dependence of

the oxidation peak potential upon log v and upon pH points to a unit value for $(\vec{n}+\delta\alpha)$ and to a unit value for h, in view of Eqs. (1) and (2). This implies that the rate determining step is a chemical step ($\delta=0$) following both the uptake of the first transferring electron ($\vec{n}=1$) and an elementary deprotonation step ($h=1$). Therefore, the possible mechanism for terbinafine electrooxidation is as follows:

$$R_1R_2MeNH^+ \underset{\text{in quasi-equilibrium}}{\rightleftharpoons} R_1R_2MeN + H^+$$

$$R_1R_2MeN \underset{\text{in quasi-equilibrium}}{\rightleftharpoons} R_1R_2MeN^+ + e^-$$

$$R_1R_2MeN^+ \xrightarrow{\text{r.d.s.}} \text{intermediate product}$$

The intermediate product of the rate-determining step (r.d.s.) undergoes further elementary steps in quasi-equilibrium, yielding the final product.

3.4. Calibration curve and detection limit

Under the optimum detection conditions, the anodic peak currents were proportional to the terbinafine concentrations in the range of 8.0×10^{-8}–5.0×10^{-5} M. The linear equation was I_p (10^{-6}/A)=3.04+7.04C (C/μM) with the correlation coefficient r=0.998. The calibration curve deviated from the linear relationship when the terbinafine concentration was more than 5.0×10^{-5} M. In this study, the detection limit of terbinafine was 2.5×10^{-8} M in terms of the role of signal to noise ratio of 3:1 (S/N=3).

3.5. Reproducibility and recovery test

The lifetime of the cysteic acid/CNTs/GCE was examined, and it demonstrated that the electrode could retain 97.5% of its initial response after five-month storage. Such electrode stability seemed to be acceptable for most practical applications. Because the modified electrode could adsorb terbinafine, it was necessary to renew the electrode surface after each determination. But this process was easily accomplished by soaking the modified electrode in 0.5 M H_2SO_4 solution to sweep between +0.6 and +1.5 V about 10 cycles by cyclic voltammetry. The 1.0×10^{-6} M terbinafine solution was determined repeatedly with the same electrode for 9 times. The average current was 10.38 μA with the relative standard deviation (RSD) of 3.5%. It was indicated that the modified electrode possessed a good reproducibility. The recovery tests of terbinafine in the range from 2.00×10^{-7} to 2.50×10^{-5} M were performed. The results are listed in Table 1. The recoveries varied in the range from 95.4 to 104.0% and the RSD was 3.8%.

Table 1
Recovery of terbinafine

Added (M)	Found (M)	Recovery (%)
1.00×10^{-7}	9.65×10^{-8}	96.5
5.00×10^{-7}	5.19×10^{-7}	103.8
1.00×10^{-6}	9.88×10^{-6}	98.8
5.00×10^{-6}	4.85×10^{-6}	97.0
1.00×10^{-5}	1.04×10^{-5}	104.0
5.00×10^{-5}	4.77×10^{-5}	95.4

Table 2
Influence of potential interferents on the voltammetric response of 5.0×10^{-6} M terbinafine

Interferent	Concentration (mM)	Signal change (%) ($i_{terbinafine}$=100%)
L−alanine	5.0	−2.9
glucose	5.0	+1.5
D−fructose	5.0	− 3.1
Tartaric acid	5.0	−1.3
Citrate	5.0	−2.3
Urea	5.0	+2.2
DL−tyrosine	5.0	−2.7
Vitamin B_1	5.0	−1.7
Vitamin B_2	5.0	+1.3
Vitamin B_6	5.0	−2.5
$NaNO_3$	5.0	+1.0
$(NH_4)_2SO_4$	5.0	−2.6
$Ca(NO_3)_2$	5.0	+2.5
KCl	5.0	+1.1
$MgCl_2$	5.0	−2.0
$BaCl_2$	5.0	+1.8
$Fe(NO_3)_3$	5.0	−2.4

3.6. Interference

Under the optimum experimental conditions, when the concentration of terbinafine was 5.0×10^{-6} M, no interferences were observed in the presence of 5.0×10^{-3} M of urea, tartaric acid, D-fructose, citrate, glucose, DL-tyrosine, L-alanine, vitamin B_1, vitamin B_2, vitamin B_6, $NaNO_3$, $(NH_4)_2SO_4$, $Ca(NO_3)_2$, KCl, $MgCl_2$, $BaCl_2$, and $Fe(NO_3)_3$. The results obtained are listed in Table 2.

For the following special detection in serum samples, the other conceivable concomitant in serum samples, such as ascorbic acid

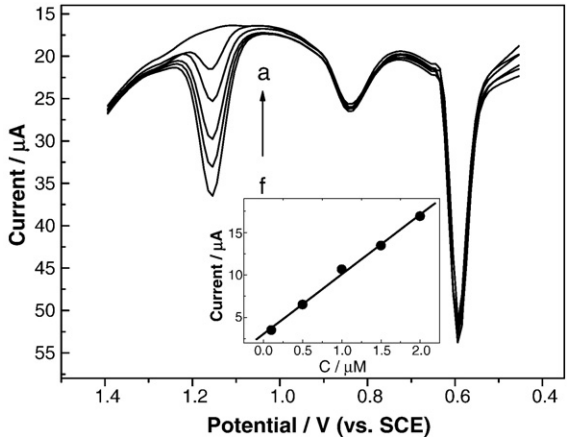

Fig. 8. The DPV of serum sample spiked with different terbinafine concentration. (a) blank serum sample; terbinafine: (b) 1.0×10^{-7} M, (c) 5.0×10^{-7} M, (d) 1.0×10^{-6} M, (e) 1.5×10^{-6} M, (f) 2.0×10^{-6} M. Inset is the plot of the anodic peak currents versus the concentration of terbinafine. The experimental parameters are similar to Fig. 3.

Table 3
Determination of terbinafine in serum samples at the modified electrode

Serum	Spiked (M)	Detected (M)	Recovery (%)
Sample 1	1.00×10^{-7}	9.71×10^{-7}	97.1
Sample 2	5.00×10^{-7}	5.12×10^{-7}	102.4
Sample 3	1.00×10^{-7}	1.05×10^{-7}	105.0
Sample 4	1.5×10^{-6}	1.46×10^{-6}	97.3
Sample 5	2.00×10^{-6}	4.84×10^{-6}	96.8

(AA), dopamine (DA) and uric acid (UA) were examined. There are no interferences from UA, DA and AA for the measurement because oxidation peak potentials of three components (less than +0.6 V) are far away from that of terbinafine (+1.156 V). In addition, some conventional medicaments, such as 1.0×10^{-3} M of atropine, penicillin, and theine, did not interfere with the determination.

3.7. Detection of terbinafine in human serum samples

The developed DPV method for the terbinafine determination was applied to human serum samples. The serum samples were obtained from volunteers. The recoveries from human serum were measured by spiking drug-free human serum with known amounts of terbinafine. The serum samples were diluted 50 times with the 0.04 M B–R buffer solution before analysis without further pretreatments. Fig. 8 shows the differential pulse voltammograms for serum samples detection. A qualitative analysis can be carried out by adding the standard solution of terbinafine into the detect system of human serum sample, and then the peak at +1.156 V linearly increased in height. The peak at +0.585 V was proved to be response of uric acid in the human serum sample. Another anodic peak appears at +0.844 V attributed to other unknown endogenous chemicals presented in serum, but they do not interfere with the determination of terbinafine. Standard addition method was employed under above optimal experimental conditions. The detection results of five human serum samples obtained were listed in Table 3. And the recovery determined was ranged from 96.8 to 105.0% and the RSD was 3.8%.

4. Conclusion

A glassy carbon electrode modified by cysteic acid and CNTs based on electrochemical oxidation of *L*–cysteine was fabricated. Terbinafine could be sensitively determined by voltammetry because cysteic acid with cation exchange property drastically increased the current response of terbinafine. In 0.04 M B–R buffer solution (pH 1.81). The peak current obtained from the DPV was linearly dependent on terbinafine concentration in the range of 8.0×10^{-8}–5.0×10^{-5} M in 0.04 M B–R buffer solution (pH 1.81), with a correlation coefficient of 0.998 and a detection limit of 2.5×10^{-8} M. The modified electrode was applicable in the detection of terbinafine in human serum samples with excellent sensitivity and selectivity. The success of this strategy suggests that cysteic acid/CNTs film will have significant electroanalytical utility in the future. The novel material can be easily applied to other types of substrate electrodes and surfaces, and this will further broaden the potential for applications. This film is considered to be a promising, low-cost, and steady material in the modification of electrodes. It is hope that the cysteic acid/CNTs modified electrode will be a good application for further sensor development.

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