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Short communication

Voltammetric sensor for tinidazole based on poly(carmine) film-modified electrode and its application

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Abstract

A poly(carmine) film-modified glassy carbon electrode (GCE) was fabricated and the electrochemical behavior of tinidazole at the modified electrode was investigated by electrochemical methods. A well-defined reduction peak was observed at 0.61 V and was applied for the determination of tinidazole. Compared with that at a bare GCE, the reduction peak potential of tinidazole shifted negatively and the reduction peak current increased significantly. The influences of some parameters on the reduction of tinidazole were also examined. Based on the experimental data, a possible mechanism was proposed for the electrochemical reaction of tinidazole at the modified electrode. It was found that the reduction peak current was proportional to the concentration of tinidazole in the range from 1.0×10^{-7} to 5.0×10^{-5} mol L^{-1}. The detection limit was about 1.0×10^{-8} mol L^{-1} after accumulation 90 s at a constant potential of 0.0 V. The proposed method was applied to determine tinidazole in drugs and the result was satisfied.

Keywords: Tinidazole; Carmine; Chemically modified electrode; Voltammetric determination

1. Introduction

Tinidazole is an anti-parasitic drug used as a treatment for a variety of amebic and parasitic infections [1,2]. Because of its important role in numerous pathological processes, tinidazole has been widely studied in recent years. To date, various methods have been employed in the quantitative determination of tinidazole, such as spectrophotometric [3,4], chromatographic methods [5,6] and electrochemical method [7,8]. To our knowledge, the detection of tinidazole at poly(carmine) film electrode has not been reported yet.

Electroactive polymeric films have acquired wide popularity, since they are easier to be modified on the electrode surface than monolayer. A number of studies indicated that polymer film-modified electrodes show an enhanced response for the determination of various important biological and clinical species. Many researches demonstrated that electropolymerization is a very convenient way to immobilize polymers on electrode surface and the thickness, permeation and charge transport characteristics of the polymeric films can be controlled by the potential and current applied. Dye molecules have been widely used as mediators to study the electrochemical reduction of nitro compounds [9,10], including methylene blue [11,12], methylene green [13] and brilliant cresyl blue [14]. In general, the derivatives of dyes formed via bonding a dye molecule to an aromatic ring covalently could decrease their proton-donor ability and improve their adsorptive activity [15]. Polymerization of dyes can form a cross-linked oligomer which leads to the enhancement of its adsorptive ability [16].

As we know, carmine is a valuable dye obtained from the bodies of the female of insect coccus cacti [17]. However, carmine, which is invariably applied in conjunction with a mordant, has not been reported as a mediator for the catalysis of any electrochemical reactions. In this paper, carmine is used to develop a polymer film-modified electrode by a constant potential technique. This modified electrode shows excellent enhancement effects on the electrochemical reduction of tinidazole. The reduction peak current increases remarkably and peak potential shifts positively slightly at this modified electrode. The experimental results show that the sensitivity for the determination of tinidazole increases markedly at the poly(carmine) film-coated GCE. Furthermore, some experiment parameters are optimized and an electrochemical method for the determina-

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tion of tinidazloe is proposed. This method is convenient and available because of its rapid response, high sensitivity, good selectivity, low detection limit, excellent reproducibility, simplicity and low cost.

2. Experimental

2.1. Apparatus and reagents

Electrochemical data were obtained with a three-electrode system using a CHI 660A electrochemical workstation (Shanghai Chenhua Co., China). A three-electrode system was developed including the polymer-modified electrode working electrode, a platinum wire counter electrode and a saturated calomel electrode (SCE) reference electrode, respectively.

Tinidazole (obtained from Sigma Chemical Company) was dissolved in ethanol to form 1.0×10^{-3} mol L^{-1} standard solution and stored at 277.15 K in dark to avoid any decomposition. Carmine was purchased from Flyka. Other chemicals used were analytical reagents. All the chemicals were used without further purification and all solutions were prepared with doubly distilled water.

2.2. Preparation of modified electrode

Electropolymerization of carmine was carried out in 1.0 mol L^{-1} H_2SO_4 solution containing 1.0×10^{-3} mol L^{-1} carmine at a constant potential of 1.6 V for 20 min. The modified electrode was washed with ethanol and distilled water in turn.

2.3. Analytical procedure

A certain volume of phosphate buffer solution (PBS, pH 5.7) was used as the supporting electrolyte. After the addition of tinidazole, the solution was deoxidized with pure nitrogen for 10 min. The accumulation was carried out at 0.0 V with stirring for 90 s. The voltammograms were recorded by linear sweep voltammograms (LSV) in the potential range from 0.0 to −1.0 V. The reduction peak current was measured at 0.61 V. After each measurement, the modified electrode was refreshed by successive cyclic voltammetric scans in a phosphate buffer (pH 5.7) between 0.0 and −1.0 V at 100 mV/s to get a reproducible electrode surface.

2.4. Sample preparation

Tinidazole tablet (10 mg, obtained from Guangzhou Qiaoguang Pharmaceutical Co. Ltd., China, No. 40060) was weighed accurately and dissolved in ethanol, then stirred magnetically for 15 min to dissolve completely. Then, it was filtered and the filtrate was diluted to 10 ml with ethanol and stored at 277.15 K in dark. The standard addition method was used to evaluate the content of tinidazole in the tablet.

The suitable content of one ampoule (1 ml of solution contains 4 mg tinidazole and 8.5 mg sodium chloride) was diluted with doubly distilled water to 50 ml and stored at 277.15 K in dark. The content of the tinidazole was determined by the standard addition method.

3. Results and discussion

3.1. Electrochemical behavior of tinidazole

Fig. 1 shows cyclic voltammograms of 1.0×10^{-4} mol L^{-1} tinidazole at the poly(carmine) film-modified GCE in 0.01 mol L^{-1} phosphate buffer solution (pH 5.7). A well-defined reduction peak appeared at 0.61 V when potential initially swept from 0.2 to −1.2 V and no peak was observed in the reversal scan, revealing that the electrode reaction of tinidazole is a totally irreversible process. According to the accepted mechanism [18], the reduction reaction should be attributed to a four-electron reduction of the nitro group via the derivation of the hydroxylamine. However, the reduction peak current decreases remarkably in the second scan. During following successive cyclic scans, the reduction peak current decreases continuously with the increase of scan number, resulting from the fact that the electrode surface has been blocked by the adsorption of the reduction products.

The voltammetric responses of 1.0×10^{-4} mol L^{-1} tinidazole at different electrodes are illustrated in Fig. 2 in pH 5.7 phosphate buffer after accumulation 90 s at 0.0 V. A flat reduction peak was observed at −0.65 V at the bare glassy carbon electrode (Fig. 2c). However, at the modified electrode the reduction peak potential shifts positively about 40 mV (Fig. 2a) and the peak current of tinidazole increased significantly. This remarkable enhancement was undoubtedly attributed to the extraordinary properties of polymer, such as high aspect ration and strong adsorption ability.

3.2. Effect of scan rate on the peak current

The influences of scan rate on the electrochemical behavior of tinidazole at the poly(carmine) film-modified GCE in pH 5.7

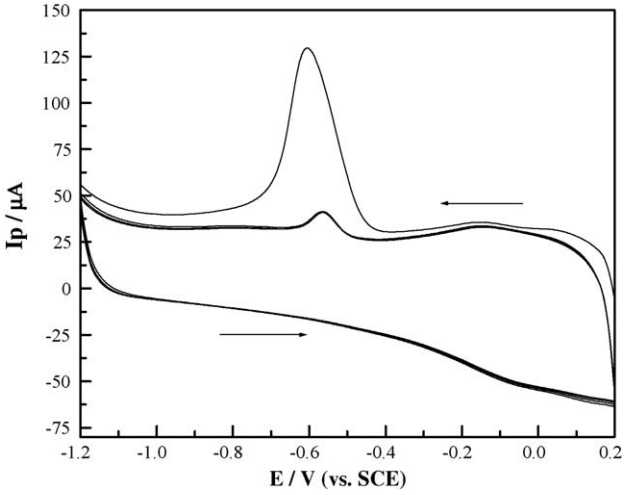

Fig. 1. Cyclic voltammograms of 1.0×10^{-4} mol L^{-1} tinidazole at the poly(carmine) film-modified GCE in 0.01 mol L^{-1} phosphate buffer solution (pH 5.7). Scan rate, 100 mV/s; accumulation potential, 0.0 V; accumulation time, 90 s.

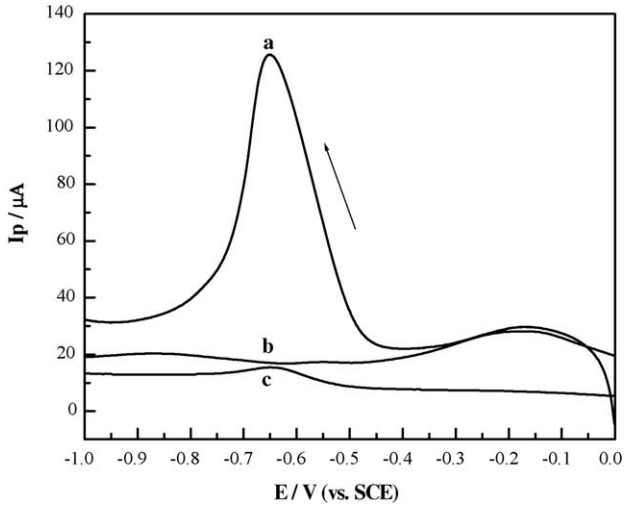

Fig. 2. Linear sweep voltammograms of 1.0×10^{-4} mol L^{-1} tinidazole at the poly(carmine) film-modified GCE (a); bare GCE (c). Curve b presents the voltammogram at the modified electrode in phosphate buffer solution in the absence of tinidazole. Scan rate, 100 mV/s; accumulation potential, 0.0 V; accumulation time, 90 s.

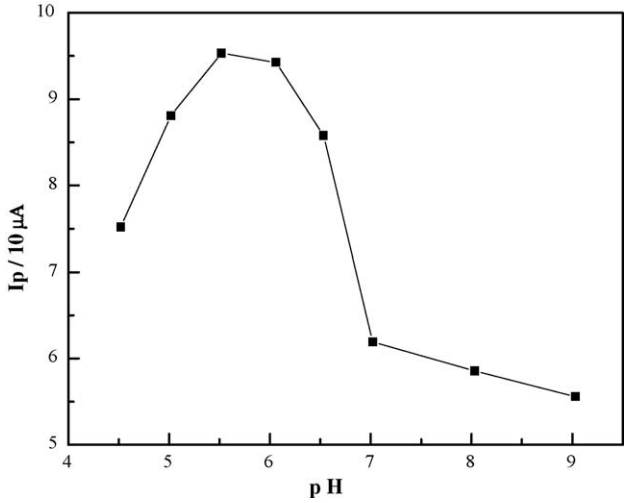

Fig. 3. Effect of pH on the reduction peak current of 1.0×10^{-4} mol L^{-1} tinidazole at the poly(carmine) film-modified GCE. Scan rate: 100 mV/s. Other conditions are the same as in Fig. 2.

phosphate buffer solution were also investigated. Cyclic voltammetry was applied for the study of the electrochemical behavior of tinidazole at different scan rates from 20 to 240 mV/s. The reduction peak current (i_p) of tinidazole increased with scan rate increasing and a linear relationship was observed. The corresponding linear regression equation can be expressed as follows: $i_p(10\,\mu A) = 2.7760 + 0.0700v$ (mV/s) ($r = 0.9929$). The result indicated that the electrode process of tinidazole at the modified electrode is controlled by an adsorption step. The reduction peak should correspond to a four-electron reduction of nitro group to the corresponding hydroxylamine according to the current accepted mechanism for the electroreduction of aromatic and heteroarmoatioc nitro compounds [19].

3.3. Effect of pH on the peak current and peak potential of tinidazole

The effects of pH of solution on the tinidazole reduction at the poly(carmine) film-modified GCE were investigated by cyclic voltammetry. The reduction peak potential shifted negatively as pH increased from 4.50 to 9.03, and obeyed the following equations: $E_p(V) = -0.289 - 0.061$pH ($r = 0.9899$). The slope for the linear regression equation is -0.061, suggesting that the number of the electrons transferred in the reduction of tinidazole is equal to that of the protons. The relationship between the peak current and solution pH was shown in Fig. 3. A maximum peak current was obtained at pH 5.7, and then decreased. Thus, a phosphate buffer (pH 5.7) was chosen for supporting electrolyte for investigation of the reduction of tinidazole at the modified electrode.

3.4. Optimization of accumulation conditions

The reduction peak current of 1.0×10^{-4} mol L^{-1} tinidazole remained almost unchanged as the accumulation potential shifted from 0.4 to 0.0 V. However, the peak current increased gradually when accumulation potential shifted negatively in the range of 0.0 to −0.5 V. Thus, an accumulation potential of 0.0 V was employed.

As shown in Fig. 4, the accumulation time significantly affected the reduction peak current of tinidazole. The reduction peak current enhanced greatly with the increase of the accumulation time within first 90 s and then almost stable value could be obtained. This may be attributed to the saturated adsorption of tinidazole on the poly(carmine) film-modified GCE surface.

3.5. Calibration graph

Under the optimized experimental conditions, the calibration curve for the determination of tinidazole at the poly(carmine) film-modified GCE was characterized by LSV

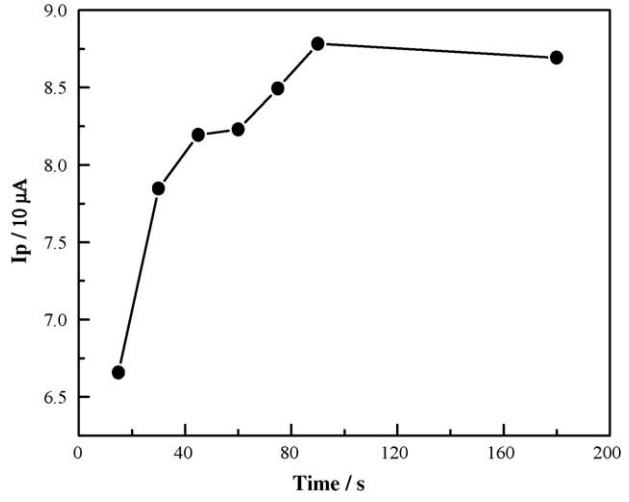

Fig. 4. Effect of the accumulation time on the reduction peak current of 1.0×10^{-4} mol L^{-1} tinidazole. Other conditions are the same as in Fig. 2.

Table 1
Determination of tinidazole in commercial available tablets ($n = 7$)

Codeine-tablet	Original detected value (μM)	Spike (μM)	Detected value after spike (μM)	Recovery (%)
#1	17.77 ± 0.12	15.00	32.53 ± 0.07	98.4
#2	7.461 ± 0.21	15.00	22.14 ± 0.03	97.9
#3	27.49 ± 0.17	10.00	37.94 ± 0.11	107.5

Table 2
Determination of tinidazole in commercial available ampoule ($n = 7$)

Codeine-ampoule	Original detected value (μM)	Spike (μM)	Detected value after spike (μM)	Recovery (%)
#1	4.99 ± 0.16	15.00	19.34 ± 0.04	95.7
#2	1.16 ± 0.03	15.00	16.17 ± 0.09	100.1
#3	3.11 ± 0.11	10.00	13.24 ± 0.06	101.3

and the linear range was comprised between 1.0×10^{-7} and 5.0×10^{-5} mol L^{-1} in terms of the relationship between the reduction peak current and tinidazole concentration. The corresponding linear regression equation can be expressed as follows: i_p (μA) = 2.7494 + 0.9286c ($r = 0.9969$) and the detection limit was 1.0×10^{-8} mol L^{-1}.

The stability of the modified electrode was evaluated by the current responses of 1.0×10^{-5} mol L^{-1} tinidazole at room temperature. During measurements, the modified electrode was keep at 4 °C. The experimental results indicated that the peak current maintains 96.84% of that of the initial response at the modified electrode after 2 weeks, suggesting that the film electrode possesses good stability.

3.6. Interference

Under optimized experimental conditions described above, the influences of some potential interference towards the current responses of 1.0×10^{-5} mol L^{-1} tinidazole have been evaluated. The results showed that 500-fold of Cd^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, Fe^{2+} and Al^{3+} do not affect the determination of tinidazole. Some organic molecules such as 10-fold of dopamine, ascorbic acid, acetaminophen, tyrosine, Vitamin B, glucose, cholesterol have almost no influences on the current responses of 1.0×10^{-5} mol L^{-1} tinidazole (signal change below 6%) (error <5%). However, some compounds containing nitro group, such as nitrofurantoin, azathioprien and nitrophenols, have serious influences on the determination of tinidazole. Because they contain the same electroactive groups as tinidazole and affect the reduction peak current of tinidazole.

3.7. Tinidazole assay in pharmaceutical formulation

The proposed method was applied for determination of tinidazole in tablets by LSV. The data are shown in Table 1. The recoveries in this method were investigated and the values are varied between 98.4 and 107.5%, which indicated that the determination of tinidazole using the poly(carmine) film-modified GCE was effective and sensitive.

3.8. Determination of tinidazole in pharmaceutical formulation

Standard addition method was used for the determination of tinidazole in a tinidazole ampoule. The analytical procedure was the same as that described above. The results are shown Table 2. The recoveries were varied in the ranged of 95.7–101.3%, which can be considered to be satisfying. These results were consistent with that obtained by HPLC.

4. Conclusion

Carmine was easy to be cast onto the surface of glassy carbon electrode to form a film-modified electrode. The modified electrode displayed a strong adsorptive activity. Based on this property, the concentration of tinidazole in solution could be determined directly with excellent sensitivity by voltammetry. Sufficient experimental results demonstrated that the poly(carmine)-modified electrode was a good electrochemical sensor for the direct measurement of tinidazole.

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