



Electrocatalytic Reduction and Voltammetric Determination of Metronidazole at Inlaying Ultra-Thin Carbon Paste Electrode

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In this paper, nichrome was adopted as a substrate, to fabricate an inlaying ultra-thin carbon paste electrode (IUTCPE). Cyclic voltammetry and linear scan voltammetry were used to investigate the electrochemical responses of metronidazole. The results revealed that, in 0.1 mol L⁻¹ Britton-Robinson buffer (pH 3), the inlaying ultra-thin carbon paste electrode showed a good electrocatalytic reduction towards metronidazole. Under optimized conditions, the well-defined reduction peak currents increased linearly with increasing the contents in the range of 7×10^{-7} - 1.17×10^{-3} mol/L. The detection limit (S/N = 3) was 2.8×10^{-7} mol/L. Inlaying ultra-thin carbon paste electrode has the advantages of simple procedure, good stability and low cost. The proposed method was successfully applied to metronidazole determination in tablets. The recovery was from 96.0 to 104.7 %.

Keywords: Inlaying ultra-thin carbon paste electrode, Metronidazole, Voltammetry, Tablets analysis.

INTRODUCTION

Metronidazole (MTZ) is one of the most important 5-nitroimidazole derivatives. It is effective against trichomonas, anaerobic bacteria, giardiasis and amoebiasis. The mechanism of its main biological function depends on the reduction of the nitro group which produces intermediate species to interact with the anaerobic micro-organisms DNA¹. It has an obvious killing and inhibiting effect on the majority of anaerobic bacteria when the metronidazole concentration in serum is in the range of 2-8 µg/mL². At present, different methods have been used for the determination of metronidazole, such as HPLC³, GC⁴, TLC⁵, spectrometry⁶⁻⁹, flow injection analysis^{10,11}, polarography^{12,13}, voltammetry¹⁴⁻¹⁸ etc. Voltammetric approaches have many advantages including short experimental time, simple procedure, high sensitivity as well as wide linear range, which have drawn a lot of attentions in the fields of drug analysis and environmental monitoring.

La-Scalea *et al.*¹ studied the reduction process of metronidazole at mercury electrodes in acid medium. They demonstrated that metronidazole completely reduced to the hydroxylamine derivative involving four electrons in two steps. Lu *et al.*¹⁶ reported that multi-walled carbon nanotubes (MWNT) which dispersed into water in the presence of a hydrophobic surfactant was used to modify the glassy carbon electrode, a

well-defined reduction peak of metronidazole was observed. Besides, many other work electrodes have been reported, such as silver electrode¹⁵, carbon nanotubes modified glassy carbon paste electrode^{16,19}, carbon paste electrode modified with incorporated metalloporphyrin¹³, etc. In searching of a new substrate material with lower cost and stable physical characteristics has been another hot issue in the electrochemistry field. As a kind of typical engineering material, nichrome alloy has been widely used due to its low price, high strength and anti-corrosion properties. However, it cannot be served as the working electrode because of its high resistivity. In this paper, a novel inlaying ultra-thin carbon paste electrode (IUTCPE) was constructed at nichrome alloy substrate, which was introduced to study the catalytic reduction of metronidazole as a new method. A well-defined reduction peak of metronidazole was obtained and the peak currents increased linearly with the increasing contents. The proposed method has been successfully applied to the detection of metronidazole tablets. The recovery was in the range of 96.0-104.7 %.

EXPERIMENTAL

Cyclic voltammograms and linear sweep voltammetry (LSV) were carried out by CHI832 electrochemical workstation (Shanghai Chenhua Instrument Company, China). A PFS-80 digital pH meter (Shanghai Dazhong Analysis Instrument

Company, Shanghai, China) was used for preparation of the buffer. A three-electrode system comprised the IUTCPE, platinum counter-electrode and a saturated calomel reference electrode (SCE). All the potentials in the text are quoted *versus* this reference electrode. A personal computer was used for data storage and processing.

Graphite powder (The purity is 99.85 %, purchased from Shanghai, China) and liquid paraffin (C.P. purchased from Xinxiang, China) were used for the preparation of IUTCPE. Metronidazole/NaCl injection was adopted as standard solution: the concentration of metronidazole was 1.169×10^{-2} mol/L (Shanghai Huazhong Pharmaceutical Ltd.). Metronidazole tablets (Kangmei Pharmaceutical Co.Ltd.)

All other chemicals were at least analytical reagent grade and all solutions were prepared with doubly distilled deionized water. All experiments were performed at ambient conditions.

Electrode preparation: A nichrome rod with 4 mm diameter (0.126 cm^2) and a known length was sealed in a plastic tube of matching length. One end was used as electrode connection held out of the plastic tube, the other as the working electrode interface. The surface was polished with alumina slurry ($0.05 \mu\text{m}$) and subsequently cleaned ultra-sonically in HNO_3 (1:1), absolute ethanol, doubly distilled water and then allowed to dry in the air.

A 1:0.25 (w/w) mixture of graphite powder and paraffin was blended in an agate mortar and pestle to obtain a homogeneous paste. The pretreated nichrome substrate was rubbed in the carbon paste for inlay. The carbon paste out of the tube was cleaned and rinsed with distilled water before use.

The electrochemical experiments were performed in the 10 mL volume of the Britton-Robinson buffer solution (pH 3) containing an appropriate volume of standard metronidazole solution with three electrodes. The voltammetric scanning step was initiated after 10s equilibrium time. The cyclic voltammogram (CV) curves and the linear scan voltammetry (LSV) curves were recorded separately.

RESULTS AND DISCUSSION

Electrochemical characteristic of IUTCPE: The electrochemical characteristic of the IUTCPE was examined by cyclic voltammetry in 0.1 mol/L H_2SO_4 solution containing 1×10^{-3} mol/L $\text{K}_4[\text{Fe}(\text{CN})_6]$. Fig. 1 showed the cyclic voltammograms of $\text{K}_4[\text{Fe}(\text{CN})_6]$ measured with carbon paste electrode (CPE), pre-anodized carbon paste electrode (PACPE) and IUTCPE. At the carbon paste electrode, a pair of oxidation-reduction peaks was observed with the redox peak potential difference of 221 mV (curve a). At the pre-anodized carbon paste electrode, the peak currents increased obviously. The oxidation peak appeared at 0.381 V, the reduction peak potential shifted to 0.306 V (curve b). Compared with the carbon paste electrode and pre-anodized carbon paste electrode, a pair of sharp oxidation-reduction peaks of $\text{K}_4[\text{Fe}(\text{CN})_6]$ was observed at the IUTCPE (curve c). The peaks separation approached 85 mV and the ratio of oxidation-reduction peaks currents approximately equaled 1. The peak currents were about 5 times higher than that of curve b. The results showed that the IUTCPE possessed more sensitive detection performance than carbon paste electrode and pre-anodized carbon paste electrode.

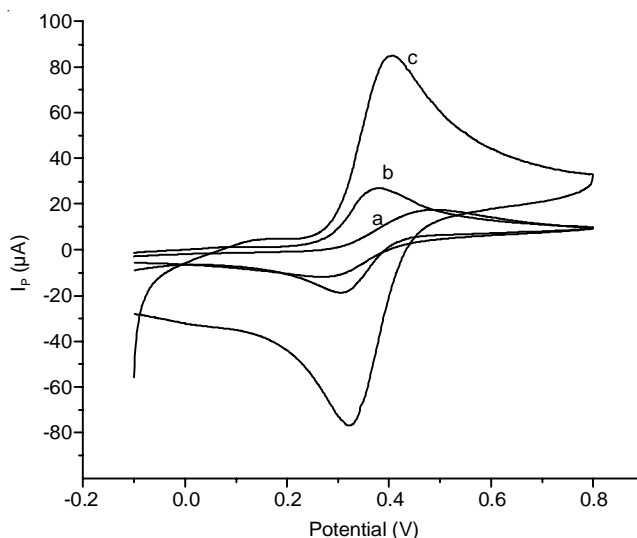


Fig. 1. Cyclic voltammograms of 1×10^{-3} mol/L $\text{K}_4[\text{Fe}(\text{CN})_6]$ in 0.1 mol/L H_2SO_4 at three different electrodes; (a): carbon paste electrode; (b): pre-anodized carbon paste electrode; (c): IUTCPE. Scan rate: 100 mV/s; pre-anodized carbon paste electrode: carbon paste electrode was anodized by successive cyclic voltammetry for 30 cycles from -0.3 to +2.1 V with a scan rate of 100 mV/s in NaOH (0.2 mol/L)

Wang *et al.*^{20,21} have reported that there was long and narrow grooved structure distributing on the nichrome alloy surface. The electrochemical inertia of nichrome substrate could be greatly changed after inlaying a layer of 100 nm thick carbon paste film. Compared with conventional carbon paste electrode, the IUTCPE had the advantage of wide potential window as well as remedied the defect of poor repeatability caused by loose structure.

Electrochemical responses of metronidazole at different electrode: As mentioned before, the IUTCPE was constructed by surface inlaying. Its surface properties such as energy levels, electronic structure and reactivity could be quite different from the conventional CPE²¹. Fig. 2 showed the comparison results for three different electrodes, *i.e.*, bare nichrome electrode, carbon paste electrode and IUTCPE by linear scan voltammetry. It can be found that there were no signals at three electrodes in 0.1 mol L^{-1} Britton-Robinson (pH 3) buffer (Fig. 2 (a), (c), (e)). Upon addition of 1.17×10^{-3} mol/L metronidazole, bare nichrome electrode presented no catalytic reduction activity to the metronidazole, which indicated that the nichrome was not suited to be a working electrode (Fig. 2 (b)). As can be seen from Fig. 2 (d), a low and broad reduction peak was observed at about -0.65 V at the carbon paste electrode over the same potential range. By contrast, Fig. 2 (f) showed the cathodic peak potential for metronidazole positively shifted to -0.58 V and the current dramatically increased. The result demonstrated that IUTCPE exhibited significant catalytic reduction ability to the metronidazole, its sensitivity to the reduction response was much higher than carbon paste electrode. It was because the thickness of the carbon paste film was at the nanometer scale²², which made the electrode surface own the special property similar to that of nano-materials. IUTCPE could be used for the quantitative determination of metronidazole.

Electrochemical reduction of metronidazole at IUTCPE: Voltammetric methods have been adopted to study

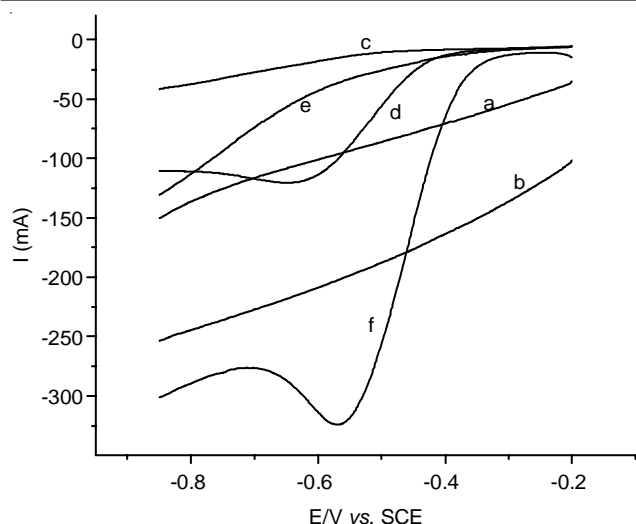


Fig. 2. Linear sweep voltammograms of 1.17×10^{-3} mol/L metronidazole in pH 3 Britton-Robinson buffer at three different electrodes. (a, b): bare nichrome electrode; (c, d): carbon paste electrode; (e, f): IUTCPE. Scan rate: 200 mV/s

the reduction of metronidazole^{16,19}. It is a complex process for the reduction of metronidazole to form a corresponding amine which accompanies by a six-electron transfer²³. The formation of the nitroderivatives (R-NO) and hydroxylamine (R-NHOH) requires four electrons and four protons²⁴. In the protonated form, reduction of the hydroxylamine to the corresponding amine requires two electrons¹⁷.

The electrochemical behaviours of metronidazole at IUTCPE have been examined using cyclic voltammetry (CV). Fig. 3 showed the first 3 scans of cyclic voltammograms responses of metronidazole at the IUTCPE in pH 3 Britton-Robinson (B.R.) buffer solution. During the first cathodic sweep from -0.2 to -0.85 V metronidazole exhibited significant reduction currents starting around -0.4 V vs. SCE. A sensitive and well-defined reduction peak appeared at -0.58 V. There was no corresponding oxidation peak could be observed on the anodic scan. This suggested that the electrode reaction of metronidazole was totally irreversible. As the increasing of scans, the peak currents at -0.58 V decreased remarkably in the second cyclic sweep and decreased slightly afterwards.

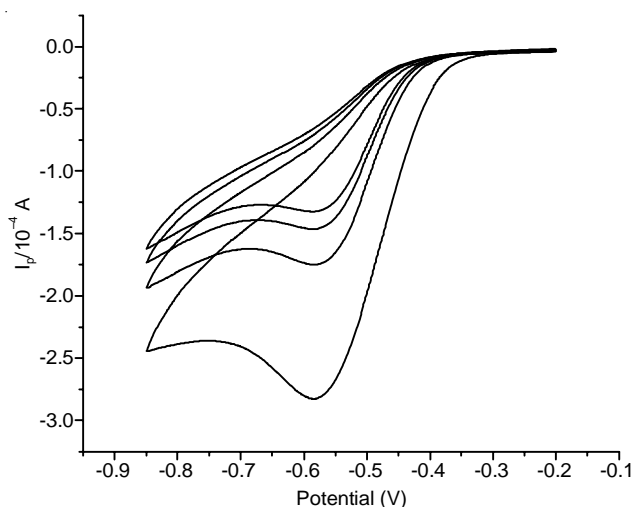
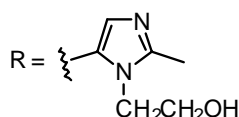
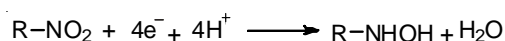


Fig. 3. Cyclic voltammograms of 1.17×10^{-3} mol/L metronidazole in pH 3 Britton-Robinson buffer at the scan rate of 200 mV/s

The phenomenon above can be explained by the adsorption of metronidazole or its reductive product occurred at the electrode surface, so as to retard the action of the electrode surface¹⁷.

According to the currently accepted mechanism for the electro-reduction of aromatic and heteroaromatic nitro compounds, this reduction peak was attributed to the four-electron reduction of nitro group to the corresponding hydroxylamine²³⁻²⁵:



Influences of supporting electrolytes and pH: The choice of supporting electrolytes is very important to the response of analyte. Various supporting electrolytes such as 0.1 mol/L $\text{CH}_3\text{COOH-CH}_3\text{COONa}$, 0.1 mol/L Britton-Robinson (B.R.), 0.1 mol/L $\text{NH}_3\text{-NH}_4\text{Cl}$, 0.1 mol/L citrate buffer, 0.1 mol/L HCl, 0.1 mol/L KH_2PO_4 , 0.1 mol/L NaOH were tested by linear scan voltammetry in the experiments. The results revealed that in the alkaline and neutral solution, the reduction peaks were broad and low. The experiment should be performed in the acidic conditions. An excellent reduction response was obtained in 0.1 mol/L Britton-Robinson buffer for metronidazole where the peak shape was well-defined and the peak current was higher.

The reduction of metronidazole is dependent on pH in acid and neutral media and independent of pH in alkaline media²⁶. In acid 0.1 mol L⁻¹ Britton-Robinson buffer, the effects of pH on the reduction peak potential were examined by linear scan voltammetry. As shown in Fig. 4, The best reduction peak with high peak current was obtained at pH 3. A lower pH less than 3.0 could cause a decrease of the current response. Brett *et al.*²⁷ reported that the metronidazole was prone to protonize if pH was lower than pKa (2.5). When pH > 3, the peak currents decreased obviously. The lower the pH of the electrolyte, the

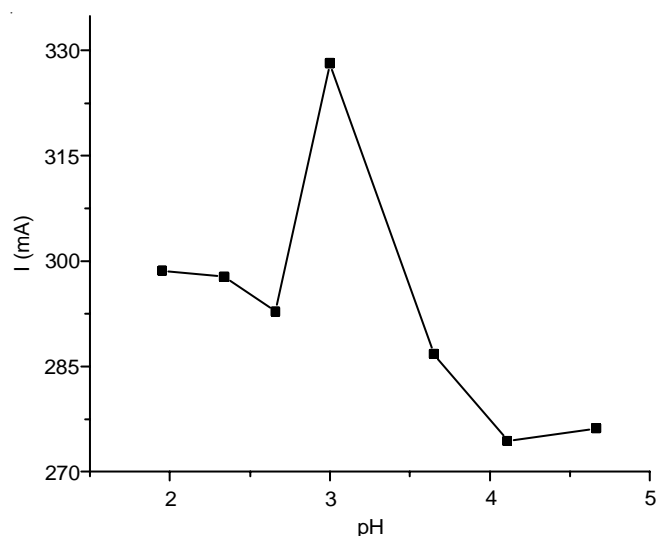


Fig. 4. Linear relationship of I_p vs pH $c_{\text{MTZ}} = 1.17 \times 10^{-3}$ mol/L, scan rate: 200 mV/s, supporting electrolyte: 0.1 mol/L Britton-Robinson

TABLE-1
DETERMINATION OF SAMPLE RESULTS AND RECOVERIES (n = 5, t 0.05, 4 = 2.78)

Samples	Contents (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	RSD (%)
1	10.00	5.00	15.17	101.7	3.2
2	10.00	10.00	19.96	96.0	2.6
3	20.00	5.00	25.22	104.4	2.1
4	20.00	8.00	27.72	96.5	3.4
5	20.00	12.00	32.56	104.7	4.1

more favorable for the reduction of the nitroimidazole¹⁴. The reduction peak potential shifted negatively with the increase of pH. Thus, 0.1 mol/L Britton-Robinson (pH 3) was chosen as the optimum electrolyte for further experiments.

Influence of scan rate: The influence of scan rate on the reduction of metronidazole at the IUTCPE was also studied by linear scan voltammetry. It was found that the potential (E_{pc}) shifted negatively with the increase of the scan rate. When scan rate was lower than 260 mV/s, the reduction peak currents increased linearly with the square root of scan rate. It obeyed the following equations: $I_p(\mu A) = 74.82 + 16.85 v^{1/2}$, $r = 0.9932$, which accorded with the Randles-Ševcik equation. When scan rate was in the range of 260-410 mV/s, $I_p-v^{1/2}$ deviating from the linear relationship and upwarp. The results suggested that the electro-reduction of metronidazole was mainly a diffusing controlled process, while it was a characteristic of adsorption process at high scan rate. Scan rate of 200 mV/s was chosen for the further studies.

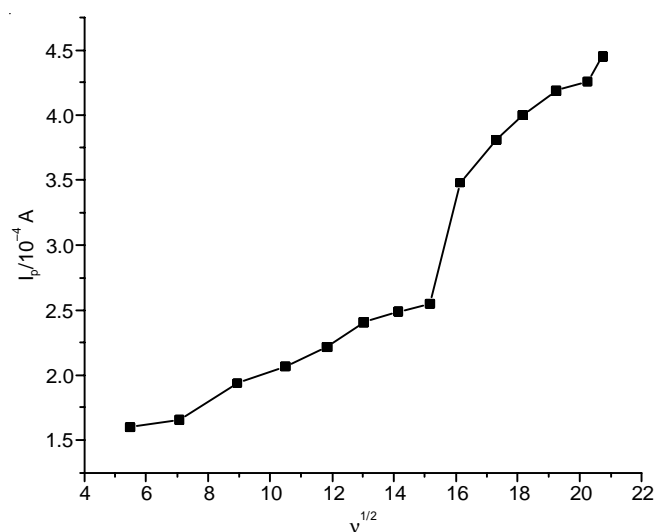


Fig. 5. Relationships between reduction current of 1.05×10^{-3} mol/L metronidazole and the $v^{1/2}$ in 0.1 mol L⁻¹ Britton-Robinson buffer (pH 3)

Influence of starting scanning potential: The electrocatalytic reaction of metronidazole was totally irreversible and the reaction rate constant is a function of potential, so the proper initial scan potential can increase the rate of electrode reaction. Changing starting scanning potential at the range of (-0.4 to 0.2 V), the reduction response was investigated by linear scan voltammetry. The peak current was higher and the peak shape was better when the starting scanning potential was -0.2 V.

Effect of interferences: Various possible interferences were examined for their effect on the determination of metronidazole. Measurements of the peak currents for each solution were

repeated three times and the average current values were obtained. If the presence of an interferent altered the average current signal by less than $\pm 5\%$, we considered that caused no interference. The results showed that 1000-fold of Na⁺, Cl⁻, CH₃COONa, citric acid, urea, thiourea, 100-fold of Mg²⁺, SO₄²⁻, Ca²⁺, L-Cys, glucose, starch did not interfere the determination of metronidazole. While 1000-fold of F⁻ and 100-fold of Cu²⁺ had serious interference on the metronidazole, the reduction signal of metronidazole decreased 10 times.

Regeneration, stability and repeatability of IUTCPE:

In the solution contained 1.17×10^{-3} mol L⁻¹ metronidazole, the calculated R.S.D. of the responses at the same electrode was 4.8% (10 measurements). After the IUTCPE was stored in the room temperature for 20 days, the reduction peak current decreased less than 5% of the initial signal. The electrode exhibited a good repeatability and Stability. Because the reaction interface of the IUTCPE was ultra thin, analyte could not diffuse into the interior of the film to produce memory effect, so the repeatability was better than carbon paste electrode²³.

Linear range and detection limit: Under optimized conditions, linear scan voltammetry was used for quantitative determination of metronidazole. The peak currents of linear scan voltammetry were linearly proportional to the concentrations of metronidazole. In the range of 7×10^{-7} - 1.17×10^{-3} mol/L, the linear regression equation was $I_p(\mu A) = 3.029 + 0.2626c$ (mmol), $r = 0.9960$. The detection limit was 2.8×10^{-7} mol/L.

Application: To demonstrate the applicability of the proposed method for the analysis of real sample, the IUTCPE was used to determine the metronidazole in the tablets.

The mass of five tablets were determined and finely powdered. After drying under vacuum at 60 °C, 0.2524 g powder was accurately weighed and dissolved in the water. The obtained suspension should be centrifuged and washed 4 times, then the filtrate was combined and transferred into a 100 mL volumetric flask to prepare the sample solution. Appropriate solutions were prepared by taking suitable volume of the clear filtrate and diluting them with supporting electrolyte mentioned above. The quantitative determination was performed by standard-addition method. The results were shown in Table-1:

Conclusion

In this paper, nichrome alloy was introduced to prepare an inlaying ultra thin carbon paste electrode as a new electrode substrate. The results indicated that the IUTCPE facilitated determination of metronidazole with good sensitivity and reproducibility compared to the conventional carbon paste electrode. Under the optimized experimental conditions, IUTCPE was successfully applied in the determination of tablets, which obtained stable and reliable results. The proposed

electrode offered a new approach for the electrochemical determination of pharmaceuticals with the significant advantages such as easy preparation, low cost, wide potential window, non-toxic and non-polluting.

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