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Determination of ornidazole in pharmaceutical dosage forms based on reduction at an activated glassy carbon electrode[1]

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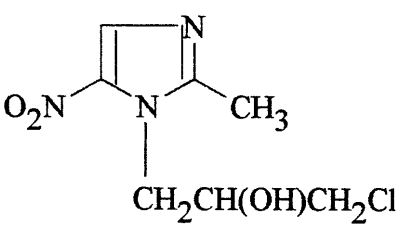
Abstract

The electrochemical reduction of ornidazole was studied at a glassy carbon electrode activated by applying a new pretreatment. The dependence of intensities of currents and potentials on pH, concentration, scan rate, nature of the solvent (aqueous media, mixed aqueous-organic systems) and surfactant was investigated. Linear calibration plots were obtained over the concentration ranges 4×10^{-6}–6×10^{-4} and 6×10^{-6}–6×10^{-4} mol l^{-1} in 0.2 M H_2SO_4 and acetate buffer (pH 4.7), respectively. The method was applied to the determination of ornidazole in different drug formulations. © 1997 Elsevier Science B.V.

Keywords: Ornidazole; Determination; Voltammetry; Activated glassy carbon electrode

1. Introduction

Certain derivatives of nitroimidazole are known to possess antibacterial, antiprotozoan and anti-cancer activity. Ornidazole, 5-nitroimidazole derivative, acts selectively against anaerobic and microaerophilic bacteria and protozoa, with a half-life longer than that of metronidazole, that is most widely used therapeutically.



The mechanism of action of nitroimidazoles, as ornidazole, is thought to involve interference with DNA by a metabolite in which the nitro group has been reduced. In-vivo, any amino metabolite of such drugs has not yet been detected on free

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form. It seems that their cytotoxicity is not due to the final reduction products, but to an unstable intermediate, possibly the formation of the anion radical, at a lower reduction level than would correspond to a complete reduction (Edwards, 1986). The studies on the mechanism of action of nitroimidazoles showed that the reduction pathway is more complex than is usually postulated for the electroreduction of aromatic nitrocompounds (Declerck and de Ranter, 1987). For these reasons, elucidation of the mechanism of the electroreduction of ornidazole and related compounds is of evident biological interest.

Very few electrochemical studies have been made on this compound. Sankar and Reddy (1989) reported that ornidazole produces a single cathodic wave in all the inert electrolytes used by polarography. Warowna et al. (1991) studied the polarographic reduction of ornidazole, metronidazole and tinidazole on dropping mercury electrode to carry out their direct determination in pharmaceutical dosage forms. Lopez Fonseca's group (1993) described the electrochemistry of ornidazole by normal pulse and reverse pulse polarography in aqueous and non-aqueous media. Studies of the electroreduction of ornidazole at solid electrodes have been limited only to its voltammetric investigation in non-aqueous media. Barety et al. (1984) studied the electroreduction of various nitroimidazole derivatives in dimethylsulphoxide where a radical anion is formed in a first electron uptake at the platinum rotating disc electrode.

According to our knowledge, no examination has appeared at carbon-based electrodes in the literature. The aim of this study was to investigate the electrochemical behavior of ornidazole at the glassy carbon electrode activated by applying a new pretreatment, to throw more light on the mechanism of the electroreduction reaction. The application of the method to the analysis of the drug in pharmaceutical formulations was also assessed.

2. Experimental

2.1. Apparatus

Voltammetric studies were carried out with a Tacussel type PRG 3 polarograph coupled with an EPL-2 recorder (Tacussel). All the potentials were referred to the saturated calomel electrode (SCE) and a platinum wire was used as counter electrode. The working electrode was a glassy carbon stationary electrode (Tacussel XM 540; area:1.013 cm^2). For the application of the pretreatment to the glassy carbon electrode, a Wenking Model HP 70 potentiostat and exact-type 250 function generator were used.

2.2. Reagents

Stock solutions of ornidazole (Roche Product, used without further purification) were prepared daily by direct dissolution in selected supporting electrolytes. Four different supporting electrolytes, namely sulphuric acid (0.2 M), acetate buffer (pH 3.6–5.6; 0.2 M), phosphate buffer (pH 6.5–10.5; 0.2 M) and sodium hydroxide (0.2 M) were prepared in doubly distilled water. Desoxygenation was accomplished by passing purified nitrogen through the cell.

2.3. Pre-treatment of the glassy carbon electrode

The electrode was pretreated by cycling a square-wave potential with a frequency of 350 Hz between the potential limits of ± 6 V followed by the application of a triangular potential sweep between ± 6 V (frequency 3500 Hz) in 0.1 M potassium nitrate solution. Finally, the electrode was subjected to an electrochemical pretreatment by applying a potential of $+1.5$ V for 5 min and then -1.0 V for 2 s in 0.1 M potassium nitrate solution.These steps were repeated until the voltammetric response of the electrode became reproducible. At the end of this procedure, the electrode surface was so stable that for ca. 40 measurements, the electrochemical pretreatment alone was sufficient before each scan (Özkan et al., 1994).

2.4. Analysis of dosage forms

Ten tablets were weighed and ground to a fine powder. An accurately weighed amount of the powder corresponding to a stock solution of concentration *ca.* 1×10^{-3} mol l^{-1} was transferred into a 100 ml standard flask and diluted to the mark with the selected supporting electrolyte. The

contents of the flask were stirred magnetically for 15 min to effect complete dissolution and then filtered through a fine pore filter paper. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and diluting them with the same supporting electrolyte.

No pretreatment for the injection solution was done except for dilution with the selected supporting electrolyte.

Voltammograms were recorded as in pure ornidazole.

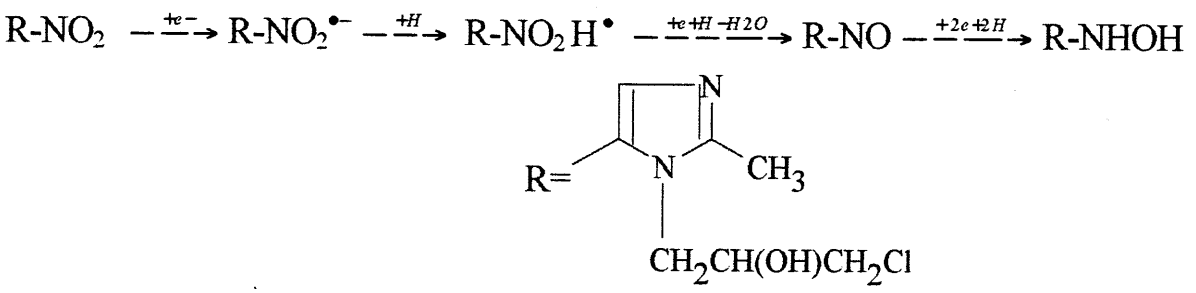
3. Results and discussion

3.1. Voltammetry at the activated glassy carbon electrode

In our previous paper (Özkan et al., 1994), a new pretreatment procedure was described for treating glassy carbon electrode which offered improved responses in drug analysis (Özkan et al., 1994; Biryol et al., 1995; Özkan and Şenturk, 1996; Biryol and Özkan, 1997; Şenturk et al., 1997). It was seen by scanning electron microscopy that this activation created scratches and holes in the glassy carbon electrode which gave a higher active area comparing to the non-activated glassy carbon electrode (Özkan et al., 1994).

In the case of activated glassy carbon electrode, the electroreduction of ornidazole was studied over pH range 1.0–12.5 in the various supporting electrolytes.

Under linear sweep voltammetry, the drug gave rise to one well-defined, sharp cathodic peak in more dilute solution and/or at lower pH (Fig. 1a). This peak is related to the four-electron reduction of the nitro group into the corresponding hydroxylamine according to a classical scheme previously described (Declerck and de Ranter, 1987; Fry, 1982; Laviron and Roullier, 1990; Zuman and Fijalek, 1990a,b; El Jammal et al., 1992).



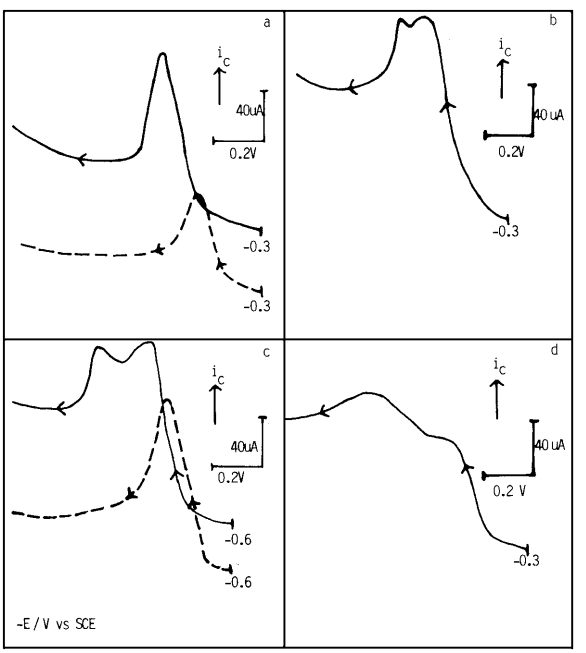


Fig. 1. Voltammograms of various concentrations of ornidazole. (a) 4×10^{-4} mol l^{-1} in acetate buffer pH 4.7; (b) 10^{-3} mol l^{-1} in acetate buffer pH 4.7; (c) 6×10^{-4} mol l^{-1} in phosphate buffer pH 9.5; (d) 4.5×10^{-3} mol l^{-1} in 0.2 M NaOH. Full line: scan rate 100 mV s^{-1}; dotted line: scan rate 25 mV s^{-1}.

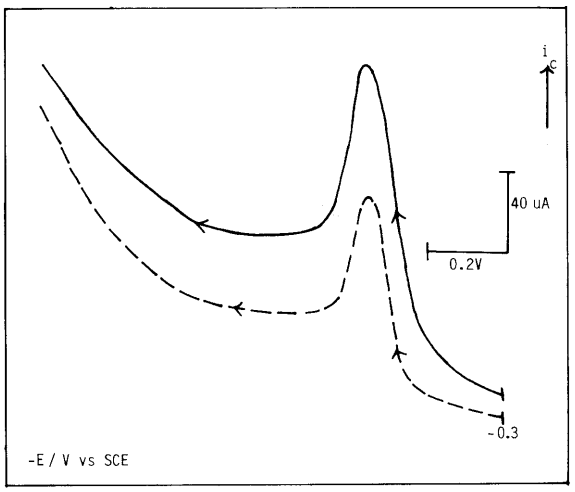


Fig. 2. Voltammograms of 4×10^{-4} mol l^{-1} ornidazole in acetate buffer pH 4.7. Scan rate 100 mVs^{-1}. Full line: activated glassy carbon electrode; dotted line: non activated glassy carbon electrode.

Though the electrochemical reduction mechanism consists of many intermediate steps, all may be less stable, thus giving only one peak. Application of the activated glassy carbon electrode caused dramatically increased current response and improvement of shape of the signal in comparison with those obtained at non-activated glassy carbon electrode polished on 0.3 μm alumina after each scan (Fig. 2). Although it seems to be the background current also after the activation procedure but the ratio of faradaic to background current at the peak potential for the activated electrode is 3.70 while in the case of non activated electrode this ratio is 1.91.

In more concentrated solutions and/or at higher pHs, the reduction peak of nitro group splitted into two peaks (Fig. 1(b, c)). This phenomenon was also affected by the pretreatment of the electrode. In the case of non-activated glassy carbon electrode, the splitting could be observed only when the concentration of ornidazole exceeded 2×10^{-3} mol l^{-1} at pH 9.5. This demonstrates the importance of surface modification which is probably involved in the electrode reaction. As pH and concentration increased, the first peak fell and the second increased, their shapes becoming broader (Fig. 1d).

As reported on the literature data, the splitting of nitro group reduction peak at solid electrodes may have several origins. This phenomenon can be attributed to the two two-electron reduction of the nitro group, nitro-nitroso and nitroso-hydroxylamine, according to a hypothesis proposed by El Jammal et al. (1992) in alkaline solutions at glassy carbon electrode. However, such a mechanism has never been reported. Some other authors have also observed a splitting on gold and glassy carbon electrodes depending on pH (Rubinstein, 1985; Nishihara and Kaise, 1983; Nishihara and Shindo, 1987). They have attributed this splitting to the formation of radical species such as $RNO_2H^{\bullet}$ or $R\text{-}NO_2^{\bullet -}$ and $R\text{-}NOH^{\bullet}$ or $R\text{-}NO^{\bullet -}$ which may be more stable in solution than when adsorbed on the electrode surface.

A single anodic peak was observed in the reverse scan in cyclic voltammetry in acidic and neutral media (e.g. at $+0.55$ V in acetate buffer pH 4.7). The anodic peak may be due to the oxidation of reduced product. In alkaline media, the peak height decreased and completely disappeared at pH values higher than 9.5. Cyclic voltammetry demonstrated the total irreversibility of this system at scan rates from 10 to 100 mVs^{-1}.

Subsequent scans with the same electrode surface resulted in a gradual decrease of the reduction peak. On the second scan and subsequent scans in dilute solutions and/or at lower pHs a new small cathodic peak was detected (Fig. 3a). On the other hand, the splitting observed in concentrated solutions and/or at higher pHs disappeared after the first scan (Fig. 3b).

As the scan rate was increased over the 10–100 mVs^{-1}, a negative shift in the peak potential was observed, with simultaneous increase in diffusion current (Fig. 1a). The splitting disappeared at lower scan rates and the first peak became again sharper (Fig. 1c). The linear increase of the reduction peak current with the square root of the scan rate indicated the electrode process to be mainly diffusion controlled.

The effect of the solution pH on the peak potential and peak current was evaluated (Fig. 4). By increasing the pH, the peak potential increased linearly, with a slope of 89 mV/pH unit, till pH

7.5, then became almost pH independent. This observation confirms the results at dropping mercury electrode (Lopez Fonseca et al., 1993). The peak intensity showed the behavior depicted in Fig. 4b; reaching a maximum at about pH 1 and between pH 4.5 and 7; hence these pH values were chosen to carry out the electroanalyiical study.

The effects of organic solvent on peak current and peak potential were also studied. As expected, the peak current in solutions containing 30% v/v dimethylformamide decreased. This effect was more pronounced in alkaline media. At all pH values, a slight decrement towards a more negative potential in peak potential was observed.

Addition of the anionic surfactant, sodium lauryl sulphate, within the range $0–2 \times 10^{-3}$ M did not resulted in considerable decrease in the peak height. The peaks became sharper than that obtained in aqueous solution without surfactant. Further, splitting into two peaks at higher concentrations and pHs decreased with addition of the surfactant, disappearing completely in higher surfactant concentrations. This observation at glassy carbon is in contrast to those obtained at mercury electrodes for nitro compounds (Lopez Fonseca et al., 1993; Kemula and Krygowsky, 1979).

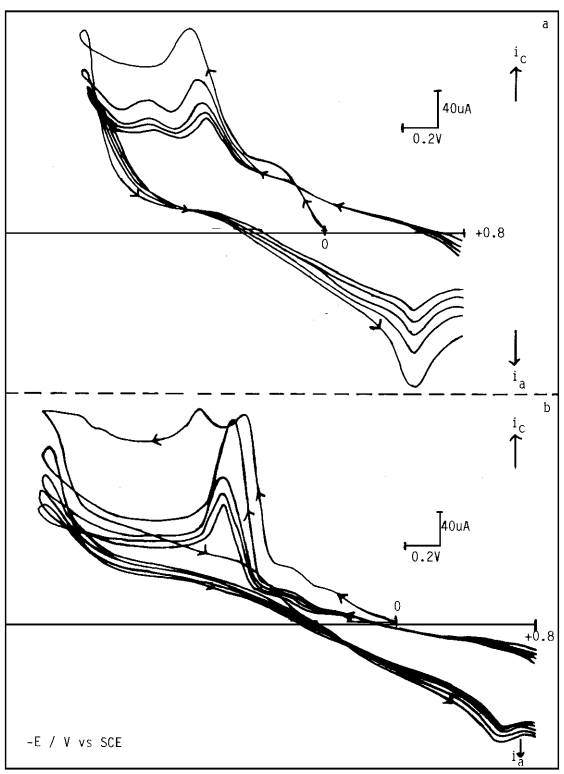


Fig. 3. Repetitive cyclic voltammograms of various concentrations of ornidazole. (a) 4×10^{-4} mol l^{-1} in acetate buffer pH 4.7; (b) 4×10^{-3} mol l^{-1} in phosphate buffer pH 8.5. Scan rate, 100 mV s^{-1}.

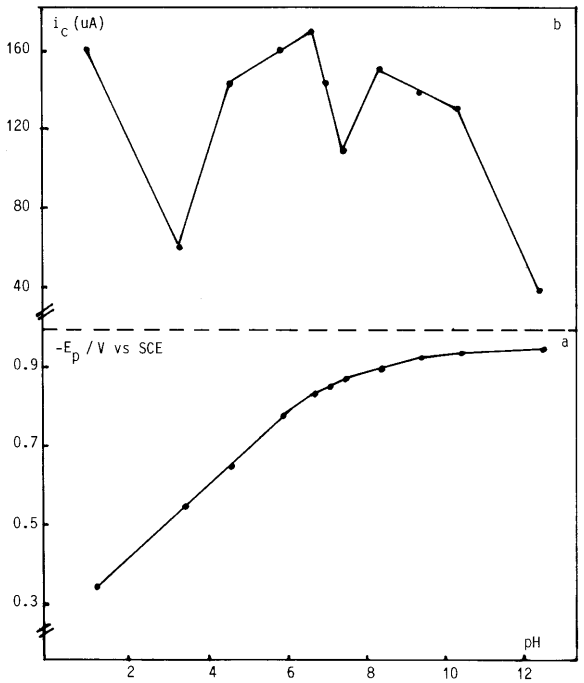


Fig. 4. Effects of the pH on the ornidazole peak potential (a) and peak current (b). Ornidazole concentration, 4×10^{-4} mol l^{-1}, Scan rate, 100 mV s^{-1}.

3.2. Analytical validity

For analytical purposes, best results (with respect to peak shape and peak current enhancement) were obtained with 0.2 M H_2SO_4 and acetate buffer (pH 4.7) at scan rate of 100 mVs^{-1}. The reproducibility of peak potential and peak current was tested by repeating four experiments on 1×10^{-4} mol l^{-1} ornidazole. The relative standard deviations were calculated to be 0.6 and 0.8% for peak potential and 1.6 and 1.4% for peak current in 0.2 M H_2SO_4 and acetate buffer, respectively. Table 1 summarizes the characteris-

Table 1
Characteristics of ornidazole calibration plots

Medium	Concentration range mol l^{-1}	Slope μA mol^{-1} per l	Intercept μA	Correlation coefficient	S.E. of slope μA mol^{-1} per l	S.E. of intercept μA
0.2 M H_2SO_4	4×10^{-6}–6×10^{-4} ($n=11$)	2.07×10^{5}	9.71	0.998	3.68×10^{3}	0.81
Acetate buffer (pH 4.7)	6×10^{-6}–6×10^{-4} ($n=10$)	2.62×10^{5}	7.28	0.999	4.13×10^{3}	0.94

Table 2
Comparative studies for ornidazole dosage forms

Medium	Voltammetric assay						Spectrophotometric assay		
	0.2 M H_2SO_4			Acetate buffer (pH4.7)					
	Vaginal			Vaginal			Vaginal		
Formulation[a]	Tablet	Tablet	Injection	Tablet	Tablet	Injection	Tablet	Tablet	Injection
Mean[b]	252.5	492.5	491.4	251.9	491.6	491.4	249.0	494.0	493.6
R.S.D.%	2.58	1.26	0.60	1.94	1.28	0.74	2.13	1.16	0.99
Student's $t^{c}_{0.05}$	1.295(2.101)	0.496(2.101)	1.152(2.101)	1.294(2.101)	0.907(2.101)	1.152(2.101)	—	—	—

[a] Tablet (250 mg per tablet), vaginal tablet (500 mg per tablet), injection solution (500 mg 3 ml^{-1}).
[b] Each value is the mean of ten experiments.
[c] Tabulated (significant) levels, at $P=0.05$, in parentheses.

Table 3
Recovery studies by proposed method at activated glassy carbon electrode

Medium	0.2 M H_2SO_4			Acetate buffer (pH 4.7)		
	Vaginal			Vaginal		
Formulation	Tablet	Tablet	Injection	Tablet	Tablet	Injection
Added (mg)	20	20	20	20	20	20
Recovered[a] (mg)	19.72	19.72	19.82	19.78	19.66	19.88
Recovery[a] (%)	98.6	98.6	99.1	98.9	98.3	99.4
R.S.D.%	1.16	1.16	0.94	1.58	1.59	1.28

[a] Each result is the average of five experiments.

tics of the calibration curves established in proposed supporting electrolytes.

On the basis of these results, the proposed method was applied to the direct determination of ornidazole in pharmaceutical dosage forms. To the best of our knowledge, there is no any official method in pharmacopoeias related to pharmaceutical preparations of ornidazole. For this reason, the spectrophotometric method (Hassan et al., 1989) was used for comparison. It is evident that the proposed method is sensitive as well as spectrophotometric procedure (Table 2).

According to the Student's t-test, the calculated t values did not exceed the theoretical value for a significance level of 0.05. These results indicate that there is no significant difference between the population means for the two procedures. On the other hand, the voltammetric assay is simple and rapid compared with the UV spectrophotometric assay. Moreover, the accuracy of the proposed method was also evaluated by recovery studies after adding known amounts of the pure drug to various preanalysed formulations of ornidazole and applying the procedure specified under Section 2. As Table 3 shows, good results demonstrate the validity of the proposed method for the determination of ornidazole in commercial dosage forms.

In summary, it is concluded that the electrochemical method for the determination of ornidazole presented in this paper has the advantage of being rapid, simple and inexpensive.

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