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Electrochemical reduction of metronidazole at activated glassy carbon electrode and its determination in pharmaceutical dosage forms[1]

S.A. Özkan [a], Y. Özkan [b], Z. Şentürk [c,*]

[a] *Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry,06100 Ankara, Turkey*
[b] *Gülhane Military Medical Academy, Department of Pharmaceutical Technology, 06018 Ankara, Turkey*
[c] *Gazi University, Faculty of Pharmacy, Department of Analytical Chemistry, 06330 Ankara, Turkey*

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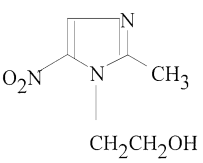
Abstract

A voltammetric method has been developed for the determination of metronidazole in dosage forms. The method is based on the electrochemical reduction of the drug at a glassy carbon electrode activated by applying a new pretreatment. The influence of pH, concentration, scan rate and presence of organic solvent and surfactant has been studied. The current is proportional to the concentration and permits the drug to be determined in the concentration range 2×10^{-6}–6×10^{-4} M in Britton-Robinson buffer (pH 10). Furthermore, results obtained by the proposed method have been compared with USP XXIII procedure which involves a HPLC method.

Keywords: Metronidazole; Voltammetry; Activated glassy carbon electrode; Drug analysis

1. Introduction

Many nitroheterocyclic compounds have antibacterial and antiprotozoan activities involving the biological reduction of the nitro group [1]. These compounds have also been used in the treatment of cancer, both as radiosensitizers and as cytotoxic agents. One representative of this important class of drug is metronidazole, 5-nitroimidazole derivative, which is an effective agent for a variety of protozoal disease including trichomoniasis, giardiasis, amoebiasis and balantidiasis [2].



Metronidazole has been determined by spectrophotometry [3–7], titrimetry [8,9], thin-layer

* Corresponding author. Gazi Üniversıtesi, Eczacılık Fakültesi, Analitik Kimya Anabilim Dalı, 06330 Etiler, Ankara, Turkey. Fax: +90 312 2131081.

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chromatography [10], gas chromatography [11–13], and high pressure liquid chromatography [14–17].

From the electrochemical point of view, several works on the electroreduction of the drug have been published. At a dropping mercury electrode, metronidazole and related compounds give rise to one cathodic wave/peak (I), or two (I and II), the first (I) corresponding to a four-electron reduction to the hydroxylamine and the second (II) to a two-electron reduction to the amine, depending on the supporting electrolyte and pH [18–23]. First attempt was made to study the electroreduction of metronidazole at solid electrodes by Bishop and Hussein [24]. The authors reported that metronidazole is reducible at rotating platinum and gold electrodes in alkaline media by two steps to the amine. A study in non-aqueous media (e.g. dimethylsulphoxide) where a radical-anion is formed in a first electron uptake at the platinum rotating-disc electrode, was also reported [25].

The cytotoxicity of nitroimidazoles is not due to the final reduction products, but to the formation of intermediates, possibly free radicals, that cause the cleavage of the DNA strand and the destabilization of the helix [26–28]. However, the reduction pathway of nitroimidazoles is more complex than previously proposed for the electroreduction of aromatic nitrocompounds [29].

In view of the above, since pharmacological activity is related to the redox processes on the nitro group,the knowledge of the voltammetric behavior of metronidazole and other 5-nitroimidazoles is of biological interest. To the best of our knowledge, the electrochemical reduction of metronidazole at carbon-based electrodes has not yet been studied. The aim of this work was to carry out an electroanalytical study of metronidazole at the glassy carbon electrode activated by applying a new pretreatment. Since the reduction mechanism of nitro group depends significantly not only on the nature of the electrode material, but for a given material (for solid electrodes) on the pretreatment [30,31], this paper presents an attempt to fill some of this gap and complements our previous works on the electrochemistry of other nitroimidazoles [32,33].

2. Experimental

2.1. Apparatus

The voltammetric measurements were performed on a PRG-3 polarograph (Tacussel) associated to an EPL-2 recorder (Tacussel). All the potentials were reported vs a saturated calomel electrode (SCE) and the auxiliary electrode was a platinum wire. The working electrode was a glassy carbon stationary electrode (Tacussel XM 540; area: 1.013 cm^2). For the electrode pretreatment a Wenking model HP 70 potentiostat and an exact-type 250 function generator were used.

High-performance liquid chromatographic experiments were carried out on a Waters liquid chromatograph (Model 510) equipped with a UV detector (Model 481). The chromatograms were analysed with a chromatographic workstation (Baseline 810).

2.2. Reagents

Metronidazole (generously provided by Eczacıbaşı Drug Inc., İstanbul, Turkey) was used without further purification. All other chemicals were of analytical reagent grade and supplied by Sigma or Merck. Stock solutions under voltammetric investigation were renewed daily and prepared in Britton-Robinson buffer (pH 1.6–10.6). Double distilled water was used throughout. Desoxygenation was accomplished by passing purified nitrogen through the cell.

2.3. Pretreatment of the glassy carbon electrode

Activation of the glassy carbon electrode was performed by polishing the electrode with 0.3 μm alumina and then by cycling a square wave potential with a frequency of 350 Hz followed by the application of a high frequency (3500 Hz) multiscan triangular potential sweep between the potential limits of ± 6 V. Finally, the electrode was subjected to an electrochemical treatment by applying a potential of $+1.5$ V for 5 min and then -1.0 V for 2 s in 0.1 M KNO_3 solution. These steps were repeated until the voltammetric response of the electrode became reproducible. At

the end of these procedures the surface was highly activated, for ca. 40 measurements, the above mentioned electrochemical treatment alone was sufficient before each scan. Details of the method which offered an improvement in sensitivity in drug analysis [32–38], were described in our previous paper [34].

2.4. Analysis of pharmaceutical formulations

The average mass of ten tablets was determined and finely powdered, then the required amount of sample to prepare a solution of ca. 10^{-3} M was transferred into a 100-ml standard flask containing 80 ml of buffer solution (pH 10). The contents of flask were stirred magnetically for 15 min and then diluted to volume with the same supporting electrolyte. The solution was filtered and the first 20 ml of the filtrate was removed. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and diluting them with the supporting electrolyte mentioned above. Ten vaginal suppositories were placed in a mortar and reduced the mass to the consistency of a paste by crushing with a pestle. A portion of the mass corresponding to a stock solution of ca 10^{-3} M was weighed accurately and proceeded as for tablets.

In the case of oral suspension, the required amount (accurately weighed) of the suspension sample, equivalent to a stock solution of concentration ca. 10^{-3} M, were transferred to a 100-ml standard flask. An 80-ml volume of buffer solution (pH 10) was added. The contents were ultrasonicated for a few minutes then made up to the mark with the same electrolyte. A portion of the mixture obtained was centrifuged and then aliquots from the upper clear layer were taken and diluted with the same buffer for preparing the appropriate solutions.

No pretreatment for the metronidazole injection was done except for dilution with the buffer solution (pH 10).

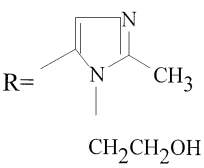
Voltammograms were recorded as in pure metronidazole. The content of metronidazole was calculated from the regression equation.

3. Results and discussion

3.1. Electrochemical reduction at the activated glassy carbon electrode

Under linear sweep voltammetry at the activated glassy carbon electrode, a single reduction peak of metronidazole was observed at the pH values between 1.6 and 10.6 (Fig. 1). This peak is attributed to the four-electron reduction of nitro group to the corresponding hydroxylamine according to the currently accepted mechanism for the electroreduction of aromatic and heteroaromatic nitro compounds [29,39,40].

$$\text{R-NO}_2 + 4\text{e}^- + 4\text{H}^+ \rightarrow \text{R-NHOH} + \text{H}_2\text{O}$$



On comparison of the voltammograms with those obtained at non-activated glassy carbon electrode polished on 0.3 μm alumina after each scan, a dramatically increased current response was found (Fig. 1). Although it seems to be background current also after the activation procedure, however, the ratio of faradaic current to

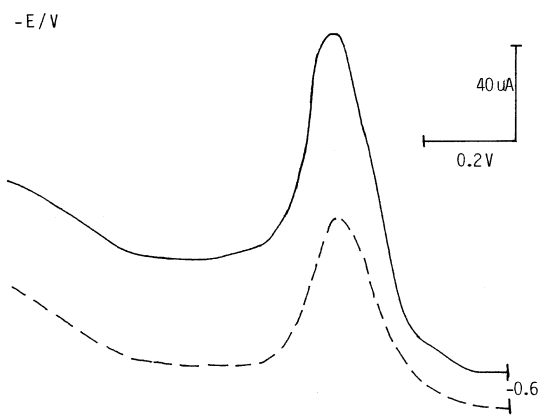


Fig. 1. Linear sweep voltammetry at (——) an activated and (- - -) a non-activated glassy carbon electrode. Metronidazole 4×10^{-4} M, Britton-Robinson buffer (pH 10), scan rate 100 mV s^{-1}.

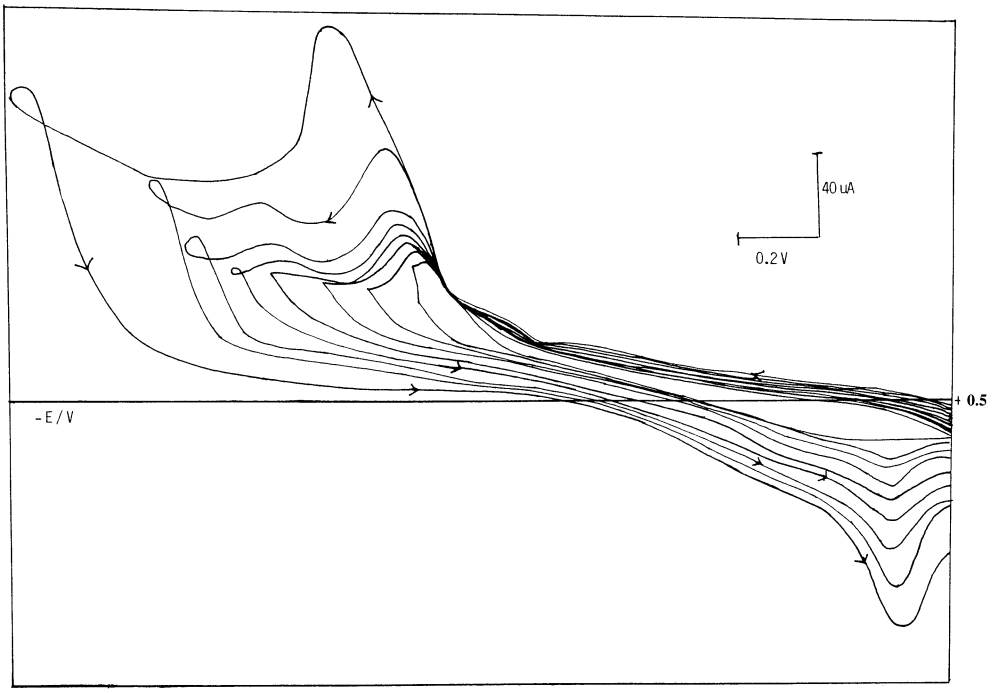


Fig. 2. Cyclic voltammograms of 4×10^{-4} M metronidazole in Britton-Robinson buffer (pH 6.5). Activated glassy carbon electrode, scan rate 100 mV s^{-1}, continuous record of curves by returning from different potentials.

background current at the peak potential for activated electrode is 3.88 while in the case of non-activated electrode this ratio is 2.66.

Our previous work on the electrochemistry of other structurally related compound, ornidazole, includes a report [33] of a single peak splitting into two peaks in more concentrated solutions and/or at higher pHs. This splitting may be attributed to the formation of radical species. However, this phenomenon could not be observed with metronidazole, probably due to the nature of the substituent group on the heterocylic ring.

In acidic and neutral media, a single anodic peak was observed during the reverse scan of cyclic voltammograms (e.g. at about $+0.4$ V in Britton-Robinson buffer pH 6.5), which is attributed to the oxidation of the corresponding hydroxylamine. The separation of the cathodic and anodic peak potential values reveals that the redox couple is not reversible at scan rates from 10 to 100 mV s^{-1}.

Fig. 2 shows repetitive cyclic voltammograms obtained by returning from less negative potentials. On subsequent scans, the reduction peak height decreased gradually and peak moved to less negative potentials. On the second scan and subsequent scans, another small cathodic peak was observed at more negative potential than the major reduction peak.

A plot of the reduction peak potential versus the square root of the scan rate showed a linear relationship between 10 and 100 mV s^{-1} ($r = 0.999$) which is a typical of a diffusion-controlled current.

The peak potential was pH dependent below pH 6.5, with a slope of 88 mV/pH unit, then remained constant with pH (Fig. 3a). This observation is in accordance with that obtained at glassy carbon electrode for other nitroimidazoles, tinidazole and ornidazole [32,33]. On the other hand, the peak current showed the behavior depicted in Fig. 3b in acidic media up to pH 4.5, then increased sharply between 4.5 and 5.5 and

remained practically constant up to 9.5 and decreased sharply again.

Addition of methanol or dimethylformamide shifted the peak potential to more negative values and resulted in a decrease in the peak height. The effect of anionic surfactant, sodium lauryl sulphate, on peak current and peak potential were also studied. In contrast to the behavior of the related compounds at mercury electrode [41,42], the splitting of the nitro group reduction peak could not be observed in alkaline solution in the presence of surfactant. On the contrary, the peaks became sharper than those obtained in aqueous solution without surfactant.

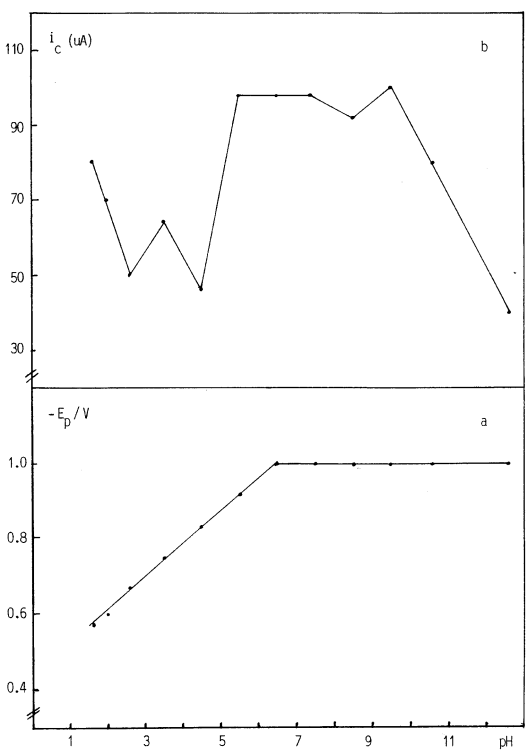


Fig. 3. Influence of pH on (a) peak potential and (b) peak current for linear sweep voltammetry at activated glassy carbon electrode. Metronidazole 4×10^{-4} M; scan rate 100 mV s^{-1}.

3.2. Calibration plots

On the basis of these results, the determination of metronidazole was carried out in aqueous media at the activated glassy carbon electrode. Useful voltammograms (with respect to peak current intensity and the reproducibility of the experiments) were obtained in Britton-Robinson buffer pH 10 at scan rate of 100 mV s^{-1}.

The voltammograms were recorded over the range of applied potential from $+0.1$ to -1.5 V. The reproducibility of peak potential and peak current was tested by repeating five experiments on 2×10^{-4} M metronidazole. The relative standard deviations were calculated to be 0.9 and 1.7% for peak potential and current, respectively.

The plot of the peak current versus metronidazole concentration was seen to be linear over the concentration range 2×10^{-6}–6×10^{-4} M ($r = 0.997$) with a slope of 24.5×10^{4} μA M^{-1}, intercept of 8.9 μA, standard error of slope 56.1×10^{2} μA M^{-1} and standard error of intercept 1.2 μA. The detection limit was found to be 1.1×10^{-6} M, according to the $3\,s/m$ definition [43], where s is the standard deviation ($n = 10$) of the signal from 3×10^{-6} M metronidazole aliquots and m is the slope of the calibration graph.

3.3. Application to pharmaceutical formulations

The above results may allow the determination of metronidazole in commercial formulations at the activated glassy carbon electrode. The proposed method can also be used for the successful estimation of metronidazole without prior separation of miconazole nitrate employed in vaginal suppository. Since miconazole nitrate is inactive at glassy carbon electrode, it did not interfere with the assay of metronidazole.

Results obtained by voltammetry were compared with those determined by HPLC system with UV detection proposed in USP XXIII [44]. The results (Table 1) showed a good agreement. Further, in order to establish the suitability of the proposed method, known amounts of the pure drug were added to the analytical solution of the pharmaceutical products and the procedure was applied. Recoveries indicate the accuracy and re-

Table 1
Assay of metronidazole by voltammetric and HPLC method

	Voltammetric method				HPLC method [44]			
	Tablet (500 mg)	Oral suspension (125 mg 5 ml^{-1})	i.v. infusion solution (0.5%)	Vaginal suppository[a] (500 mg)	Tablet (500 mg)	Oral suspension (125 mg 5 ml^{-1})	i.v. infusion solution (0.5%)	Vaginal suppository[a] (500 mg)
Mean[b]	503.3	126.0	501.2	502.4	506.0	125.4[c]	503.2	504.5[d]
R.S.D. (%)	1.74	2.58	1.27	1.89	1.72	2.19	1.86	2.06
t-Test of significance	0.702	0.460	0.534	0.466	($p = 0.05$, $t = 2.101$)			

R.S.D., relative standart deviation.
[a] In the presence of 100 mg miconazole nitrate per suppository.
[b] Mean of ten experiments.
[c] Analysed by HPLC described in [15].
[d] Analysed by the official method [44] as for tablets.

Table 2
Recovery studies by proposed method at activated glassy carbonelectrode

Formulation	Recovery[a] (%)	R.S.D. (%)
Tablet	98.4	0.56
Oral suspension	99.4	1.92
i.v. infusion solution	99.5	0.66
Vaginal suppository	98.9	0.92

[a] Mean of three determinations.

peatability of the proposed voltammetric method (Table 2).

As a conclusion, results obtained in the determination of metronidazole using activated glassy carbon electrode showed an improvement in sensitivity of linear sweep voltammetry and its possibilities of application to pharmaceuticals.

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