Electroanalytical determination of metronidazole in tablet dosage form

SELEHATTIN YILMAZ[1], ESRA BALTAOGLU[1], GULSEN SAGLIKOGLU[1],
SULTAN YAGMUR[1], KAMRAN POLAT[2] and MURAT SADIKOGLU[3]*

[1]*Onsekiz Mart University, Faculty of Science and Arts, Department of Analytical Chemistry, 17020, Canakkale, Turkey,* [2]*Ankara University, Faculty of Science, Department of Chemistry, 06100, Ankara, Turkey and* [3]*Gaziosmanpasa University, Faculty of Education, Department of Science Education, 60100, Tokat, Turkey*

Abstract: In this study, the electrochemical reduction and determination of metronidazole were easily realized in Britton–Robinson buffer (pH 4.01) using an UTGE by cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques. In this acidic medium, one irreversible and a sharp cathodic peak were observed. A linear calibration curve for DPV analysis was constructed in the metronidazole concentration range 3×10^{-6}–9×10^{-5} mol L^{-1}. The limit of detection (*LOD*) and limit of quantification (*LOQ*) were 1.42×10^{-7} and 4.76×10^{-7} mol L^{-1} respectively.

Keywords: metronidazole; determination; voltammetry; UTGE; dosage form.

INTRODUCTION

Metronidazole is a nitroimidazole anti-infective medication (Fig. 1) used mainly in the treatment of infections caused by susceptible organisms, particularly anaerobic bacteria and protozoa.[1–7]
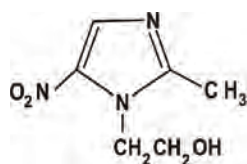

Fig. 1. Chemical structure of metronidazole.

Methods for the assay of metronidazole in pharmaceutical dosage forms are usually based on high performance liquid chromatographic (HPLC)[8] and spectrophotometric[9] techniques. For such applications, however, the analyses are time consuming and are of high cost.

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*Corresponding author. E-mail: seletyilmaz@hotmail.com

Hitherto, only a few papers have been published about the electroanalytical determination of metronidazole based on its reduction behaviour.[1–7] The determination of metronidazole on an ultra trace graphite electrode (UTGE) based on its detailed electrochemical reductive behaviour has to date not yet been reported. Therefore, it was considered of interest to investigate the properties of the reduction process and determination of metronidazole in tablet dosage form using a UTGE.

EXPERIMENTAL

Apparatus

A Model Metrohm 757 VA trace analyzer (Herisau, Switzerland) was used for the voltammetric measurements, with a three-electrode system consisting of an ultra trace graphite working electrode (UTGE, disc diameter; R = 2 mm, Metrohm), a platinum wire auxiliary electrode and Ag/AgCl (KCl 3 mol L^{-1}, Metrohm) reference electrode. Firstly, deoxygenation of the supporting electrolyte solutions was performed with argon gas for 5 min before all experiments. Then, the argon gas was also passed through the solutions for 60 s after the addition of each sample solution in the experiments. All pH measurements were made with Model Metrohm 744 pH meter (Herisau, Switzerland).

Reagents

Metronidazole as pure active material and its Nidazole$^{®}$ tablets (labelled as containing 250 mg metronidazole per tablet) was kindly supplied by I. E. Ulugay (Istanbul, Turkey). A stock solution of 1.0×10^{-2} mol L^{-1} of metronidazole was prepared by dissolving an accurate mass of the drug in an appropriate volume of ethanol and kept in a refrigerator. Britton–Robinson buffer solutions (0.04 mol L^{-1}, pH 2.09–12.00); acetic acid, Riedel, Seelze, Germany, 100 mas. %; boric acid, Merck, Darmstadt, Germany, and phosphoric acid, Carlo Erba, Rodeno, France, 85 mas. %, were used for the supporting electrolyte solution.

Calibration graph for quantitative determination

The stock solution of metronidazole was diluted with ethanol to obtain different metronidazole concentrations. Using the optimum conditions described in the experimental section, a linear calibration curve for DPV analysis was constructed in the metronidazole concentration range 3×10^{-6}–9×10^{-5} mol L^{-1} (Fig. 2). The repeatability, accuracy and precision were checked (Table I).

Working voltammetric procedure of spiked tablet dosage forms

Ten tablets were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration 1×10^{-2} mol L^{-1} was weighed and transferred into a 10 mL volumetric flask and the volume was adjusted with ethanol. The contents of the flask were centrifuged for 20 min at 4000 rpm to affect complete dissolution and then diluted to volume with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with selected supporting electrolyte solutions. Each solution was transferred into the voltammetric cell. The nominal content of the corresponding regression equations was compared with previously plotted calibration plots (Table II).
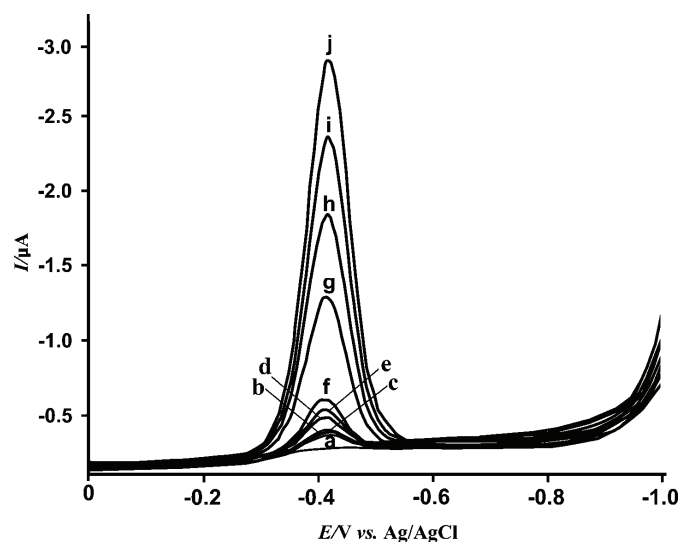
Fig. 2. The calibration voltammograms at different concentrations (b–j) of metronidazole in 0.04 mol L^{-1} BR buffer (pH 4.01) on an UTGE by DPV; a) blank; b–j) increasing concentrations of metronidazole.

TABLE I. Regression data of the calibration lines for the quantitative determination of metronidazole. The calibration plots were obtained in 0.04 mol L^{-1} BR buffer (pH 4.01) on an UTGE using the DPV technique

Parameter	Results
Measured potential, mV	–0.432
Linear concentration range, mol L^{-1}	3.0×10^{-6}–9.0×10^{-5}
Slope, µA M^{-1}	2.96×10^{4}
Intercept, µA	1.35
Correlation coefficient, r	0.0233
SE of slope	16.48
SE of intercept	0.9989
Number of measurements, N	10
LOD / mol L^{-1}	1.42×10^{-7}
LOQ / mol L^{-1}	4.76×10^{-7}
Repeatability of the peak current, RSD / %	0.47
Reproducibility of the peak current, RSD / %	0.41
Repeatability of the peak potential, RSD / %	0.69
Reproducibility of the peak potential, RSD / %	0.61

RESULTS AND DISCUSSION

Electrochemical reduction behaviour of metronidazole

The electrochemical reduction process and determination using this electrode were first realized using the CV and DPV techniques. The CV measurements performed with a 1×10^{-4} mol L^{-1} metronidazole solution at scan rates

between 10–1000 mV s^{-1} on a UTGE in 0.04 mol L^{-1} BR buffer (pH 4.01) are shown in Fig. 3.

TABLE II. Application of the DPV technique for the assay of metronidazole in commercial spiked Nidazole® tablets and mean recoveries on an UTGE

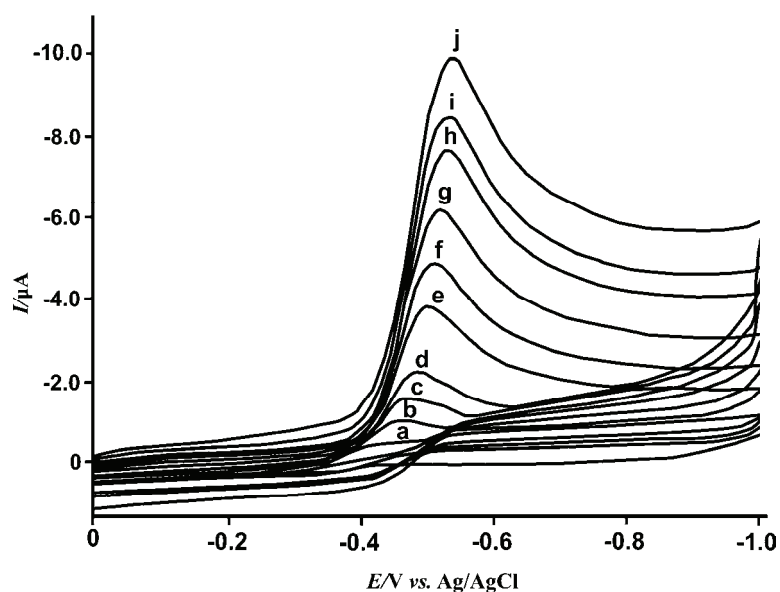| Parameter	Results
Labelled, mg	250.00
Amount found, mg	255.14
RSD / %	1.24
Bias, %	2.05
Metronidazole spiked, mg	20.00
Found, mg	19.20
Recovery, %	96.00
RSD of recovery , %	0.29
Bias / %	4.00

Fig.3. The cyclic voltammograms of 1×10^{-4} mol L^{-1} metronidazole in 0.04 mol L^{-1} Britton–Robinson (BR) buffer (pH 4.01) on an UTGE. Scan rate, mV s^{-1}: a) blank, b) 10, c) 25, d) 50, e) 100, f) 150, g) 250, h) 500, i) 750 and j) 1000.

As can be seen from Fig. 3, metronidazole exhibited one well-defined cathodic peak at different scan rate values. By reversing to 1.00 V, no anodic oxidation peak corresponding to the cathodic response was observed in the anodic region. This indicated that the reduction process of metronidazole had an irreversible nature.

Scan rate studies were then performed to assess whether the processes on the UTGE were under diffusion or adsorption control.[10–20] Two tests were employed for this procedure. One of them was the linear relationship obtained on the UTGE between the peak current and square root of the scan rate between 10–1000 mV s^{-1} as follows:

$$I_p/\mu A = 0.2423\nu^{1/2} \ (mV \ s^{-1}) + 0.04 \ (r = 0.991) \tag{1}$$

Correlation coefficient was found very close to 1.0 showing that the reduction process was diffusion-controlled.

Another important test is the plot of the logarithm of the peak current *vs.* the logarithm of the scan rate that gave a straight line with a slope of 0.52, which is nearly the same as the theoretical value of 0.5 that is expected for an ideal reaction of solution species.[10–20] The equation obtained on the UTGE was:

$$\log I_p / \mu A = 0.5221 \log \nu \ (mV \ s^{-1}) - 0.6358 \ (r = 0.998) \tag{2}$$

Therefore, a diffusion component must be taken into account. Other studies were conducted in line with this phenomenon.

Next, in order to obtain the optimum experimental conditions, the effect of pH on peak potential and peak intensity were studied on the UTGE using DPV techniques. The DPV results for the reduction reaction of metronidazole are given as *E*–pH and *I*–pH graphs in Figs. 4a and 4b, respectively, for the UTGE. The voltammetric response was strongly pH dependent. The peak potential of the cathodic peak was shifted to more positive values with increasing pH (Fig. 4a).

As can be seen from Fig. 4a, highly linear segments of potential were found between pH values of about 2–8. In acid and neutral media, the reduction was pH dependent, although for pH values higher than 8, the reduction was pH independent, in agreement with Brett and Leach.[6,7]

This indicates that metronidazole shows basic properties in acidic and neutral media (pH 2–8). The differential pulse and cyclic voltammograms of metronidazole at pH 4.01 are shown in Figs. 2 and 3, respectively. This peak corresponds to reduction of the nitro group to form the hydroxylamine, involving 4 electrons and 4 protons followed by a two-electron reduction of the hydroxylamine to amine.[6] However, metronidazole shows weakly basic (or strong acid) properties at pH values higher than 8, meaning a decrease in the protonation of hydroxylamine with increasing pH.[6]

Validation parameters for the quantitative analysis

Validation of the procedure for the quantitative determination of metronidazole was examined by the DPV technique *via* evaluation of the limit of detection (*LOD*), limit of quantification (*LOQ*), repeatability, reproducibility, accuracy and precision (Tables I and II).
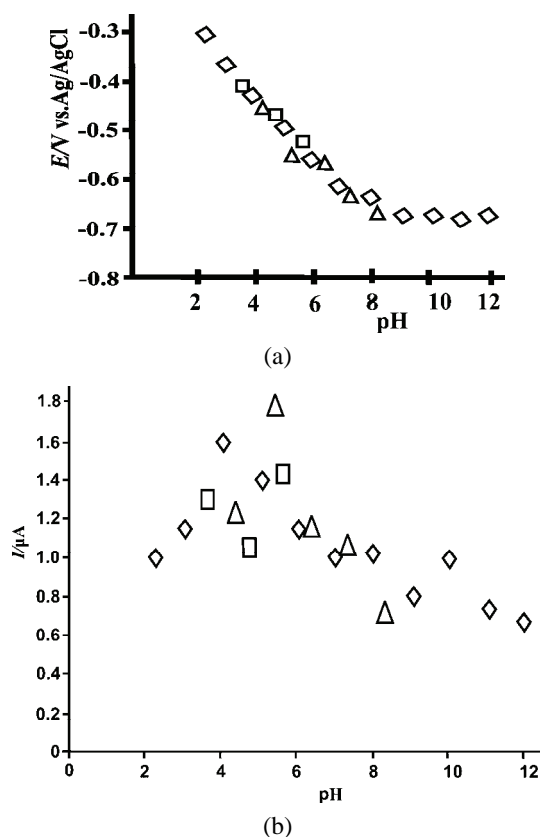
(a)

(b)

Fig. 4. Effect of pH on the DPV peak potential (a) and peak current (b) of 5×10^{-5} mol L^{-1} metronidazole; □) acetate (0.2 mol L^{-1}); Δ) phosphate (0.067 mol L^{-1}); ◊) Britton–Robinson buffer (0.04 mol L^{-1}).

LOD and *LOQ* were calculated from the electro-reduction peak current using the following equations: *LOD* = 3 *s*/*m* and *LOQ* = 10 *s*/*m* (*s* is the standard deviation of the peak currents (ten runs), *m* is the slope of the calibration curve):[10–20]

$$LOD = 3\ s/m = 3\times1.4\times10^{-3}/2.96\times10^{4} = 1.42\times10^{-7}\ M \qquad (3)$$
$$LOQ = 10\ s/m = 10\times1.4\times10^{-3}/2.96\times10^{4} = 4.73\times10^{-7}\ M \qquad (4)$$

The precision and accuracy of the developed method were checked by recovery studies in tablet dosage form. The procedures are given in the pharmaceutical application section (Table II).

A UTGE and three different electrodes (a DNA-modified glassy carbon electrode, a mercury thin film electrode and a glassy carbon electrode) showed a similar trend in the reduction mechanism for metronidazole, dependent on pH in acid and neutral media and independent in alkaline media.[6] One irreversible and a sharp cathodic peak at –0.432 V were observed in agreement with Brett and Leach (–0.465 V).[6,7]

Pharmaceutical applications

The amount of metronidazole in nidazole commercial tablets was calculated by reference to the appropriate calibration plots. The results obtained are given in Table II.

To determine whether excipients in the tablets interfered with the analysis, the accuracy of the proposed methods were evaluated by recovery tests after the addition of a certain amount of pure drug to pre-analyzed formulations of metronidazole (Table II). The results showed the validity of the proposed techniques for the quantitative determination of metronidazole in tablets.

ИЗВОД

ЕЛЕКТРОАНАЛИТИЧКО ОДРЕЂИВАЊЕ МЕТРОНИДАЗОЛА
У ТАБЛЕТНИМ ДОЗИРАНИМ ОБЛИЦИМА

SELEHATTIN YILMAZ[1], ESRA BALTAOGLU[1], GULSEN SAGLIKOGLU[1], SULTAN YAGMUR[1], KAMRAN POLAT[2]
и MURAT SADIKOGLU[3]

[1]*Onsekiz Mart University, Faculty of Science and Arts, Department of Analytical Chemistry, 17020, Canakkale, Turkey,* [2]*Ankara University, Faculty of Science, Department of Chemistry, 06100, Ankara, Turkey u* [3]*Gaziosmanpasa University, Faculty of Education, Department of Science Education, 60100, Tokat, Turkey*

У овом раду су приказани електрохемијска редукција и одређивање метронидазола у Бритон–Робинсоновом пуферу (pH 4,01) применом цикличне волтаметрије (ЦВ) и диференцијалне пулсне волтаметрије (ДПВ). У киселој средини запажен је иреверзибилан и оштар катодни пик. Конструисана је калибрациона крива за ДПВ, линеарна у опсегу 3×10^{-6}–9×10^{-5} mol L^{-1} метронидазола. Граница детекције (ГД) и квантификације (ГК) износе $1,42\times10^{-7}$ одн. $4,76\times10^{-7}$ mol L^{-1}.

REFERENCES

1. E. Bishop, W. Hussein, *Analyst* **109** (1984) 759
2. S. A. Özkan, Y. Özkan, Z Şentürk, *J. Pharm. Biomed. Anal*. **17** (1997) 3299
3. M. A. La-Scalea, S. H. P. Serrano, I. G. R. Gutz, *J. Braz. Chem. Soc.***10** (1999)127
4. P. N. Bartlett, E. Ghoneim, G. El-Hefnawy, I. El-Hallag, *Talanta* **66** (2005) 869
5. E. Baltaoglu, master thesis, Çanakkale Onsekiz Mart University, Institute for Natural & Applied Sciences. Canakkale, 2007
6. A. M. O. Brett, S. H. P. Serrano, I. G. R. Gutz, M. A. La-Scale, *Electroanalysis* **9** (1997) 110
7. S. C. Leach, R. D. Weaver, K. Kinushita, W. Lee, *J. Electroanal Chem.* **129** (1981) 213
8. B. M. Tashtoush, E. L. Jacobson, M. K. Jacobson, *Drug Dev. Ind. Pharm.* **34** (2008) 840
9. O. Adegoke, O. Umoh, *Acta Pharm.* **59** (2009) 407
10. S. Yilmaz, B. Uslu, S. A. Ozkan, *Talanta* **54** (2001) 351
11. B. Uslu, S. Yılmaz, S. Ö. Özkan, *Pharmazie* **56** (2001) 629
12. B. T. Demircigil, S. A. Özkan, Ö. Çoruh, S. Yılmaz, *Electroanalysis* **14** (2002) 122

13. S. A. Ozkan, B. Uslu, *Anal. Bioanal. Chem.* **372** (2002) 582
14. S. Skrzypek, W. Ciesielski, A. Sokołowski, S. Yilmaz, D. Kazmierczak, *Talanta* **66** (2005) 1146
15. M. Çıtak, S. Yılmaz, Y. Dilgin, G. Türker, S. Yagmur, H. Erdugan, N. Erdugan, *Current Pharm. Anal*. **3** (2007) 141
16. S. Skrzypek, W. Ciesielski, S. Yilmaz, *Chem. Anal. (Warsaw)* **52** (2007) 1071
17. S. Yılmaz, S. Skrzypek, Y. Dilgin, S. Yagmur, M. Coskun, *Curr. Anal. Chem*. **3** (2007) 41
18. S. Yilmaz, M. Sadikoglu, G. Saglikoglu, S. Yagmur, G. Askin, *Int. J. Electrochem. Sci.* **3** (2008) 1534
19. S. Yılmaz, *Colloids Surfaces., B* **71** (2009) 79
20. M. Sadikoglu, G. Saglikoglu, S. Yagmur, E. Orta, S. Yilmaz, *Curr. Anal. Chem.* **7** (2011) 130.