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Short communication

Electrochemical study on the determination of tinidazole in tablets

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Abstract

The electrochemical reduction of tinidazole has been carried out in aqueous solution in the pH range 1.8–11.3 by differential-pulse (DP) polarography. Tinidazole exhibits one or two reduction peaks depending on pH. In strongly acidic solution (pH < 4.5), one reduction peak was obtained and it was suitable for analytical purposes. A method for the determination of tinidazole by DP polarography in Britton–Robinson buffer of pH 3.0, which allows quantification over the range 0.03–7.30 μg/ml, was proposed. The method was successfully applied to the determination of tinidazole in tablets with mean recovery and relative standard deviation of 98.7 and 3%, respectively. Excipients did not interfere in the determination. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Tinidazole determination; Differential-pulse polarography; Pharmaceutical formulations

1. Introduction

Tinidazole (TNZ) is a 5-nitroimidazole derivative which is highly effective as a treatment for Giardiasis. The oral single doze of TNZ is equivalent to a 3-day therapy with metronidazole without substantial side effects [1].

Several methods have been reported for the determination of TNZ, including spectrophotometry [2–5] and chromatography [6–9]. Little attention has been paid to the determination of TNZ using the electrochemical techniques [10,11]. The electrochemical techniques have been successfully applied for the determination of some pharmaceutical formulations and biological fluids [12–14]. On the other hand, there is no official method that has been adopted in the pharmacopoeias for the determination of TNZ.

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In this work, the optimum conditions for the voltammetric and polarographic determination of TNZ in raw material and tablets are described. The suggested method will be compared with the non-aqueous titration method [15], which is used in the quality control laboratories of pharmaceutical companies. This method is based on the non-aqueous titration of TNZ using perchloric acid solution.

2. Experimental

2.1. Apparatus

Differential pulse (DP) voltammetry and polarography were carried out using an EG&G 264A Voltammetric Analyzer with a model 303A static mercury dropping electrode operated in the HMDE mode. The three-electrode system was completed using a platinum auxiliary electrode and a Ag–AgCl reference electrode. The pH measurements were made with a Hanna pH meter, model HI 8424.

2.2. Reagents

Tinidazole was obtained from Pfizer through a local pharmaceutical company (Jerusalem Pharmaceutical Company, Palestine). A 1.0×10^{-3} M solution in ethanol was prepared freshly every 3 days. The supporting electrolyte was a Britton–Robinson (BR) buffer solution. All solutions were prepared from doubly distilled water and analytical-reagent grade chemicals (BDH and Merck).

2.3. Proposed procedure

The general procedure used to produce DP polarograms and voltammograms was as follows: An aliquot (9.5 ml) of BR buffer of pH 3 in the presence of 5% ethanol was placed in a dry, clean voltammetric cell and deoxygenated with highly pure nitrogen for 10 min. A negatively directed DP scan was initiated between 0.0 and -0.7 V. The operational parameters of the scan were: scan rate, 5 mV s^{-1}, pulse amplitude, 50 mV; and drop time, 1 s. After the background polarogram had been obtained, aliquots of the required amounts of standard TNZ solution were added.

2.4. Official procedure

First, 0.15 g TNZ were dissolved in 25 ml anhydrous acetic acid. Then titration with 0.10 M perchloric acid was performed, to determine the end point potentiometrically. Each millilitre of 0.10 M perchloric acid is equivalent to 24.75 mg TNZ.

2.5. Analysis of tablets

The TNZ drug is commercially available as Fasigyn (Pfizer, USA) and Tinogyn (JEPH, Jerusalem, Palestine). The excipients used in Tinogyn and Fasigyn formulations are starch, talc powder and magnesium stearate. Ten tablets of each drug were weighed and finally powdered separately. An accurate weight equivalent to one tablet was transferred quantitatively to a 250 ml volumetric flask; 200 ml doubly distilled water and 12.5 ml ethanol added, and the mixture shaken well for 30 min, then diluted to volume with doubly distilled water. A 1.25 ml volume of the supernatent liquid was diluted to 100 ml with doubly distilled water. Then 0.5 ml of this solution was transferred into the voltammetric cell containing deaerated BR buffer (pH 3.0). The polarograms were recorded under the optimum experimental conditions. The amount of TNZ was calculated from the resulting current values using the already constructed calibration graph.

2.6. Controlled potential electrolysis

Mercury-pool electrolysis was carried out in two buffers (pH 3.0 and 7.0) containing 200 mg TNZ. The electrolysis cell was a 250 ml conical flask in which the reference (Ag/AgCl), auxiliary and the gas inlet were inserted through a cork.

At pH 3.0, the potential was controlled at -0.20 V (first wave), and at pH 7.0, the potential was controlled at -0.55 and -0.96 V for the first and second wave, respectively (i.e on the limiting current plateau of the waves). The progress of electrolysis was followed by recording the

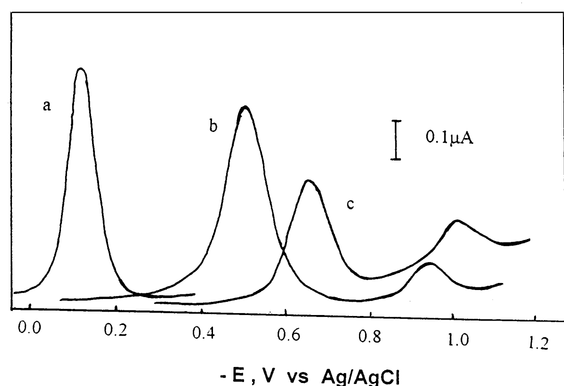

Fig. 1. Differential-pulse voltammograms for 2.50×10^{-5} M TNZ at (a) pH 3, (b) pH 7 and (c) pH 11. Pulse amplitude, 50 mV; scan rate, 5 mV s^{-1}; $\mu = 0.06$; 5% ethanol.

decrease in current with time, and the number of electrons involved in the electrode process was computed from the I–t curve following the procedure outlined by Lingane [16].

3. Results and discussion

Most of studies on the electrochemical reduction of imidazole derivatives [17–19] have been done using the mercury electrodes. The effect of pH on the peak potential at a concentration 2.5×10^{-5} M TNZ was studied over the pH range 1.8–11.3. TNZ gives rise to one or two reduction peaks, depending on pH. In strongly acidic solution (pH < 4.5), one reduction peak was obtained which refers to the six-electron reduction of the nitro group to the corresponding amine. Above pH 4.5, TNZ exhibits two reduction peaks; the height of the first is twice that of the second. The first peak corresponds to the reduction of the nitro group to form intermediate hydroxylamine, transferring four electrons, while the second peak corresponds to reduction of hydroxylamine to amine, transferring two electrons. Confirming this hypothesis is the fact that the controlled potential electrolysis applied at potentials on the plateau of the first polarographic wave (pH 3.0) verified the consumption of approximately six electrons per molecule. On the other hand, the results for the controlled potential electrolysis were also applied for the first and second wave at pH 7.0, indicating the consumption of approximately four and approximately two electrons per molecule, respectively. Similar results were obtained for ornidazole [18] and some nitroimidazoles [20–23]. The differential pulse voltammogram (DPV) at different pH values for the reduction of TNZ, at a concentration of 2.50×10^{-5} M, using a HMDE is shown in Fig. 1. The values for the first peak currents and potentials at different pH values are listed in Table 1. The half-wave potential ($E_{1/2}$) of the polarographic waves shifted toward more negative potential with an increase in pH. The $\Delta E_{1/2}/\Delta pH$ relation of the first and second waves are straight lines with a slope equal to -52 and -61 mV, respectively, indicating that the H^+ ion is consumed in the reduction process.

Table 1
Effect of pH on the DP voltammograms of 5×10^{-5} M TNZ[a]

pH	Peak potential (−V) versus Ag/AgCl	Peak current (μA)
2	0.16	1.70
3	0.22	2.35
5	0.35	2.25
7	0.48	2.30
9	0.65	2.20
11	0.75	1.45

[a] Scan rate, 5 mV s^{-1}; pulse amplitude, 50 mV; ionic strength, 0.06; in presence of 5% ethanol.

On the other hand, the voltammetric response of TNZ was examined in different buffer solutions, e.g. Britton–Robinson, mclliviane and acetate buffer. The Britton–Robinson buffer was chosen as the best, due to more uniform peak shape, less tailing, less broadening of peak and normal baseline start. The peak half width ($W_{1/2}$) in Britton–Robinson buffer was found to be less than the other two buffers. From these results, the quantitative determination of TNZ was performed in Britton–Robinson buffer of pH 3.0, where a single peak of highest and reproducible value is obtained.

The peak current is linearly related to the pulse amplitude between 10 and 100 mV. A pulse amplitude of 50 mV was chosen as optimum as there is a loss of resolution at high pulse amplitude

Table 2
Optimum conditions and characteristics of calibration curve for DP polarographic determination of TNZ

Optimum pH	3.0 (Britton–Robinson)
Pulse amplitude (mV)	50
Scan rate (mV s^{-1})	5
Drop time (s)	1
Ionic strength (μ)	0.06
Ethanol percentage (%)	5
Linear range (μg ml^{-1})	0.03–7.30
Slope (μA μg^{-1} ml)	0.21
Regression coefficient	0.997
RSD (%)	2–3.2
Recovery range (%)	98.4–99.0
Detection limit (μg m^{-1})	0.03

values. The differential pulse voltammograms of 2.5×10^{-5} M TNZ at pH 3.0 are recorded at various scan rates. At scan rates higher than 5 mV s^{-1}, the width of the peak increases and its height decreases. At a slower scan rate than 5 mV s^{-1}, the peak height is small compared with the 5 mV s^{-1} scan rate. For DPV experiments, a scan rate of 5 mV s^{-1} was used.

The reason for studying the effect of ethanol concentration on the wave height and $E_{1/2}$ is because TNZ was prepared in ethanolic solution, since it is practically insoluble in water. It was found that the minimum ethanol content required for solubilizing TNZ is 5% (V/V) ethanolic solution. The effect of ethanol content in the range 5–60% on the voltammetric peak of fixed amount of TNZ at pH 3.0 was investigated. The results obtained showed that the peak height decreased and the $E_{1/2}$ shifted to more negative potentials by increasing the percentage of ethanol. This behaviour may be attributed to the formation of an adduct between TNZ and ethanol, which decreases the rate of electron transfer and, hence, the peak current will be decreased and $E_{1/2}$ shifted to more negative potential [24]. Good signals were obtained in the presence of 5% ethanol.

The addition of different concentrations of Na_2SO_4 solution to the supporting electrolyte (pH 3.0) was investigated to study the effect of ionic strength on the voltammetric behaviour of 2.5×10^{-5} M TNZ. It was found that, the solution of $\mu = 0.06$ ionic strength tends to give sharper peaks and so it was suggested as the most suitable medium to attain a constant ionic strength.

No significant interference was observed from the excipients commonly used in the formulations, such as glucose, sucrose, starch, magnesium stearate or talc powder.

3.1. Preparation of the calibration graph

The applicability of both methods, differential pulse polarography (DPP) and voltammetry (DPV) as an analytical technique for the determination of TNZ was tested under the optimum conditions.

3.2. DPP determination of TNZ

The maximum response and higher resolution of the DPP peak of the investigated compound can be obtained at the following conditions: Britton–Robinson buffer (pH 3), 50 mV pulse amplitude, 5 mV s^{-1} scan rate and 1 s drop time.

Under the aforementioned conditions, the height of the peak increases gradually with increasing concentration of TNZ and the response of the peak height (i_p) as a function of concentration (C, μM) is linear. The calibration curve was recorded over the concentration range 0.03–7.30 μg ml^{-1}, with a regression coefficient of 0.997 and standard deviation of 2–3.2%. The validity of the method is supported by the constancy of i_p/C. The detection limit is 0.03 μg ml^{-1}, which was determined as the concentration that gives a signal to background noise ratio equal to 3 [25]. The optimum conditions for determination of TNZ as well as the characteristics of the calibration curve are summarized in Table 2. Typical DP polarograms showing successive enhancement of peak current with increasing TNZ concentration are shown in Fig. 2.

The reproducibility of the DPP method was checked by performing 10 measurements on a 3.65 μg ml^{-1} solution. A relative standard deviation of 2–5% was obtained.

Table 3
Determination of TNZ in pharmaceutical formulations by proposed and official methods

Drug (trade name)	Labelled amount (mg)	DPP method			Official method		
		Found[a]	Recovery (%)	RSD (%)	Found[a]	Recovery (%)	RSD (%)
Fasigyn[b]	500	495	99.0	2.9	504	100.8	2.7
Tinogyn[c]	500	492	98.4	3.0	506	101.2	3.5

[a] Average of five determinations.
[b] Pfizer, USA.
[c] JEPH, Jerusalem.

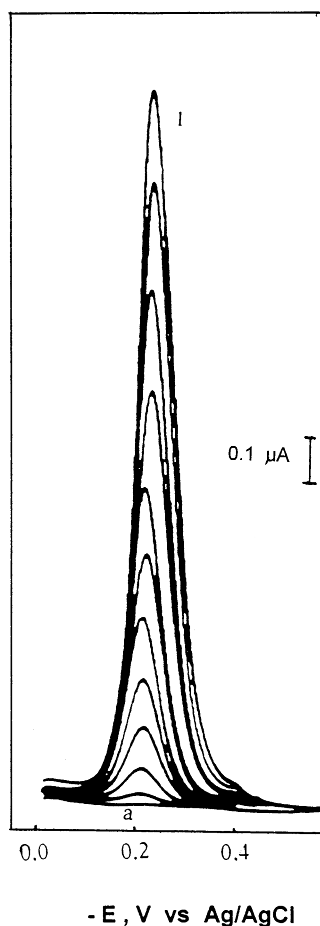

Fig. 2. Concentration dependence of the differential pulse polarograms of TNZ at pH 3: (a) background; (b) 1.05×10^{-6} M; (c) 3.15×10^{-6} M; (d) 7.30×10^{-6} M; (e) 1.24×10^{-5} M; (f) 1.80×10^{-5} M; (g) 2.46×10^{-5} M; (h) 3.06×10^{-5} M; (i) 4.04×10^{-5} M; (j) 4.52×10^{-5} M; (k) 5.47×10^{-5} M; (l) 5.90×10^{-5} M of TNZ. Other conditions as Fig. 1.

3.3. Application to analysis of pharmaceutical formulations

The validity of the DPP technique for the determination of TNZ in pharmaceutical formulations was investigated by assaying Fasigyn and Tinogyn (each is labelled to contain 500 mg TNZ per tablet). Five analyses of five different samples of each drug gave a mean recovery of 99.0 and 98.4% for Fasigyn and Tinogyn, respectively. The results obtained for the formulations are listed in Table 3 and compared with the non-aqueous titration method, which has been used in the quality control laboratories of the drug companies.

4. Conclusion

The advantages of this method for analytical purposes lie in the rapid determination of TNZ in pharmaceutical formulations, easy preparation of the sample, fair enough reproducibility and use of inexpensive instrumentation. On the other hand, the suggested method is cheaper and faster than high-performance liquid chromatography methods, more accurate and highly reproducible than the non-aqueous titration method, and more simple and practical than the voltammetric methods using the pretreated glassy carbon electrode [10]. Therefore, the presented method can be recommended for routine analysis of TNZ in pharmaceutical formulations and may be adopted in the pharmacopoeias.

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