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Short communication

Polarographic determination of benznidazole in DMSO

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1. Introduction

Chagas' disease, a protozoan infection caused by the kitenoplastid *Tripanosoma cruzi*, constitutes a major public health problem for developing nations. According to the World Health Organization [1] an estimated 20 million people are infected with this parasite.

Benznidazole (*N*-benzil-2-nitroimidazolylacetamide) is a chemotherapeutic agent currently used for the treatment of *T. cruzi* infections in the chronic and acute phases. This drug belongs to the nitroimidazole class of compounds, which have attracted much attention in chemotheraphy [2].

Some investigators [2,3] have demonstrated that many pharmacological effects of nitroimidazoles can be correlated with their reductive metabolism the implication being that a product containing the reduced nitro group is the biologically active species.

Electrochemical techniques play an important role in the understanding of the reduction mechanism and chemical stability of the reduction products [4,5] in addition to the possibility of developing analytical methods for the determination of these compounds in biological fluids and dosage forms [6–8].

Regarding few papers occur in the literature about the determination of benznidazole. Raaflaub and Ziegler [9] investigated the bioavailability of the compound in plasma using polarography. Nothenberg [10] used UV spectrophotometry to determine benznidazole in the concentration range $0.95–9.15 \times 10^{-5}$ mol l^{-1} in methanol. Walton and Workman [11] determined benznidazole and its amine metabolised in biological fluids by HPLC/UV.

In this communication an attempt has been made to study the direct current and differential pulse polarographic behaviour and to develop a

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polarographic method for the analysis of benznidazole in pharmaceutical formulations (i.e. tablets). In particular the determination has been made in dimethylsulfoxide which is a suitable solvent for other tests on their compounds.

2. Experimental

The polarographic measurements were carried out with a Metrohm E506 polarograph. The conventional three-electrode cell contained a working electrode (dropping mercury) an auxiliary electrode (a platinum rod) and a saturated calomel reference electrode (SCE). Dry nitrogen was passed through the solution to remove oxygen (20 min) and during the runs it was maintained flowing over the solution.

The tablets (Rochagan®) and the pure drug were kindly provided by Roche. All the other chemicals were of analytical reagent grade.

The polarographic behaviour of benznidazole was studied in 0.1 mol l^{-1} tetrabutylammonium tetrafluorborate (Bu_4NBF_4) in dimethylsulfoxide (DMSO) solution using the direct current (DC) and differential pulse (DP) techniques, in the -0.4– -1.4 V potential range.

The pharmaceutical tablets (250 mg containing 100 mg benznidazole) were dissolved directly in 50 ml of DMSO. For the differential pulse polarographic (DPP) determination, 1.0 ml of the sample solution and 10 ml of a 0.1 mol l^{-1} Bu_4NBF_4 in DMSO solution were introduced into the polarographic cell. The benznidazole concentration was determined by the standard addition method (successive 0.5 ml additions of a 5.0×10^{-3} mol l^{-1} benznidazole standard stock solution).

The ultraviolet spectrophotometric measurements were made with a Beckman DU-70 spectrophotometer and 1-cm quartz cells at 314 nm [10]. The pharmaceutical tablets were dissolved in 100 ml of methanol. The benznidazole concentration was obtained using the standard addition method (0.1 ml of this solution and successive additions of a 2×10^{-4} mol l^{-1} benznidazole standard stock solution in methanol).

3. Results and discussion

3.1. Polarographic behaviour

Benznidazole exhibited a single well-defined DP peak and DC wave (Fig. 1) in DMSO/0.1 M Bu_4NBF_4, with a peak potential at -0.97 V and a $E_{1/2}$ of -1.0 versus SCE (for a 1.0×10^{-3} mol l^{-1} solution).

Logarithmic analysis of the obtained DC polarogram using the criteria of E versus log $(i_d/i_d - i)$ showed a linear dependence with a slope of 68 mV, which suggests that the electrode process has some irreversible character ($\alpha n = 0.87$), with the transfer of one electron [12]. The plot of the diffusion current as a function of the square root of the mercury height was a straight line, indicating that the reduction process is controlled by diffusion [13].

Fig. 2 shows the variation of i_{p1}/i_{p2} (ratio of peaks currents using different drop times 2 and 0.4 s, respectively) with the pulse amplitude (ΔE). The increasing values indicate that the electron transfer can be either irreversible, or a reversible transfer followed by a chemical reaction [14].

The distinction between a reversible or irreversible electron transfer can be made by the variation of $\alpha n W_{1/2}$ with $\alpha n \Delta E$. For reversible

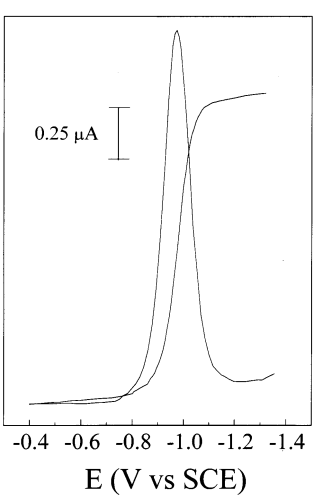

Fig. 1. DC and DP polarograms of 1.0×10^{-3} mol l^{-1} benznidazole in DMSO/0.1 mol l^{-1} Bu_4NBF_4: $t_g = 0.4$ s; $\Delta E = +100$ mV.

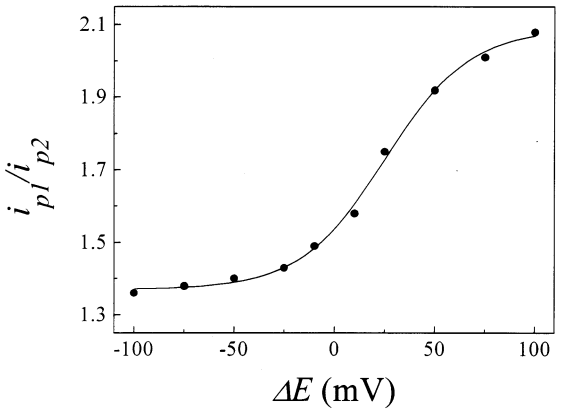

Fig. 2. Variation of i_{p1}/i_{p2} as a function of ΔE for 1.0×10^{-3} mol l^{-1} benznidazole in DMSO/0.1 mol l^{-1} Bu_4NBF_4. $t_g =$ 2.0 s (i_{p1}); $t_g = 0.4$ s (i_{p2}).

electron transfers $\alpha n W_{1/2} \rightarrow 90$ mV for $\alpha n \Delta E \rightarrow 0$, and different values for irreversible electron transfers. According to Fig. 3 the electronic transfer in benznidazole seems to be a reversible transfer followed by a chemical reaction (EC mechanism) [14] probably due to dimerization, protonation or a disproportionating of the anion radical.

3.2. Determination

The variation of peak currents with the concentration of benznidazole pure drug are linear in the 1.0×10^{-6}–5.0×10^{-3} mol l^{-1} concentration range, the experimental data fitting the

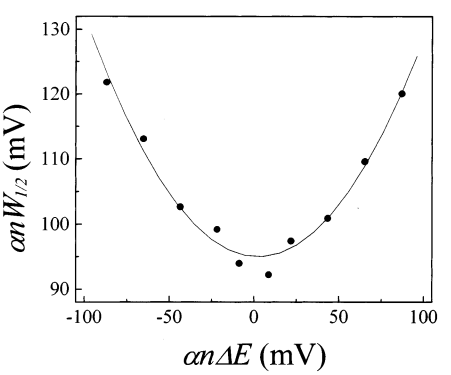

Fig. 3. Variation of $\alpha n W_{1/2}$ as a function of $\alpha n \Delta E$ for 1.0×10^{-3} mol l^{-1} benznidazole in DMSO/0.1 mol l^{-1} Bu_4NBF_4: $t_g = 0.4$ s.

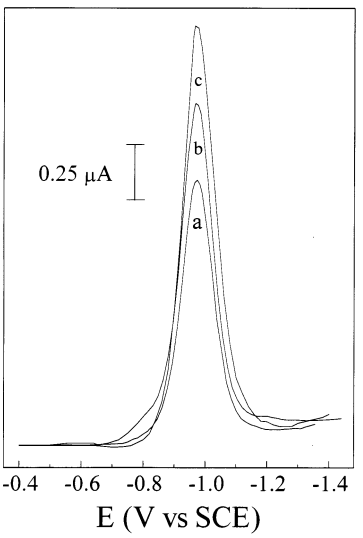

Fig. 4. DP polarograms for successive standard additions of a benznidazole stock solution obtained for the analysis of a pharmaceutical tablet: (a) 0; (b) 0.5; (c) 1.0 ml: $t_g = 0.4$ s; $\Delta E = +100$ mV.

following equation: $i_p\ (nA) = 38.1 + 1.74 \times 10^6$ C (mol l^{-1}), $r = 0.999$: with a detection limit of about 4×10^{-7} mol l^{-1} [15].

In the determination of benznidazole in pharmaceutical tablets by the standard addition method (Fig. 4) standard deviations of 9% ($n =$ s) were obtained, low relative errors (2%. compared with the value supplied by the manufacturer). Table 1 gives the assay results for the dosage form using the by polarographic method and, the ultraviolet spectrophotometric method described in literature [10].

The analysis of the pharmaceutical tablets by the proposed polarographic method were carried

Table 1
Concentration (mg/tablet) of benznidazole obtained for different samples ($n = 5$).

Sample	DPP	UV
A	102 ± 2	105 ± 3
B	99 ± 2	103 ± 3
C	101 ± 2	104 ± 3
Labelled amount	100	

out without previous treatment of the sample (such as filtration, solvent extraction, etc.), avoiding possible analyse losses. The method is fast, simple, sensitive and appropriate for routine analysis. The pharmaceutical tablets dissolves readily in dimethylsulfoxide and the excipients present caused no interference with the determination. This was verified using the peak current of the sample obtained for the standard addition method in the calibration curve: this gave the same concentration of benznidazole.

Furthermore, the results obtained for the ultraviolet spectrophotometric method [10] were identical to the polarographic ones, which indicates the efficacy of the proposed polarographic method. From these results it can be concluded that the polarographic method is sufficiently accurate and precise for it to be applied to the determination of benznidazole in pharmaceutical formulations.

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