Polarographic Study of Nifurtimox

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Abstract □ Nifurtimox is polarographically reducible over the whole pH range, the nitro group being reduced to the hydroxylamine group in a 4e process and subsequently the amine being formed in a 2e process at a pH value below 4. The C=N—N linkage is reduced by a mechanism involving reductive fission of the N—N bond. The differential pulse polarographic peaks for the reduction of the nitro group to the hydroxylamine group at pH 6 were used in developing a new polarographic method for the determination of nifurtimox in pharmaceutical forms.

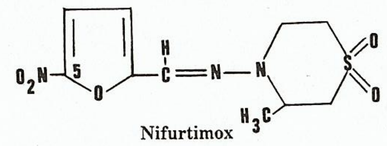
Nifurtimox, 4-[(5-nitrofurfurylidene)amino]-3-methylthiomorpholine 1,1-dioxide (see structure) is a chemotherapeutic agent derived from nitrofuran which has been successfully used in the treatment of trypanosomiasis caused by *Trypanosoma cruzi* (Chagas' disease).[1] Chagas' disease is a parasitic infection affecting several million Latin Americans.[2] The high morbidity and mortality of this disease gives rise to very important health, social, and economic problems in Latin American countries where it is endemic.

Nifurtimox is one of the most readily available drugs for the treatment of the acute phase of the disease, but it has important undesirable side effects which have impaired its use and frequently compelled cessation of the treatment.[3] Several studies[4–6] suggest that unwanted toxic effects are related to its nitroreductive biotransformation to reactive free radicals or other metabolites interacting with cellular constituents with deleterious effects.

For the determination of nifurtimox, methods including colorimetry, thin-layer chromatography, and HPLC have been published.[7,8] The aim of this work was to study the polarographic behavior of nifurtimox in order to elucidate the reduction mechanism at a dropping mercury electrode and to investigate the similarity to the metabolic pathway for the biological degradation of this nitro derivative. Furthermore, we developed a more convenient method for the analysis of nifurtimox in pharmaceutical dosage forms.

Experimental Section

Chemicals—Nifurtimox (4-[(5-nitrofurfurylidene)amino]-3-methylthiomorpholine 1,1-dioxide) was obtained from Laboratorio Bayer (Buenos Aires, Argentina). As a supporting electrolyte, a buffered solution was used with the following components and concentrations: 0.02 M acetic and 0.02 M phosphoric acid for pH < 8.5; and 0.02 M phosphoric acid and 0.02 M $NaHCO_3$ for pH > 8.5. The pH was adjusted with NaOH and the ionic strength was kept at 0.3 M with KCl. Nifurtimox tablets (Lampit; Laboratorio Bayer, Buenos Aires, Argentina) were obtained commercially. The drug was dissolved in buffer:10% ethanol mixtures. All chemicals were of analytical reagent grade. The solutions were polarographed after deoxygenation by the passage of a stream of purified nitrogen for 10 min. Peak potentials (E_p) were measured against a saturated calomel electrode (SCE).



Nifurtimox

Apparatus—Both direct current and differential pulse polarograms were recorded on a Tacussel assembly similar to the one previously described.[9] The SCE and platinum wires were used as a reference and auxiliary electrodes, respectively. Measurements were performed using a thermostatic cell. For d.p. polarography, the following parameters were used: scan rate, 5 mV/s; drop time, 1.0 s; pulse amplitude, 60 mV.

Results and Discussion

The bactericidal properties of nitrofurans were demonstrated in 1944[10] and this group of compounds has been widely used as antibacterials ever since. There are a wide variety of derivatives from the basic nitrofuran structure. However, the nitro group in the 5 position is essential for activity and is therefore common to all drugs in the series. The presence of the nitro group, which is susceptible to polarographic reduction, renders this class of compounds suitable for polarographic analysis. Several polarographic methods[11–15] for determining nitrofurans such as nitrofurazone, furazolidone, and nitrofurantoin have been described. Nifurtimox possesses a similar pattern to the above nitrofurans. However, no polarographic study has been described.

Nifurtimox gives rise to four peaks i^1, i^2, i^3, and i^4 in acid media (pH 1–4). At pH 4, peaks i^3 and i^4 coalesce, and peak i^2 disappears. Above pH 4, only two peaks appear. The variation of the peak height with pH is showed in Figure 1, and the variation of the peak potential, E_p, with pH for the five different peaks is shown in Figure 2. The main peak,

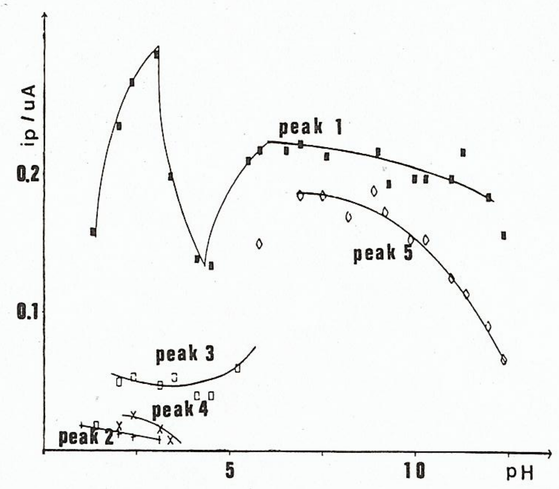


Figure 1—Variation of the peak current with pH.

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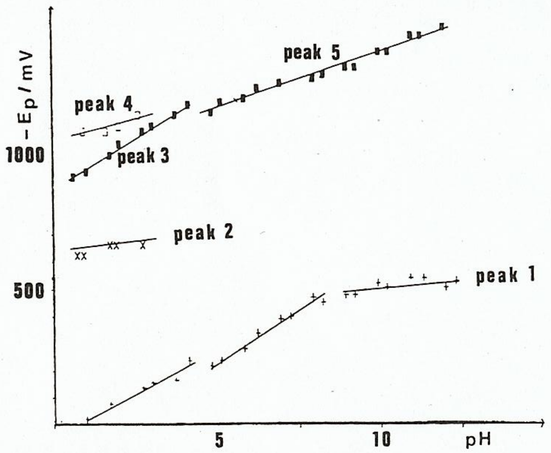


Figure 2—Variation of the peak potential with pH.

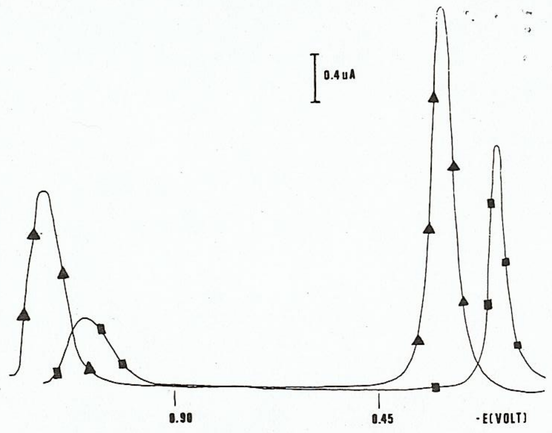
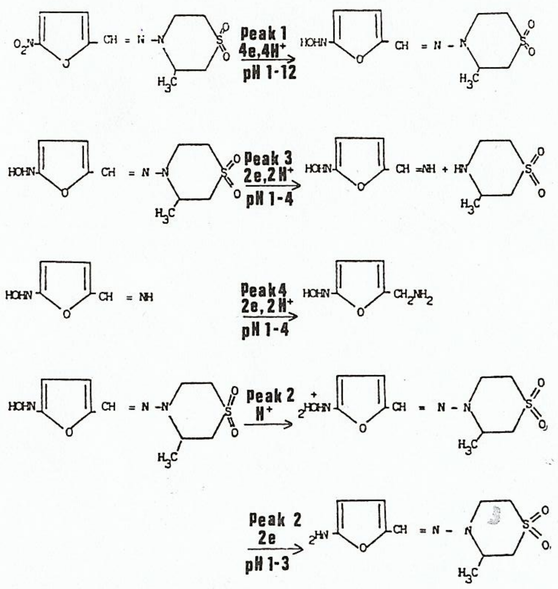


Figure 3—Differential pulse polarograms of nifurtimox solutions at pH 6 (▲) and pH 4 (■).

which appears throughout the pH range studied, is peak i^1 corresponding to the $4\bar{e}$ reduction of the nitro group to a hydroxylamine group. The subsequent reduction of the protonated hydroxylamine group to an amino group, in a $2\bar{e}$ step, results in peak i^2. Peaks i^3 and i^4 represent a process due to the reductive fission of the N—N bond in the C=N—N group, as has been observed in structurally related compounds.[16,17] Above pH 4, peaks i^3 and i^4 coalesce in peak i^5. The proposed polarographic reduction mechanism is illustrated in Scheme I.

In vivo, the nitrofuran compounds are generally metabolized to the corresponding amines via nitroso and hydroxylamine intermediates. However, in the polarographic experiment, the reduction of the nitroso group to hydroxylamine occurs at a more positive potential than the reduction of the nitro group, so the reduction is not detected. This polarographic method could be used in metabolic studies in order to monitor the hydroxylamine intermediate simultaneously with the parent compound.



Scheme I—Proposed reduction mechanism of nifurtimox.

Figure 3 exhibits differential pulse polarograms at pH 4.0 and 6.0, showing specificity and selectivity of scan in separating the nitro peak from the azomethine peak. For quantitative studies we selected the nitro peak at pH 6. The peak current shows a linear dependence on the square root of the mercury height column and a linear dependence on temperature. The temperature coefficient was 0.38% $°C^{-1}$. Furthermore, the peak current shows a linear dependence on the nifurtimox concentration between $1{\cdot}10^{-7}$ and $1{\cdot}10^{-4}$ M. The above behavior is in accord with a diffusion-controlled process. However, electrocapillary curves show absorption of the nifurtimox over mercury.

In order to develop a quantitative polarographic method, a calibration curve method was used. This curve is described by the following equation obtained by the least square linear regression method:

$$i_p\,(\mu A) = 0.109 + 24366C\,(M) \qquad (1)$$

(correlation coefficient 0.999, n = 10, concentration range 0.1–0.01 mM, pH 6, 25 °C), where i_p is the peak current and C is the nifurtimox concentration.

Table I shows the recovery study for a synthetic sample containing 120 mg of nifurtimox. The average recovery of 99.2% and the standard deviation of 1.80 indicate good accuracy and precision for the proposed assay. Individual tablet assay and composite studies are shown in Tables II and III, respectively. The method is sensitive enough to be applied to single tablet assays. Preparation of the sample is easy because the excipients cause no interferences with nifurtimox determination. In the time schedule of the analytical procedure, nifurtimox appears to be stable to daylight exposure.

We recommend this method for content uniformity studies and stability testing of nifurtimox in day-to-day routine analyses.

Table I—Recovery of Nifurtimox from Synthetic Samples[a]

Sample	Amount Found, mg	Amount Recovered, %
1	116.6	97.20
2	119.8	99.80
3	122.4	102.00
4	121.2	101.00
5	120	100.00
6	117.6	98.00
7	115.2	96.00
8	118.8	99.00
9	121.2	101.00
10	117.6	98.00
Average	119.5	92.2
SD	±2.16	1.80

[a] Each synthetic sample represents a commercially available formulation containing 120 mg of nifurtimox plus excipients according to the manufacturer's batch formulas.

Table II—Content Uniformity by Individual Tablet Assay for Dosage Forms[a] of Nifurtimox

Tablet	Amount Found, mg
1	120.4
2	119.2
3	124.1
4	120.1
5	114.9
6	117.2
7	117.0
8	116.2
9	118.6
10	119.4
Average	118.7
SD	±2.4

[a] Lampit (Laboratorio Bayer, Buenos Aires, Argentina); declared amount, 120 mg of nifurtimox; total amount, 400 mg/tablet.

Table III—Composite Analyses for Potency of Nifurtimox Tablets

Tablet[a]	Amount Found, mg
1	119.7
2	117.2
3	122.5
4	121.8
5	117.1
6	118.8
7	118.4
8	116.5
9	119.3
10	123.4
Average	119.5
SD	±2.3

[a] Each tablet contained 402.3 mg of powdered material (average weight of ten tablets).

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