Polarographic Determination of Nitrofurazone and Furazolidone in Pharmaceutical Formulations and Urine

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In pyridine - formic acid buffer and tetramethylammonium chloride solution over the pH range 0–8.5, nitrofurazone, furazolidone and other nitrofuran derivatives are reduced in a six-electron process, giving rise to a well defined polarographic reduction wave at a dropping mercury electrode. The current is diffusion controlled and proportional to the concentration from about 5.88×10^{-3} M to the limit of detection of 1.24×10^{-6} M. The proposed method permits these drugs to be determined, without any prior separation or extraction, in pharmaceutical formulations and in urine at levels at which the unchanged drugs are excreted.

Other drugs commonly used as therapeutic agents, such as nitro derivatives of 1,4-benzodiazepines, chloramphenicol, metronidazole and tinidazole, are reduced in a single four-electron process at more negative potentials, which makes simultaneous determinations possible.

Keywords: *Nitrofurazone determination; furazolidone determination; polarography; pharmaceutical formulations; urine*

Nitrofurazone [5-nitro-2-furaldehyde semicarbazone] (**I**) and furazolidone [3-(5-nitrofurfurylideneamino)-2-oxazolidone] (**II**) are synthetic nitrofuran derivatives with a nitro group at the 5-position on the furan ring. They have been widely used in the treatment of caecal coccidiosis in chickens and necrotic enteritis in swine. They are generally added to animal feeds to prevent various poultry and swine diseases. In man these drugs are therapeutically effective as antibacterial and bactericidal agents. Structures of the compounds are shown in the preceding paper, p. 965.

The exact mechanism of action of nitrofurazone is not known. It appears, however, that the drug acts by inhibiting bacterial enzymes involved in carbohydrate metabolism. Furazolidone is bactericidal owing to its interference with several bacterial enzyme systems, possibly including prevention of acetylation of coenzyme A. Furazolidone also acts as a monoamine oxidase inhibitor.

These compounds have been determined by spectrophotometry,[1] conductimetry,[2] gas - liquid chromatography,[3] high-performance liquid chromatography[4] and iodimetry.[5] A survey of the literature indicates that very little attention has been paid to the polarographic determination of these drugs.[6–9] This paper reports a method for the polarographic determination of nitrofurazone and furazolidone in pharmaceutical formulations and urine.

Preliminary experiments showed that, in well buffered solutions and using a donor-active solvent, the reduction of aromatic nitro compounds in a single four- or six-electron process depends on the nature and position of the substituents on the aromatic ring, as discussed in the preceding paper.[10]

Experimental

Reagents

All chemicals were analytical-reagent grade reagents.

Stock solutions (10^{-2} M) of nitrofurazone and furazolidone (Sigma Chemical, St. Louis, MO, USA) were prepared by dissolving the appropriate amount of each drug in dimethylformamide (DMF). Tablets containing these drugs were dissolved in DMF and assayed polarographically. Gelatine solution (0.5%) was used to eliminate the polarographic maxima. The supporting electrolyte used contained 0.1 M tetramethylammonium chloride solution and pyridine - formic acid buffer (pH 4.5) and was prepared as described previously.[11]

Apparatus and Conditions for Polarographic Analysis

Polarographic analysis was performed using a Polariter PO4 instrument (Radiometer, Copenhagen, Denmark). A saturated calomel electrode (SCE) was used as a reference electrode together with a dropping mercury electrode (DME) as the working electrode. Dissolved air was removed from the solutions by bubbling oxygen-free nitrogen through the cell for 10 min. All measurements were performed at 25 ± 1 °C.

Procedure

Aliquots of the stock solutions were diluted in 15 ml of supporting electrolyte, 1 ml of gelatine solution was added and the solution was purged with oxygen-free nitrogen for 10 min and subjected to polarography in the d.c. mode.

pH Studies

The effect of pH in the range 1–14 on the limiting current for **I** and **II** at a concentration of 0.124 mM was studied. From these polarographic data, the optimum pH for the determination of the drugs was chosen.

Calibrations Graphs

Aliquots of the pure drugs dissolved in DMF were diluted in the supporting electrolyte and polarographed. The standard additions method was employed in all instances and the results were used in the preparation of limiting current *versus* concentration graphs.

Determination of I and II in Pharmaceutical Formulations

An ophthalmic solution containing a nominal 20 mg per 100 ml of **I** was used for analysis. Portions of 1.0 ml of the ophthalmic solution were transferred into separate 10-ml calibrated flasks and diluted to the mark with the supporting electrolyte and assayed polarographically.

Tablet formulations containing a nominal 100 mg of **II** in a total mass of approximately 378 mg were analysed in order to examine the applicability of the proposed method. Ten tablets were thoroughly ground and mixed. Samples of approximately 20 mg of **II** were accurately weighed, dissolved in

Table 1. Effect of nitrofurazone and furazolidone concentrations on i_d values

Concentration (C)/ 10^{-6} M	Nitrofurazone		Furazolidone	
	i_d/μA	i_d/C	i_d/μA	i_d/C
1.24	0.024	19.35	0.023	18.55
2.44	0.048	19.67	0.045	18.44
3.61	0.066	18.28	0.067	18.56
12.4	0.23	18.54	0.225	18.30
24.4	0.45	18.44	0.45	18.44
36.1	0.675	18.70	0.65	18.00
58.8	1.10	18.71	1.08	18.37
124	2.30	18.60	2.29	18.46
361	6.84	18.94	6.51	18.03
588	10.9	18.53	10.5	17.85

DMF, transferred into separate 10-ml calibrated flasks and diluted to the mark with DMF. The contents of the flasks were shaken for 20 min and then allowed to settle. A 0.1-ml aliquot of the clear supernatant liquor was diluted to 25 ml with the supporting electrolyte and a portion of this solution was subjected to polarography. By reference to calibration graphs the concentrations of the **I** and **II** in each sample were calculated.

Determination of II in Urine

A calibration graph was constructed in accordance with the limits at which the unchanged drug is excreted. Various amounts of **II** were added to a fixed volume of urine and aliquots of these spiked urine samples were diluted with the suppoting electrolyte and polarographed. The calibration graph in the range 3–60 μg ml^{-1} was a straight line passing through the origin.

Results and Discussion

Polarograms of nitrofurazone and furazolidone recorded in the proposed supporting electrolyte (pH 4.5) exhibit a single well defined wave with half-wave potentials of −0.18 and −0.17 V, respectively.

The mercury flow-rate, m, and drop time, t, were determined at various heights of the mercury column, h. This was repeated for several concentrations and the value of $ih^{-\frac{1}{2}}$ was found to be constant, indicating that the current is diffusion controlled. The relationship between limiting current and concentration of each depolariser was found to be linear over a wide range of concentration (Table 1). The limits of detection were 0.24 and 0.28 μg ml^{-1} for **I** and **II**, respectively. Above 1×10^{-3} M of either drug the i_d/c relationship was not linear, probably owing to adsorption of the drugs at the mercury drop surface. At concentrations below 1.5 μg ml^{-1} the supporting electrolyte was used as a blank. No maximum was observed at concentrations of the drugs below 1×10^{-5} M and no gelatine was added.

The precision of the polarographic method was tested with solutions of similar concentration and the standard deviation of the ratio of diffusion current to sample mass was found to be satisfactory.

For **I** and **II**, the best defined and differentiated waves for analytical purposes were obtained at $3 < \text{pH} < 5$ in the proposed supporting electrolyte.[10]

On the basis of these results, **I** and **II** were determined in pharmaceutical formulations. Ten polarographic assays on an ophthalmic solution containing 20 mg per 100 ml of **I** gave a mean value of 19.78 mg per 100 ml with a relative standard deviation of 1.39%. Tablets containing a nominal 100 mg of **II** were also analysed. Ten assays were carried out, giving a mean value of 98.66 mg per tablet with a relative standard deviation of 1.05%.

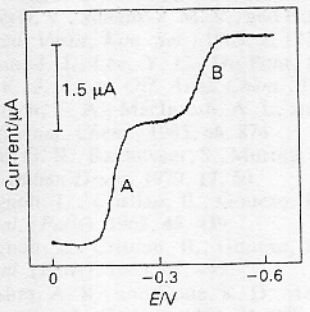

Fig. 1. Polarographic reduction waves of nitrofurazone and chloramphenicol, each at 0.122 mM. (A) Nitrofurazone, $E_{\frac{1}{2}}$ −0.18 V, i_d 2.10 μA. (B) Chloramphenicol, $E_{\frac{1}{2}}$ −0.41 V, i_d 1.42 μA. V_i = 0.0 V

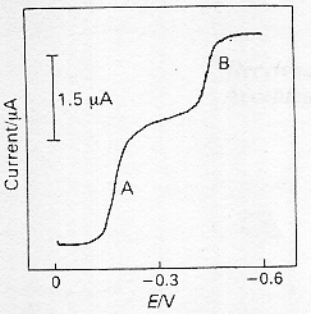

Fig. 2. Polarographic reduction waves of furazolidone and chloramphenicol, each at 0.122 mM. (A) Furazolidone, $E_{\frac{1}{2}}$ −0.17 V, i_d 2.09 μA. (B) Chloramphenicol, $E_{\frac{1}{2}}$ −0.41 V, i_d 1.42 μA. V_i = 0.0 V

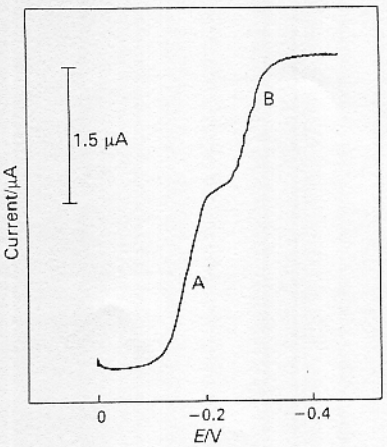

Fig. 3. Polarographic reduction waves of furazolidone and clonazepam, each at 0.122 mM. (A) Furazolidone, $E_{\frac{1}{2}}$ −0.17 V, i_d 2.07 μA. (B) Clonazepam, $E_{\frac{1}{2}}$ −0.28 V, i_d 1.43 μA. V_i = 0.0 V

The precision of the method for the determination of **II** in urine was checked using different spiked urine samples over a wide concentration range. The results showed a relative standard deviation of 3.2% at the lowest concentration levels and 1.92% at concentrations of 10 μg ml^{-1} and higher.

Urine samples were obtained from patients at specific time intervals during single- and multiple-dose administration. It was observed that after administration of a single oral dose of 200 mg the apparent drug concentration in urine increases until it reaches 8% of the initial dose at 2 h and then begins to decrease. On administration of 200 mg three times daily, approximately 15–20% of a total daily dose is excreted as unchanged drug. After 8–10 h the polarographic signal disappears and only non-nitro metabolites are excreted. The main non-nitro metabolites are voltammetrically active amines that exhibit well defined anodic waves. The differences

between polarography and voltammetry arise because polarography determines only the nitro moiety whereas voltammetry permits the determination and detection of other metabolites.[10]

Because of the carcinogenicity and other adverse effects of nitrofurazone when administered in large oral doses, the determination of this drug in normal urine samples was not possible. However, the calibration graph constructed using spiked urine samples was linear in the range 3–60 μg ml^{-1}.

When a solvent - buffer system containing pyridine and formic acid in conjunction with tetramethylammonium chloride solution is used as the supporting electrolyte, these nitrofurans are reduced in a single six-electron wave whereas other nitro derivatives are reduced in a single four-electron wave. Reduction of these nitrofurans occurs at lower negative potentials than for other nitro compounds commonly used as therapeutic agents, such as chloramphenicol, tinidazole, metronidazole and some nitro derivatives of 1,4-benzodiazepines. The large difference in $E_{\frac{1}{2}}$ values makes simultaneous determinations possible, as can be seen in Figs. 1–3.

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