Polarographic Behaviour and Determination of Nitrofurantoin, Chloramphenicol and Related Compounds

Alfonso Morales, Maria I. Toral and Pablo Richter
Departmento de Química, Facultad de Ciencias Básicas y Farmacéuticas, Universidad de Chile, Las Palmeras 3425, Casilla 653, Santiago, Chile

The polarographic behaviour of nitrofurantoin and chloramphenicol have been studied in pyridine - formic acid buffer and tetramethylammonium chloride solution at pH 4.3. The $E_{\frac{1}{4}}$ *versus* pH and i_{lim} *versus* pH relationships show that the $E_{\frac{1}{4}}$ values are pH dependent and that the current is independent of pH except when acid - base equilibria affect it. The difference in $E_{\frac{1}{4}}$ values (230 mV) makes simultaneous determination possible. Simultaneous determinations of both drugs were performed in a concentration range suitable for application to pharmaceutical formulations and body fluids. Other nitro derivatives can also be determined without interference. The standard additions technique provided results as satisfactory as the use of a calibration graph. The proposed method has the advantage over previously used electrochemical methods of not requiring prior separations.

Keywords: *Nitrofurantoin determination; chloramphenicol determination; polarography; pharmaceutical formulations; urine*

All of the available polarographic methods for the determination of nitrofurantoin, chloramphenicol and other nitro derivatives are based on the facility of reduction of the nitro group. However, compounds having the same electroactive group often interfere mutually when they are present together in samples. A brief survey of the literature indicates that polarography has been applied over the years to determine these drugs in pharmaceutical preparations or in body fluids in which one of them is the only reducible species.[1–6] Further, the variable number of reduction waves described for each compound, depending on the supporting electrolyte, pH and maximum supressors employed, illustrate the complexity of the electrode reactions involved.

This paper reports a polarographic study of nitrofurantoin, chloramphenicol and related compounds and a method is suggested for their determination with enhanced selectivity.

Experimental

Reagents

All chemicals used were AnalaR-grade reagents.

Nitrofurantoin USP and chloramphenicol RS (Reference Standard) were used for basic studies. Standard solutions (10^{-2}–10^{-3} M) were prepared in pyridine. Stock solutions of *p*-nitrophenol and chloramphenicol in either ethanol - water mixture or pyridine were also prepared. Capsules and tablets containing these drugs were dissolved in pyridine and assayed polarographically. Gelatin solution (0.5%) was used as a maximum suppressor.

The supporting electrolyte was prepared by diluting 20 ml of pyridine (12.3 M) and 10 ml of formic acid (98–100%) with 120 ml of 0.1 M tetramethylammonium chloride (TMAC) solution. The resulting solution had a pH of 4.3. On varying the ratio of formic acid to pyridine the pH changed from 2.6 to 5.1. Extension of the pH range was achieved by using dilute hydrochloric acid or sodium hydroxide solution.

Techniques

Aliquots of the stock solutions were diluted with the supporting electrolyte, de-oxygenated with oxygen-free nitrogen and subjected to polarography in the d.c. mode using a Polariter PO4 instrument (Radiometer). A saturated calomel electrode was used as the reference electrode. The mercury flow-rate, m, and the drop-time, t, were determined at various heights of the mercury column, h. This was repeated for several concentrations and the value $ih^{-\frac{1}{2}}$ was constant. A linear dependence of the limiting current on depolariser concentration was established.

The effect of pH in the range 1–14 on the half-wave potential and limiting current for nitrofurantoin and chloramphenicol at a concentration 0.125 mM was studied. From these polarographic data, the optimum pH for determination of the drugs was chosen.

Aliquots of the pure drug solutions were serially diluted with the supporting electrolyte and polarographed and the results were used in the construction of calibration graphs. For the graphs of limiting current *versus* concentration, the standard additions method was alternatively employed when a solvent other than pyridine was used.

The precision of the method was checked with solutions of similar concentration and the standard deviation of the ratio of diffusion current (i_d) to sample mass was found to be satisfactory. The method was applied to the analysis of individual formulations containing nitrofurantoin (I) and chloramphenicol (II) by reference to the calibration graphs. The same procedure was used for urine without modification. Calibration graphs were obtained by adding known amounts of these drugs to blank urine.

Determination of I and II in Pharmaceutical Formulations

Tablet formulations containing a nominal 100 mg of compound I in a total tablet mass of approximately 210 mg were analysed in order to examine the applicability of the method. Not less than ten tablets were thoroughly ground and mixed. Portions equivalent to about 20 mg of I were accurately weighed, dissolved in pyridine and transferred into separate 10-ml calibrated flasks. The contents were allowed to settle after shaking. 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 subjected to polarography. The amount of I in the portion of the sample taken was calculated by reference to the calibration graph. A similar method of quality control on simple capsules containing a nominal 250 mg of II in a total mass of approximately 300 mg was attempted.

Determination of I in Urine

A calibration graph was constructed in accordance with the limits at which the unchanged drug is excreted. Different amounts of I were added to a fixed volume of urine. Aliquots

Table 1. Polarographic data for nitro compounds at the same concentration (0.124 mM) in pyridine - formic acid and TMAC as supporting electrolyte

Compound	No. of runs	$E_{\frac{1}{2}}$/V	i_d/μA	I*
p-Nitrophenol	7	−0.50	2.38	8.57
o-Nitrophenol	5	−0.40	2.30	8.28
m-Nitrophenol	6	−0.40	2.10	7.56
Nitrofurantoin	8	−0.18	2.40	8.64
Chloramphenicol	9	−0.41	1.62	5.83
Metronidazole	6	−0.42	1.62	5.83
Nitrazepam	5	−0.34	1.62	5.83
Parathion	8	−0.32	1.64	5.90

* $I = i_d/cm^{2/3}t^{1/6}$, where I = diffusion current constant; i_d = diffusion current (μA); c = concentration of electrolysed substance (mmol l^{-1}); m = flow-rate of mercury (mg s^{-1}); and t = drop time of the D.M.E. (s).

of these spiked urine samples were diluted with the supporting electrolyte and the polarograms recorded. The calibration graph ranging from 3 to 60 μg ml^{-1} was a straight line passing through the origin. Using the same procedure, calibration graphs were constructed for II and *p*-nitrophenol.

Results and Discussion

Preliminary experiments indicated that at pH 4.3 a variety of nitro-containing compounds give well defined polarographic waves when a solvent - buffer system in conjunction with tetramethylammonium chloride solution was used as the supporting electrolyte (Table 1). The possibility of simultaneous determinations is based on the difference in $E_{\frac{1}{2}}$ values and any sample containing a pair of members of the compounds listed can be polarographically determined. A pre-wave that precedes the discharge for the background supporting electrolyte was observed and corresponds in nature and origin to that reported for pyridinium ion reduction.[7] Under the same experimental conditions the nitro-substituted furan ring of I behaves polarographically in the same way as *p*-nitrophenol, giving rise to only one well defined wave corresponding to a six-electron reduction. A similar polarographic behaviour of structurally related compounds has been reported recently.[8] When equimolar solutions of I and *p*-nitrophenol are subjected to polarography the ratio of the wave heights is 1.03 ± 0.04, indicating a similar process; this corresponds to the well known six-electron reduction of the nitro group to the amine.[9,10] The difference in $E_{\frac{1}{2}}$ values makes simultaneous determination possible (Fig. 1.).

Using the Ilković equation the diffusion coefficient of I was calculated to be 5.15×10^{-6} cm^2 s^{-1}.

On the other hand, II gave rise to a four-electron d.c. polarographic wave, corresponding to reduction of the nitro group to hydroxylamine, as established by Hess[11] and confirmed by other workers.[2,12] When polarograms of solutions containing similar concentrations of I and II were recorded it was evident that both drugs can be simultaneously determined and that the wave height of II is about two thirds of that for I and *p*-nitrophenol (Fig. 2).

Polarograms recorded with various amounts of these depolarisers showed that the d.c. current increased linearly with increasing concentration (Table 2). The results indicate that these compounds can be determined over a wide range of concentrations and that the limits of detection are 0.17, 0.3 and 0.4 μg ml^{-1} for *p*-nitrophenol, I and II, respectively.

On the basis of these results, I and II were determined in pharmaceutical formulations. Ten polarographic assays on tablets containing 100 mg of I gave a mean value of 100.14 mg per tablet with a relative standard deviation of 2.01%. Capsules containing a nominal 250 mg of II were also

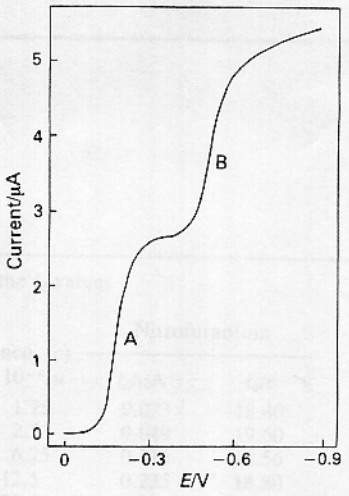

Fig. 1. Polarographic reduction waves of nitrofurantoin and *p*-nitrophenol at the same concentration of 0.124 mM. A, nitrofurantoin; $E_{\frac{1}{2}}$, −0.18 V; and i_d, 2.4, μA. B, *p*-Nitrophenol; $E_{\frac{1}{2}}$, −0.52 V; and i_d, 2.38 μA. V_i, 0.0 V

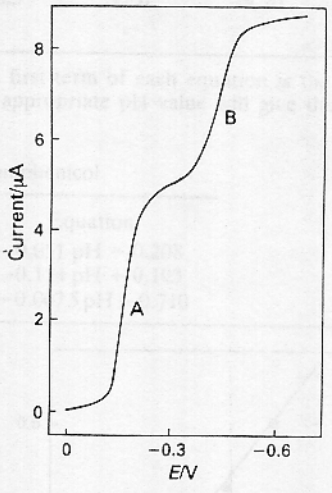

Fig. 2. Polarographic reduction waves of nitrofurantoin and chloramphenicol at the same concentration of 0.25 mM. A, Nitrofurantoin; $E_{\frac{1}{2}}$, −0.18 V; i_d, 4.7 μA. B, Chloramphenicol; $E_{\frac{1}{2}}$, −0.43 V; i_d, 3.2 μA. V_i = 0.0 V

analysed. Ten assays were carried out, giving a mean value of 248.1 mg per capsule with a standard deviation of 2.72 mg and a relative standard deviation of 1.1%.

Using a calibration graph ranging from 3 to 60 μg ml^{-1}, compound I was determined in urine. The analysis of numerous samples showed a relative standard deviation of 3% at the lowest concentration and 2% at concentrations of 10 μg ml^{-1} and higher. II was determined in urine within the same concentration range and the relative standard deviation at the lowest concentration was 1.92%. Simultaneous determination of both drugs was then carried out within the same range. The technique is sufficiently sensitive to permit analysis at the concentration encountered after therapeutic dosage. Other commonly used drugs containing a nitro group or their metabolites do not interfere.

The method can be used to determine *p*-nitrophenol in urine in cases of parathion poisoning.

The linearity of the calibration graphs holds over a wide range of concentrations. Above 1×10^{-3} M of I or II the linear relationship between concentration and current no longer applies, probably owing to adsorption of the electroactive species at the D.M.E. 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 0.01 mM and no gelatin was added.

Table 2. Effect of chloramphenicol, *p*-nitrophenol and nitrofurantoin concentrations on the i_d values

Concn. (*c*) × 10^{-6}/M*	Chloramphenicol i_d/μA	Chloramphenicol i_d/c	*p*-Nitrophenol i_d/μA	*p*-Nitrophenol i_d/c	Concn. (*c*) × 10^{-6}/M	Nitrofurantoin i_d/μA	Nitrofurantoin i_d/c
1.24	0.018	14.52	0.024	19.35	1.25	0.023	18.40
2.44	0.032	13.11	0.045	18.44	2.5	0.049	19.60
5.88	0.079	13.44	0.115	19.56	6.25	0.116	18.56
12.4	0.162	13.06	0.23	18.55	12.5	0.235	18.80
24.4	0.32	13.11	0.47	19.26	25.0	0.48	19.20
58.8	0.77	13.10	1.15	19.56	62.5	1.18	18.88
124	1.62	13.06	2.38	19.19	125	2.43	19.44
244	3.12	12.79	4.61	18.89	250	4.75	19.00
588	7.78	13.23	11.2	19.05	625	12.0	19.20

* These concentrations apply to both chloramphenicol and *p*-nitrophenol.

Table 3. Equations of the half-wave potentials of I and II at different pH values. The first term of each equation is the slope of the line and the second is the intercept of the line with the potential axis. Substitution of the appropriate pH value will give the $E_{\frac{1}{2}}$ value at that pH

Nitrofurantoin pH	Nitrofurantoin Equation	Chloramphenicol pH	Chloramphenicol Equation
0–5.02	−0.049 pH + 0.031	0–4.90	−0.051 pH − 0.208
5.02–8.26	−0.101 pH + 0.29	4.90–7.55	−0.114 pH + 0.103
8.26–~~13~~ 14	−0.003 4 pH − 0.514	7.55–14	−0.007 5 pH − 0.710

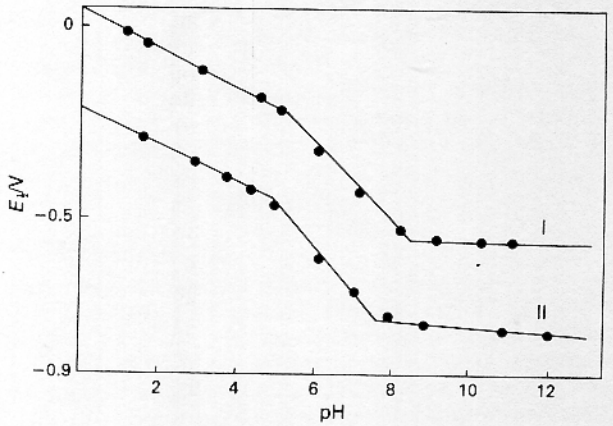

Fig. 3. Variation of $E_{\frac{1}{2}}$ with pH. I, Nitrofurantoin; and II, chloramphenicol

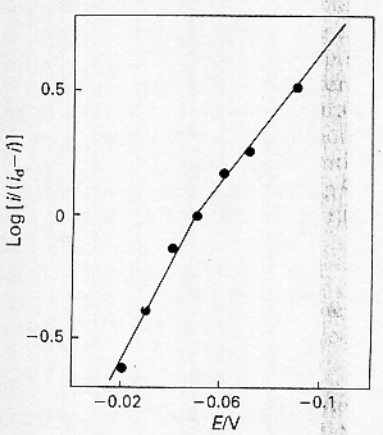

Fig. 4. Logarithmic analysis of the nitrofurantoin wave at pH 2.6. Slopes 50 and 77 mV

Variation of $E_{\frac{1}{2}}$ and i_{lim} with pH

The half-wave reduction potentials for I and II become more negative with increasing pH. The $E_{\frac{1}{2}}$ *versus* pH graphs (Fig. 3) show three intersecting linear sections so that it is not possible to express them in terms of only one equation. The equations that describe the variations of $E_{\frac{1}{2}}$ with pH were deduced from the graphs and are given in Table 3. The slight decrease in current observed for I at pH 2.6 is probably associated with the acid - base properties of its two cationic forms.[4] Further, the log[$i/(i_d - i)$] *versus* E graph at this pH has two linear portions, indicating that two reactions are occurring (Fig. 4). Above pH 5 the wave slowly begins to decay for both compounds and the first break on the $E_{\frac{1}{2}}$ *versus* pH graph (Fig. 3) relates to the pH at which the hydroxylamine intermediate in the reduction of the nitro group is no longer protonated and therefore cannot be easily reduced. At about pH 8, the current falls sharply and the wave breaks up into two waves. This fall is accompanied by a change in the slope of the $E_{\frac{1}{2}}$ *versus* pH graph (Fig. 3), indicating that an acid - base equilibrium can affect the reduction mechanism of the molecule as a whole. For I the current became constant in both $E_{\frac{1}{2}}$ and i_{lim} above pH 11, which suggests a pK value probably associated with the above-mentioned acid - base equilibrium that occurs at pH 8.

The second ill-defined and highly irreversible wave was also unaltered in the values of $E_{\frac{1}{2}}$ and of limiting current over the pH range 11–14. The pK_a values obtained polarographically compare reasonably well with those determined by UV spectrophotometry, indicating that nitrofurantoin exists in four different forms.[4] For all these compounds, including *p*-nitrophenol, the best defined and differentiated waves for analytical purposes were obtained at pH $\leqslant$ pK_1.

The number of protons (p) involved in the rate-determining step of the electrode reaction is given by

$$\Delta E_{1/2}/\Delta\text{pH} = -0.059p/\alpha n_a$$

where α is the transfer coefficient. The value of αn_a for the irreversible process was calculated from the equation

$$E = E_{1/2} - (0059/\alpha n_a) \log [i/(i_d - i)]$$

The αn_a and p values corresponding to the rate-determining step of the reaction were calculated at different pH values. At pH 4.3 an αn_a value of 1.14 and a p value of 0.98 were calculated for I. Values of αn_a lying between 1 and 2 suggest that two electrons take part in the rate-determining step of the electrode reaction. The half-wave potential indicates some nitroso character, while the easy reduction in a single wave may be a consequence of the rapid elimination of water from

the hydroxylamine, giving a highly reducible intermediate imine. Such behaviour has been shown to occur in nitro and nitrosophenols.[13,14] The αn_a and p values obtained for II at the same pH were 0.89 and 0.82, respectively. For both drugs the pronounced shift in the half-wave potential with increasing pH (Fig. 3) indicates that two protons are involved in the rate-determining step of the process in the pH range 5–8.

As the half-wave potentials are pH dependent and hydronium ions are involved in the reduction process, it is necessary to buffer solutions. Polarograms of these compounds recorded in pyridine - formic acid buffer and tetramethylammonium chloride solution at pH 4.3 exhibit a single and very well defined wave. The current is diffusion controlled and proportional to the concentration from about 6×10^{-4} M to the limit of detection at about 1.2×10^{-6} M. This work offers a rapid and sensitive method of analysis for nitrofurantoin, chloramphenicol, p-nitrophenol and various other nitro-containing compounds. Determinations were performed directly without any preceding and time-consuming separations. Further, the method is selective. Nitrofurantoin can be determined in the presence of chloramphenicol or p-nitrophenol because of the wide separation in their $E_{\frac{1}{2}}$ values. Other commonly used drugs containing nitro groups do not interfere in the determination of nitrofurantoin.

The wide range of concentrations over which these drugs can be determined indicates the possible applications of the method. At higher concentrations, determinations in pharmaceutical formulations are possible. The method is sensitive enough to measure concentrations as low as those encountered after therapeutic dosage. The technique may be used for the direct determination of p-nitrophenol in urine in cases of parathion poisoning.

The proposed supporting electrolyte permits high selectivity in the polarographic determination of these compounds.

This work was financed by the Department for Research Development of the University of Chile. This financial support is greatly appreciated.

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