Voltammetric Behaviour of Nitrofurazone, Furazolidone and Other Nitro Derivatives of Biological Importance

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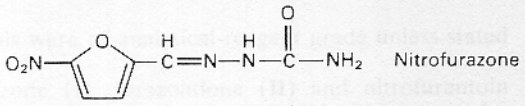
In pyridine - formic acid buffer and tetramethylammonium chloride solution of pH 4.5 at a dropping mercury or a glassy carbon electrode, nitrofurazone, furazolidone and nitrofurantoin are reduced in a single six-electron wave, while chloramphenicol and other structurally related nitro derivatives are reduced in only one four-electron wave, the nitro group being reduced to the amine or to the hydroxylamine, respectively. The electrochemical behaviour of these compounds depends mainly on the nature and position of the substituents. Reduction to the primary amine occurs when the substituents possess available π electrons to conjugate with the nitro group of the aromatic ring, which determines the transformation of the hydroxylamine into the amine via formation of a highly reducible intermediate imine or a quinonoid structure. In contrast, if the formation of the intermediate imine is made impossible by an adverse effect of the substituent, the hydroxylamine does not undergo further reduction.

Cyclic voltammograms were recorded at different pH values and at different scan rates in order to identify certain relatively unstable species. The effect of pH on the diffusion-limited current and on the $E_{\frac{1}{2}}$ values of the polarographic waves was also studied and the results obtained were compared with those obtained by cyclic voltammetry.

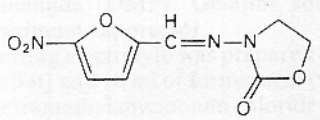
On this basis, and according to the polarographic and cyclic voltammetric data, a reduction mechanism for the nitrofuran derivatives is suggested, in which the importance of the homogeneous chemical reactions associated with the electron-transfer steps is examined.

Keywords: *Nitrofurazone, furazolidone and chloramphenicol; nitro derivatives of 1,4-benzodiazepines; polarography; cyclic voltammetry*

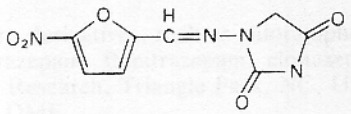
Nitrofurazone (**I**), furazolidone (**II**) and nitrofurantoin (**III**) are structurally related, with a nitro group at the 5-position on the furan ring. The electrochemical behaviour of these nitrofurans and other aromatic nitro derivatives such as chloramphenicol, clonazepam, nitrazepam, flunitrazepam and parathion is based on the ease of reduction of the nitro group at a dropping mercury or solid electrode.



Nitrofurazone



Furazolidone



Nitrofurantoin

Polarography has been widely used in order to elucidate the reduction mechanism at a dropping mercury electrode and to investigate the resemblance with the metabolic pathway for the biological degradation of these nitro derivatives. These compounds are generally metabolised *in vivo* to the corresponding amines via nitroso and hydroxylamine intermediates. However, the polarographic techniques cannot determine these metabolites owing to the ill-defined reduction wave of the hydroxylamine, the inability of the amine group to react at the dropping mercury electrode and because the reduction of the nitroso group to hydroxylamine occurs at a more positive potential than the reduction potential of the nitro group to hydroxylamine, and consequently that reduction wave is not detected. Further, the variable number of reduction waves for each compound, depending on the supporting electrolyte, pH and maximum supressors employed, illustrates the complexity of the electrode processes involved. In Britton - Robinson buffer at pH values below 5, the nitro group of the nitrofurantoin is reduced to hydroxylamine in a four-electron process and subsequently to the amine in a two-electron process.[1] Similarly, nitrofurazone shows a two-step reduction wave in different supporting electrolytes, the second wave being attributed to a simultaneous reduction of the hydroxylamine and the azomethine group.[2]

The determination of some nitrated neterocyclic compounds containing similar types of reduction sites to that of nitrofurazone was studied earlier by Vignoli *et al.*,[3] using a Britton - Robinson buffer of pH between 1.81 and 11.98. They observed two waves for the reduction of the nitro group and three waves at lower pH values when the compounds had a substituted imino group.

A polarographic method has been used to determine furazolidone in feed pre-mixes,[4] but little attention has been paid to the electrochemical behaviour of this compound.

In previous polarographic work[5] in which a solvent - buffer system containing pyridine and formic acid in conjunction with tetramethylammonium chloride solution was used as the supporting electrolyte, it was found that the nitro group of the nitrofurantoin shows only one reduction wave corresponding to a six-electron process, and that chloramphenicol and other structurally related compounds are reduced in a single four-electron wave.

The aim of this work was to study the electrochemical behaviour of molecules having the same electroactive group in order to elucidate the effects of the nature and position of the substituents on the reduction. Significant aspects involved in

Table 1. Voltammetric data for reduction of **I** and **II** in pyridine - formic acid with TMAC as supporting electrolyte

Compound	Scan rate/ V s^{-1}	i_p/μA	$i_p/Cv^{\frac{1}{2}}$
0.196 mM **I**	0.020	5.60	202
	0.050	9.20	210
	0.100	13.20	213
	0.200	17.60	201
	0.300	20.80	194
	0.400	23.60	191
0.476 mM **I**	0.020	14.4	214
	0.050	22.4	211
	0.100	33.2	220
	0.200	43.2	203
	0.300	51.6	198
	0.400	59.2	197
0.196 mM **II**	0.120	5.8	209
	0.050	9.2	210
	0.100	12.8	207
	0.200	18.0	205
	0.300	21.6	201
	0.400	24.2	195
0.476 mM **II**	0.020	14.4	214
	0.050	22.4	211
	0.100	32.0	213
	0.200	44.2	212
	0.300	55.2	212
	0.400	60.4	201

Table 2. Polarographic data for the reduction of nine nitro compounds (0.124 mM) in pyridine - formic acid with TMAC as supporting electrolyte

Compound	No. of runs	$E_{\frac{1}{2}}$/V	i_d/μA
p-Nitrophenol	7	−0.50	2.38
Nitrofurantoin	8	−0.16	2.27
Nitrofurazone	7	−0.18	2.30
Furazolidone	7	−0.17	2.29
Chloramphenicol	9	−0.41	1.62
Nitrazepam	5	−0.34	1.62
Flunitrazepam	6	−0.28	1.55
Clonazepam	7	−0.28	1.58
Parathion	8	−0.32	1.64

the reduction were examined, together with the homogeneous chemical reactions accompanying the electrode process.

Experimental

Reagents

All chemicals were of analytical-reagent grade unless stated otherwise.

Nitrofurazone (**I**), furazolidone (**II**) and nitrofurantoin (**III**) (Sigma Chemical, St. Louis, MO, USA) were used for the basic studies. Standard solutions (1.0×10^{-2} M) were prepared by dissolving the appropriate amount of each drug in dimethylformamide (DMF). Gelatine 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 4.5. On varying the ratio of formic acid to pyridine the pH could be varied over the range 2.6–5.1.[5]

Other nitro derivatives, such as chloramphenicol, *p*-nitrophenol, nitrazepam, flunitrazepam, clonazepam and parathion (EPA Research, Triangle Park, NC, USA), were also dissolved in DMF.

Apparatus

Polarographic assays were performed using a Polariter PO4 instrument (Radiometer, Copenhagen, Denmark). A dropping mercury electrode was used as the working electrode and a saturated calomel electrode (SCE) as the reference electrode.

Cyclic voltammetric experiments were performed using a CV-27 voltammograph (Bioanalytical Systems, Lafayette, IN, USA). A three-electrode assembly was used for all measurements. Glassy carbon was employed as working electrode, an SCE as the reference electrode and a platinum coil as the counter electrode.

An Orion Research Digital Ion-Analyzer 701 with glass and SCE electrodes was used for pH determinations.

Techniques

Aliquots of the standard solutions were diluted with the supporting electrolyte, de-oxygenated with oxygen-free nitrogen and analysed using the d.c. polarographic mode. The mercury flow-rate, m, and the drop time, t, were determined at various heights of the mercury column, h. The diffusion-controlled character of the current and the dependence of the diffusion-limited current on the depolariser concentration were established.

Cyclic voltammetric experiments were carried out under identical experimental conditions. All measurements were performed at 25 ± 1 °C. Dissolved air was removed from the solutions by bubbling oxygen-free nitrogen through the cell for 10 min, then passing it over the solution during the electrolysis. Voltammograms were recorded at scan rates between 0.02 and 0.4 V s^{-1}. The current function $i_p/Cv^{\frac{1}{2}}$ was found to be fairly constant for **I** and **II** (Table 1).

pH Studies

The effects of pH on the half-wave potentials and diffusion-limited current for **I** and **II** at a concentration 0.124 mM were studied over the pH range 1–14. The corresponding voltammograms were recorded under identical conditions.

Results and Discussion

Under the experimental conditions described above, the polarographic reduction of some aromatic nitro compounds of biological importance was found to give rise either to a single well defined wave corresponding to a six-electron process or to a single wave corresponding to a four-electron process. The electrochemical behaviour depends on the nature of the aromatic ring and on the nature and position of the substituents. When a solvent - buffer system (pH 4.5) containing pyridine and formic acid in conjunction with tetramethylammonium chloride solution was used as the supporting electrolyte, compounds containing a nitro-substituted furan ring behave as *p*-nitrophenol and are reduced to the corresponding amine in a six-electron reaction in a single step. Reduction of such compounds, except *p*-nitrophenol, occurs at relatively lower negative potentials than that of other nitro compounds, which indicates some nitroso character (Table 2). When equimolar solutions of **I**–**III** were polarographed using the supporting electrolyte mentioned above, the ratio of wave heights was approximately 1.00 ± 0.04, indicating a reduction process similar to that for the corresponding primary amine (Fig. 1). Using controlled potential electrolysis, Mishra and Gode[2] recently demonstrated that the ultimate reduced product of nitrofurazone is the primary amine.

Reduction by six electrons in a single step occurs only if the substituents possess available π electrons to conjugate with

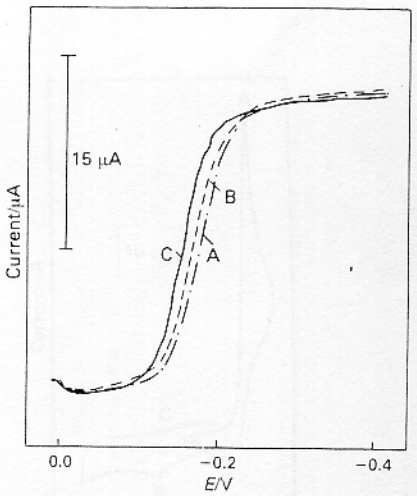
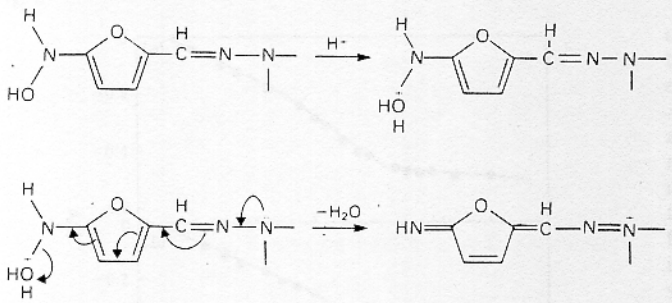


Fig. 1. Polarographic reduction waves of nitrofurazone, furazolidone and nitrofurantoin, each at 0.124 mM. (A) Nitrofurazone: $E_{\frac{1}{2}}$ −0.18 V and i_d 2.3 μA. (B) furazolidone: $E_{\frac{1}{2}}$ −0.17 V, i_d 2.29 μA. (C) Nitrofurantoin: $E_{\frac{1}{2}}$ −0.16 V, i_d 2.27 μA. V_i = 0.00 V

the nitro group of the aromatic ring, which makes the transformation of the hydroxylamine into the imine or a quinonoid structure possible. This substituted donor group must be located at the 2-position on the furan ring or in a *para* or *ortho* position on the benzene ring in order to achieve the interaction of the π systems of the aromatic ring and of the substituents with the intermediate hydroxylamine to give the corresponding imine, which is then reduced to the primary amine.

Electrochemical reduction of **I–III** in a single wave can be explained regardless of the electron transfer process by the extremely fast chemical reactions occurring, owing to the presence of the moiety >C=N–N<. The following scheme represents the mechanism of formation of the corresponding imine from the intermediate hydroxylamine.



p-Nitrophenol, *p*-nitroaniline, nitrosophenols and other aromatic nitro compounds containing similar types of reduction sites and a donor group substituent show analogous polarographic behaviour.[5–9]

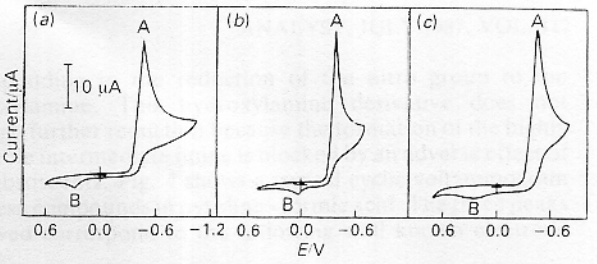
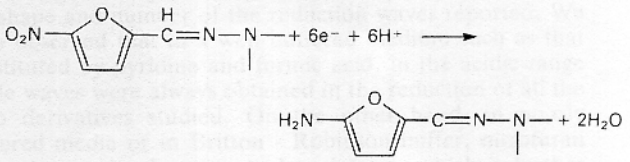


Fig. 2. Cyclic voltammograms of (*a*) nitrofurazone; (*b*) furazolidone; and (*c*) nitrofurantoin, each at 0.476 mM. pH, 4.5. Glassy carbon electrode. Scan rate. 0.1 V s^{-1}

Cyclic voltammograms of the nitrofuran derivatives (Fig. 2) were recorded under identical conditions, in order to identify intermediate species. In all instances the scan is initiated in a negative direction from 0.0 V. The initial reduction peak A corresponds to a six-electron reduction of the nitro group to the amine derivative, as shown below.



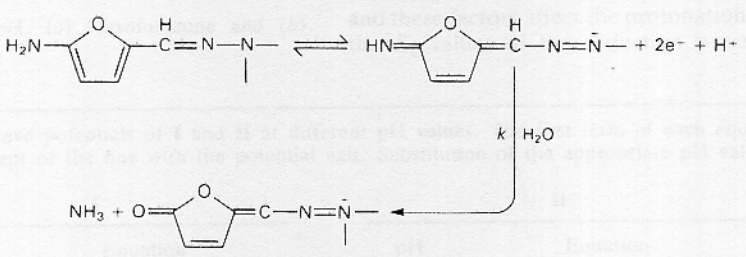
The amine thus produced is subsequently oxidised in the reverse scan at peak B to the imine or quinonoid structure intermediate. This imine is hydrolysed to a quinone derivative, which is neither oxidised nor reduced at these potentials, as shown in Scheme 1.

It can be observed that the electron transfer precedes the chemical reaction (EC reaction). Similar behaviour has been shown to occur in the oxidation of *p*-aminophenol at a platinum electrode in aqueous solutions.[10,11] Wave clipping, that is, reversal of the scan direction before peak A, causes peak B to disappear, indicating that peak B is the oxidation product of the primary amine previously formed in A.

At scan rates higher than 0.3 V s^{-1} a second cathodic peak C appears (Fig. 3), indicating that a reversible reduction of the imine derivative occurs, and that the hydrolysis of this imine is too slow to affect the reduction process. In other words, if the scan rate is very high relative to k, very little imine will be lost to the succeeding hydrolysis reaction and the electrochemical process will be reversible (see Fig. 3). Conversely, if the scan rate is low relative to k, the chemical reaction will be essentially over before the voltage scan is reversed, and the electrode process will appear totally irreversible.

The reduction potential of the highly reducible intermediate imine is more positive than the reduction potential of the nitro group to amine (peak A) and consequently this wave is not observed in normal d.c. polarography.

On the other hand, it was observed that chloramphenicol,[5,12–14] nitrazepam,[15] flunitrazepam and parathion show a different voltammetric behaviour to the nitrofuran derivatives. The former are reduced in a single four-electron wave



Scheme 1

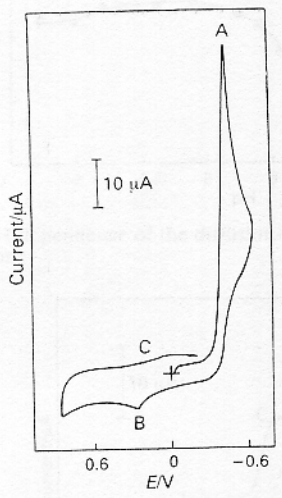


Fig. 3. Cyclic voltammogram of nitrofurazone. Scan rate, 0.3 V s^{-1}; other conditions as in Fig. 2

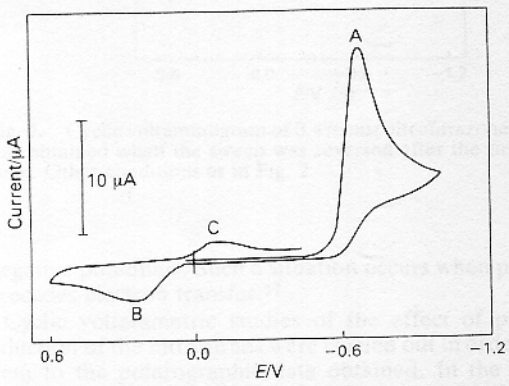


Fig. 4. Cyclic voltammogram of chloramphenicol. Conditions as in Fig. 2

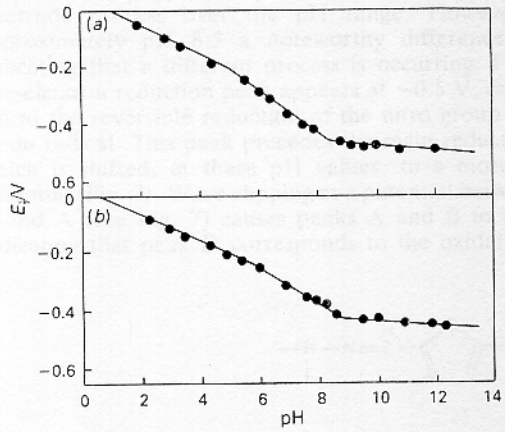


Fig. 5. Variation of $E_{\frac{1}{2}}$ with pH. (*a*) Nitrofurazone and (*b*) furazolidone, each at 0.124 mM

corresponding to the reduction of the nitro group to the hydroxylamine. This hydroxylamine derivative does not undergo further reduction because the formation of the highly reducible intermediate imine is blocked by an adverse effect of the substituents. Fig. 4 shows a typical cyclic voltammogram for these compounds in pyridine - formic acid. The three peaks observed correspond to the following well known electrode process:

Peak A: $ArNO_2 + 4e + 4H^+ \rightarrow ArNHOH + H_2O$
Peak B: $ArNHOH \rightarrow ArNO + 2e + 2H^+$
Peak C: $ArNO + 2e + 2H^+ \rightarrow ArNHOH$

Kissinger and Heineman[16] showed that the three peaks observed in the voltammogram of chloramphenicol in an acetate buffer system and using a carbon paste electrode involve more than a simple electron transfer. The irreversibility of peak A is due to the slow electron transfer occurring in the step nitro → nitroso derivative.[12–15]

In addition to these aspects, the buffer constituents of the supporting electrolyte also seem to have a significant effect on the shape and number of the reduction waves reported. We have observed that in a well buffered medium such as that constituted by pyridine and formic acid, in the acidic range single waves were always obtained in the reduction of all the nitro derivatives studied. On the other hand, in poorly buffered media or in Britton - Robinson buffer, nitrofuran derivatives are reduced to hydroxylamine, which is further reduced to the amine in a second separate wave.[1–3] According to Hess,[12] chloramphenicol is reduced in two steps in phthalate buffer of pH 4 and a similar reduction in two separate waves in acetate media has been reported.[13]

pH Studies

In d.c. polarography, the half-wave potentials for **I** and **II** are pH dependent and are shifted cathodically with increasing pH. The $E_{\frac{1}{2}}$ *versus* pH graph (Fig. 5) shows three linear portions. The equations that describe the variations of $E_{\frac{1}{2}}$ with pH were deduced from the graph and are given in Table 3.

The diffusion-limited current for both compounds is also pH dependent (Fig. 6). The slight decrease in the wave height below pH 3 is probably associated with an acid - base equilibrium as previously reported for nitrofurantoín.[1,5] Above pH 5.0 the wave slowly begins to decay for **I** and **II**, and at this pH the first break on the $E_{\frac{1}{2}}$ *versus* pH graph occurs, representing 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 approximately pH 8.5 for **I** and at pH 8.8 for **II**, each wave falls sharply and breaks up into two waves. This fall is accompanied by a change in the slope of the $E_{\frac{1}{2}}$ *versus* pH graph, indicating that a different electrode process occurs. Therefore, for all these compounds the best defined and differentiated waves for analytical purposes were obtained at $3 < pH < 5$. The scission of the polarographic wave and the change in the slope of the $E_{\frac{1}{2}}$ *versus* pH graph observed at pH 8–9 can be related to the cyclic voltammetric behaviour. Possibly an increase in pH increases the dissociation constant of the protonated species and these factors affect the protonation rate and consequently the $E_{\frac{1}{2}}$ values of the reduction wave are shifted to more

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

I		**II**	
pH	Equation	pH	Equation
0.0–5.12	$E_{\frac{1}{2}} = -0.051pH + 0.050V$	0.0–5.5	$E_{\frac{1}{2}} = -0.045pH + 0.024V$
5.12–8.43	$E_{\frac{1}{2}} = -0.075pH + 0.173V$	5.5–8.79	$E_{\frac{1}{2}} = -0.061pH + 0.112V$
8.43–14	$E_{\frac{1}{2}} = -0.0089pH - 0.384V$	8.79–14	$E_{\frac{1}{2}} = -0.0080pH - 0.354V$

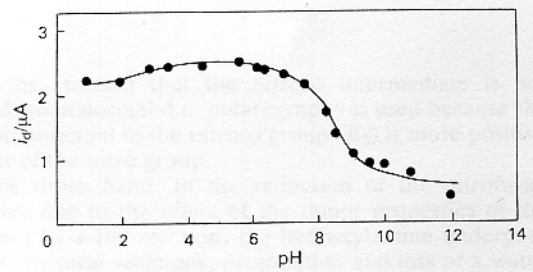


Fig. 6. pH dependence of the diffusion-limited current of 0.124 mM nitrofurazone

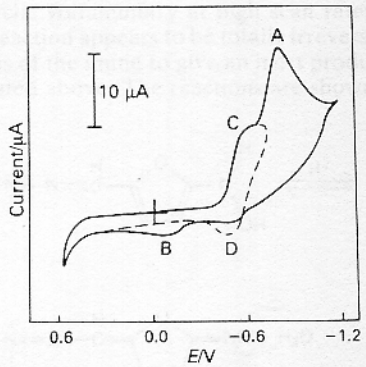


Fig. 7. Cyclic voltammogram of 0.476 mM nitrofurazone at pH 10. D was obtained when the sweep was reversed after the first reduction peak. Other conditions as in Fig. 2

negative potentials. Such a situation occurs when protonation precedes electron transfer.[17]

Cyclic voltammetric studies of the effect of pH on the reduction of the nitrofurans were carried out in order to relate them to the polarographic data obtained. In the pH range 1–8.5 no difference was observed in the shape of the cyclic voltammetric waves (Fig. 2), except that the potentials were shifted cathodically as the pH increased, indicating a similar electrode process over the pH range. However, above approximately pH 8.5 a noteworthy difference appears, indicating that a different process is occurring. For **I–III** a one-electron reduction peak appears at −0.5 V, corresponding to the reversible reduction of the nitro group to a nitro anion radical. This peak precedes the main reduction wave, which is shifted, at these pH values, to a more negative potential (Fig. 7). Wave clipping at a potential between peaks C and A (see Fig. 7) causes peaks A and B to disappear, indicating that peak D corresponds to the oxidation of the anion radical formed in C. The existence of this relatively stable anion radical in alkaline media has been recently reported in the reduction of nitrobenzene when platinum, gold and glassy carbon electrodes were used.[18] The formation of the nitro anion radical can be explained by delocalisation of the electrons in the aromatic ring due to the low proton activity in the bulk of the solution. Detection of the nitro anion radical is possible only through cyclic voltammetry.

Mechanism of the Electrode Process

Based on the polarographic and cyclic voltammetric studies, a reduction mechanism for **I–III** can be proposed. The nature of the waves and peaks was found to be diffusion controlled in the supporting electrolyte used, as shown by the $i_{lim}h^{-\frac{1}{2}}$ and $i_p/Cv^{\frac{1}{2}}$ relationships.

The irreversibility of the electrode process was verified by logarithmic analysis of the wave. The slope of the *E versus* log $(i/i_d - i)$ graph exceeds appreciably $59/n$ mV and the numerical value of $E_{\frac{1}{4}} - E_{\frac{3}{4}}$ exceeds $54.6/n$ mV.[19]

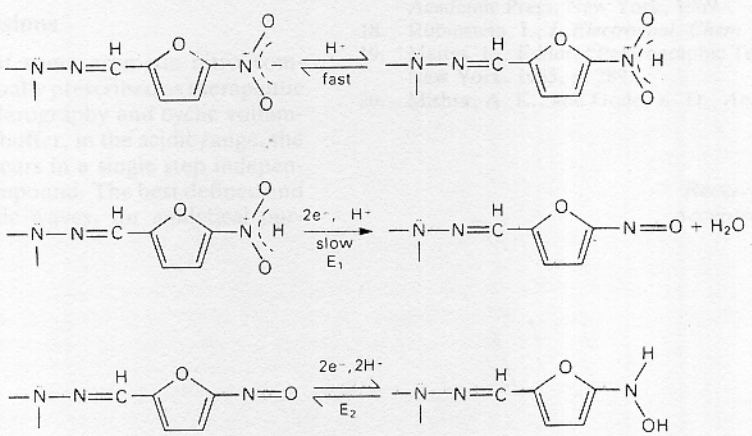
The αn_a values (where α is the transfer coefficient) and the number of protons (p values) corresponding to the rate-determining step were calculated for **I** and **II** at selected pH values. At pH 4.5 the αn_a values for **I** and **II** were found to be 1.23 and 1.15, respectively, indicating that two electrons take part in the rate-determining step of the reaction.

From the equation:

$$\frac{dE_{\frac{1}{4}}}{dpH} = \frac{-0.059}{\alpha n_a} \cdot p$$

p was found to be 0.93 and 0.88 for **I** and **II**, respectively, showing that one proton is involved in the rate-determining step of the reaction over the pH range 1–8. The participation of the hydrogen ion in the rate-determining step is due to protonation of the nitro group to form a more readily reducible species, which is reduced to the nitroso intermediate (CE reaction). A similar stoicheiometry of the rate-determining step for the reduction of nitroxazepine hydrochloride has been reported recently (Scheme 2).[20]

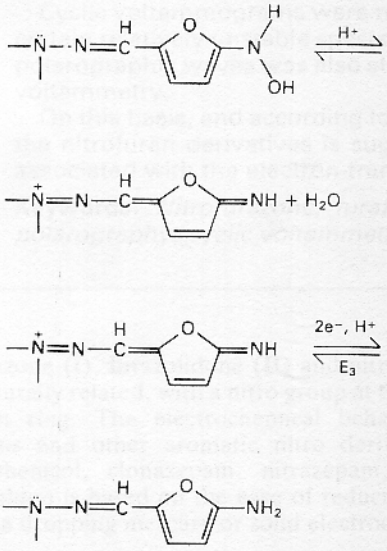
The nitroso intermediate group is rapidly reduced to the hydroxylamine, which is stabilised at this stage whenever the fast chemical reactions that would allow the transformation of this hydroxylamine into the highly reducible intermediate imine are inhibited. This inhibition takes place when the substituent does not have donor properties, in which event a four-electron reduction occurs. This was observed in compounds such as chloramphenicol, parathion, nitro derivatives of 1,4-benzodiazepines and other structurally related substances (CEE reaction). The nitroso - hydroxylamine reversible couple can be detected by cyclic voltammetry only when the hydroxylamine is the ultimate reduction product (Fig. 4).



Scheme 2

It must be stressed that the nitroso intermediate is not observed when normal d.c. polarography is used because the reduction potential of the nitroso group (E_2) is more positive than that of the nitro group.

On the other hand, in the reduction of the nitrofuran derivatives due to the effect of the donor properties of the substituent by a EC reaction, the hydroxylamine undergoes very fast chemical reactions, protonation and loss of a water molecule, giving rise to the highly reducible intermediate imine, which is then reduced to the primary amine in a reversible process (Fig. 3). This reversible couple was observed by cyclic voltammetry at high scan rates. At slow scan rates this reaction appears to be totally irreversible owing to the hydrolysis of the imine to give an inert product (Figs. 2 and 3) as indicated above. The reactions are shown below.



It must be noted that the potential E_3 is more positive than E_1 and therefore only one wave is observed. It may be concluded that the hydrolysis of the imine derivative is too slow to affect the polarographic wave. The above-mentioned processes, as already stated, take place in a well buffered medium of pH 4.5. In the pH range 9–14 the potentials of **I** and **II** are shifted cathodically and one reversible couple appears, at more positive potentials, corresponding to the reversible reduction of the nitro group to a nitro anion radical derivative (Fig. 7).

Conclusions

The voltammetric behaviour of some aromatic nitro compounds of biological interest, usually prescribed as therapeutic agents, has been studied by polarography and cyclic voltammetry. In pyridine - formic acid buffer, in the acidic range, the reduction of the nitro group occurs in a single step independently of the structure of the compound. The best defined and differentiated d.c. polarographic waves, for analytical purposes, were obtained at $3 < pH < 5$. As is apparent from Table 2, the difference in the half-wave potentials makes the simultaneous determination of different nitro derivatives possible.

Cyclic voltammetry was used to identify certain intermediates, metabolites and final products when reducing, under similar conditions, nitro derivatives having different substituents. Hence the formation of the intermediate nitroso group in the reduction of the nitro compounds, which are reduced to hydroxylamine, has been clearly demonstrated, together with the formation of the intermediate imine when the nitro compounds are reduced to amines. The donor properties of the substituent drive the reduction completely to the primary amine, whereas a substituent that is not a donor promotes reduction to hydroxylamine.

Cyclic voltammetry also proved to be useful for the diagnosis of the electrode reactions that are coupled with homogeneous chemical reactions.

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