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An electrochemical study of tinidazole at mercury electrodes

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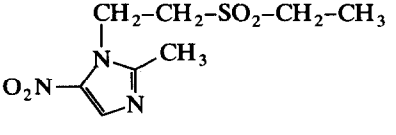
Abstract

The electrochemical behaviour of 1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole (tinidazole) was studied by normal pulse and reverse pulse polarography. In aqueous solutions of pH 2.5–4.8 containing no surfactant, tinidazole gives rise to two irreversible cathodic waves, one (I) corresponding to a four-electron electrode reaction and the other (II) to a two-electron reaction; at higher pH the limiting current of I increases at the expense of II until only a single wave for a six-electron reaction is observed at pH > 7.5. A mechanism for the reaction of wave I under the various experimental conditions is suggested. In strongly alkaline media tinidazole decomposes; the kinetics of this process were studied by normal pulse polarography and the second-order rate constant is reported. In basic solutions containing dimethyl formamide or sodium lauryl sulphate, wave I splits into two or three waves; when the splitting is complete, the electrode reaction corresponding to the first wave I′, is the reversible formation of the anion radical, and the formal potential of the tinidazole/anion radical system can be determined.

INTRODUCTION

Like other 5-nitroimidazoles, tinidazole,



possesses selective activity against anaerobic or microaerophilic bacteria and protozoa. Its half-life is longer than that of metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitro-1*H*-imidazole, the member of this class that is most widely used for therapeutic purposes [1].

Both the selective cytotoxicity of 5-nitroimidazoles and sensitization of hypoxic cells to radiation by 2-nitroimidazoles appear to involve the biological reduction of the NO_2 group in reactions which initiate the cleavage of the DNA strand and the destabilization of the helix [2,3]. According to the model put forward by Edwards [1,2], the active form of these agents is the protonated anion radical $R-NO_2H^{\cdot}$, which by accepting an electron oxidizes the DNA molecule, thereby causing the release of thymidine phosphate and the cleavage of the DNA strand. This mechanism is in keeping with the empirical observation that the activities of both 5- and 2-nitroimidazoles depend on the standard potential of the system $R-NO_2/R-NO_2^{\cdot-}$ formed by the nitro derivative and its anion radical.

In view of the above, it is of some interest to elucidate the mechanism of electrochemical reduction of 5-nitroimidazoles, especially when the common characteristics of electrochemical and enzymatic reactions are borne in mind [4]. Several articles have already appeared on the polarographic or voltammetric reduction of metronidazole and some other 5-nitroimidazoles [5–16]. In the case of tinidazole, however, little electrochemical research has been done. A study of its polarographic determination in tablets found that in aqueous media the reduction of its nitro group gives rise to a four-electron cathodic wave [17]; in dimethylsulphoxide it produces two cathodic waves at stationary or rotating platinum electrodes, of which the first corresponds to the formation of an anion radical that is stable on the voltammetric time scale [13]; and it has also been reported that complete coulometric reduction of tinidazole and other nitroimidazoles at mercury electrodes in aqueous media of pH 7.4 requires a non-integer number of electrons [18], showing that the partially reduced intermediates are reactive on the coulometric time scale. The latter finding is supported by reports that the complete reduction of metronidazole gives rise to numerous products, including the nitrite ion and compounds resulting from the fragmentation of the imidazole ring, and that increasing pH reduces the number of electrons required for complete reduction and increases the concentration of NO_2^- produced, showing that the nitrite is formed from the anion radical intermediate [19,20].

In the work described here, we studied, chiefly by normal and reverse pulse polarography, the electrochemical behaviour of tinidazole in buffered aqueous media (in the presence and absence of surfactant) and in dimethylformamide (DMF) + water mixtures. Our aims were to determine (i) the mechanism of the electrochemical reduction of tinidazole on the time scales of the techniques employed, (ii) the formal potential of the tinidazole/anion radical system in aqueous media, using an alternative to the pulse radiolysis technique used hitherto [21], (iii) the kinetics of the observed degradation of tinidazole in strongly alkaline media and (iv) optimal conditions for the analytical determination of tinidazole by differential pulse polarography.

EXPERIMENTAL

Normal pulse (NPP), reverse pulse (RPP), direct current (DCP) and tast (DCP_{tast}) polarography were carried out using a PAR 174 polarographic analyser connected to a Houston Instruments 2000 *X–Y* recorder and a PAR 174/70 drop timer. A dropping mercury indicator electrode was used, the counterelectrode was a platinum wire and the reference a saturated calomel electrode. DCP was used exclusively to study the effect of the height of the mercury reservoir on the limiting currents of the waves. The other techniques were used with the height of the mercury reservoir kept at 72.5 cm and the drop time mechanically controlled at 2.0 s; the mercury flow rate measured in water under open circuit was 1.44 mg s^{-1}.

Differential pulse polarography (DPP) for analysis was carried out using a Metrohm Polarecord E506 polarograph in conjunction with a three-electrode system of the same type as for NPP. The mercury reservoir height was maintained at 72.5 cm and the drop time at 2.0 s. The mercury flow rate was 0.73 mg s^{-1} and the DPP amplitude −50 mV. Some NP and RP polarograms were also recorded with this instrument.

The accuracy of the half-wave potentials was checked by comparison with $E_{1/2}$ for thallium(I) in 0.1 M KCl.

Except when the influence of temperature was investigated, the polarographic data were obtained at 298.0 ± 0.5 K. Solutions were deaerated by bubbling oxygen-free nitrogen through them for 10 min.

A Knik 761 pH-meter was used to measure pH.

UV–visible spectra were obtained in a Kontron Uvikon 810P spectrophotometer and ^1H nuclear magnetic resonance (NMR) spectra in a Bruker WM 250 (250 MHz) instrument using tetramethylsilane (TMS) as internal standard.

Tinidazole was a Pfizer product. A 1.25×10^{-3} M stock solution of this reagent was prepared daily by dissolving tinidazole in deaerated water. Other chemicals used were Merck reagent grade products. Water was purified using a Millipore Milli-RO-Milli-Q system.

In the polarographic studies, nitric acid + sodium nitrate, Britton–Robinson and sodium hydroxide buffers were employed. When Britton–Robinson buffers containing 40% DMF were used, care was taken to record polarograms before buffer components were precipitated. Except when its influence was studied, the ionic strength was controlled at 0.5 M using sodium nitrate.

UV–visible spectra were recorded using HCl–KCl buffers of ionic strength 0.5 M.

To identify the products of its degradation in strongly alkaline media, tinidazole was dissolved in aqueous NaOH of pH 13.1, and after standing for 1 h at room temperature the solution was extracted with chloroform. The organic extract was dried (Na_2SO_4) and filtered, the solvent was removed in vacuo and the residue was dissolved in $CDCl_3$ and analysed by ^1H NMR spectroscopy. The aqueous layer was neutralized with 1 M HCl and extracted with ethyl acetate. This extract was dried

(Na_2SO_4) and filtered, the solvent was removed in vacuo and the residue was dissolved in dimethylsulphoxide (DMSO) and analysed by ^1H NMR spectroscopy.

RESULTS AND DISCUSSION

Electrochemical reduction of tinidazole in aqueous media with no surfactant

Limiting currents at pH 2.5–4.8.

NP polarograms of tinidazole solutions show two cathodic waves, I and II; their limiting currents are independent of pH and the ratio between them is 2:1 (Figs.

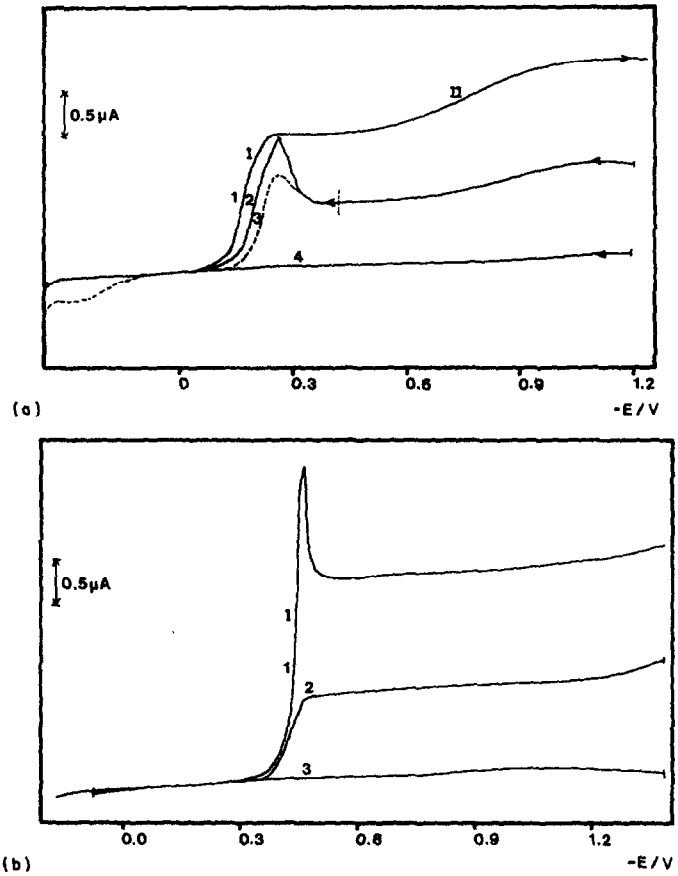


Fig. 1. (a) NP (1) and RP (2–4) polarograms of tinidazole at pH 4.1. Base potentials 0.30 V (1), −1.20 V (2,4) and −0.45 V (3). Tinidazole concentrations 1×10^{-4} M (1–3) and 0 (4). (b) NP (1) and RP (2,3) polarograms of tinidazole at pH 8.8. Base potentials 0.10 V (1) and −1.40 V (2,3). Tinidazole concentrations 1×10^{-4} M (1,2) and 0 (3).

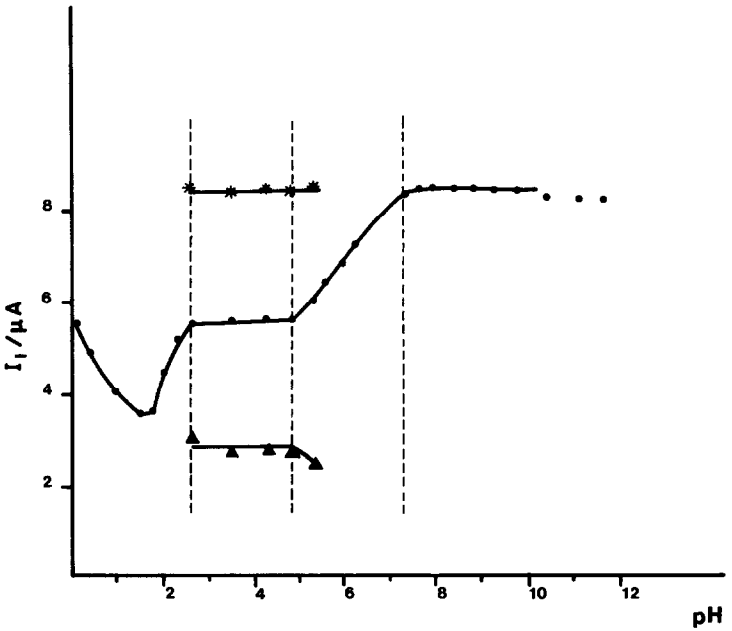


Fig. 2. Plots of I_l^I (●), I_l^{II} (▲) and I_l^{I+II} (*) against pH. Tinidazole concentration 1×10^{-4} M.

1(a) and 2). RP polarograms show two totally cathodic waves at potentials close to those at which I and II appear (Fig. 1(a)), which implies the totally irreversible nature of the corresponding electrode reactions [22]. Furthermore, at pH 4.1 the RP polarogram recorded with a base potential corresponding to the plateau of wave I shows that the product of the electrode reaction for I gives rise to an anodic wave with $E_{1/2} = 0.192$ V.

Under NPP the limiting current of I, I_l^I, and the total limiting current, $I_l^I + I_l^{II}$, are proportional to the concentration of tinidazole and both have a temperature coefficient of 1.72% $°C^{-1}$ (Table 1). Under DCP both limiting currents are

TABLE 1

Temperature coefficient of the limiting current of NPP wave I and the ratio between the limiting currents of I under NPP and DCP_{tast} at various pH

pH	Temperature coefficient/% $°C^{-1}$	I_l^I (NPP)/I_l^I (DCP_{tast})
1.5	2.66	3.82
4.3	1.72	4.34
5.6	2.41	3.98
8.5	1.59	4.44

proportional to the square root of the height of the mercury column. These findings show that under these conditions I_l^I and $I_l^I + I_l^{II}$ are diffusion controlled. The ratio between the values of I_l^I recorded under NPP and DCP_{tast}, 4.34 (Table 1), is only slightly above the value of 4.19 expected for diffusion-controlled limiting currents under the experimental conditions employed in this work (NPP electrolysis time 48.65 ms, drop time 2.0 s [23].

Under NPP at pH 2.8 the ratios between I_l^I and the limiting currents of the first waves produced by *o*-chloronitrobenzene and *p*-chloronitrobenzene are 0.93 and 0.96 respectively. Since the numbers of electrons involved in the latter reactions have been determined coulometrically [24] these ratios show that in this pH range I corresponds to a four-electron process. Furthermore, in acidic media the anodic peaks produced by aromatic nitro compounds during the reverse scan of cyclic voltammograms, which have been attributed to the reversible oxidation of the corresponding hydroxylamine [25,26], occur at potentials close to that of the anodic RPP wave produced by tinidazole when the base potential is located on the plateau of I (Fig. 1(a)). It is accordingly concluded that in this pH range the electrode reaction corresponding to I produces a hydroxylamine and therefore that wave II corresponds to the two-electron reduction of this hydroxylamine to the amine.

Limiting currents at pH 4.8–7.5

As pH rises, I_l^I increases and I_l^{II} decreases but the total limiting current remains the same as at pH 2.5–4.8 (Fig. 2). The data listed in Table 1 show that under these conditions I_l^I is partially kinetically controlled.

Limiting currents at pH > 7.5

When pH 7.5 is reached, only wave I remains, its limiting current being independent of pH and equal to 6/4 of the value of I_l^I at pH 2.5–4.8 (Figs. 1(b) and 2). At pH > 7.5 I_l^I is once more diffusion controlled, as is shown by its being proportional to the concentration of tinidazole (and under DCP to the square root of the height of the mercury column) and by the data listed in Table 1. RP polarograms show a totally cathodic wave appearing at the same potentials as I (which confirms the total irreversibility of the electrode reaction). Together with the other results, the absence of the anodic wave attributed to the oxidation of the hydroxylamine (Fig. 1(b)) shows that in these media I corresponds to the direct transfer of six electrons per molecule to form the amino derivative. The fact that, unusually, this number of electrons differs from the number determined coulometrically at similar pH (4.1 at pH 7.4) [18] shows that the polarographic techniques used allow only low concentrations of reduction process intermediates susceptible to involvement in side reactions.

At pH > 4.8 NPP wave I exhibits a maximum (Fig. 1(b)) which increases with increasing tinidazole concentration and decreasing effective pulse duration and which is accordingly attributed to the adsorption of the electroactive species on the electrode surface [27].

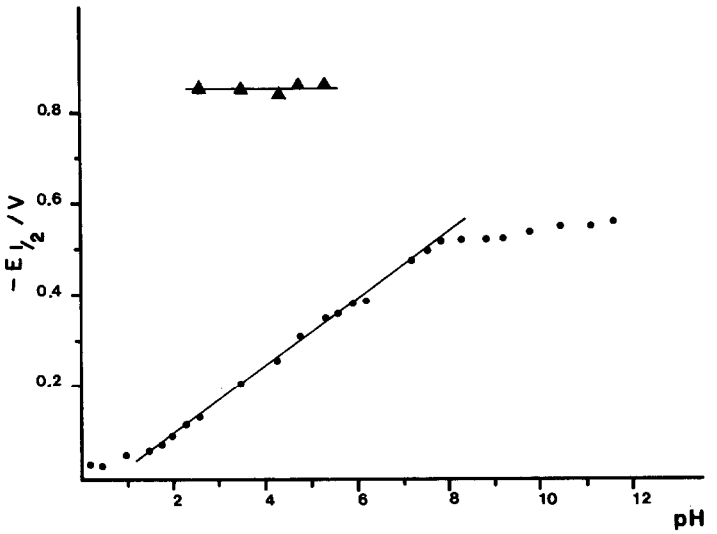


Fig. 3. Plots of $(E_{1/2})^{I}$ (●) and $(E_{1/2})^{II}$ (▲) against pH. Tinidazole concentration 1×10^{-4} M.

At pH > 12 I_l^I falls and a new wave at more negative potentials grows as the time elapsed since preparation of the tinidazole solutions increases. The kinetics of the degradation process causing this behaviour are reported later in the paper.

Limiting currents at pH < 2.5

In this pH range wave II is masked by the effect of the discharge of the proton and I_l^I depends on pH with a minimum at pH 1.6 (Fig. 2). UV–visible spectra recorded between 180 and 386 nm in 1.0×10^{-4} M tinidazole solutions of pH 0.4–3.6 show that as pH falls, the band that at pH 3.6 is located at 320 nm wanes and a new band appears which at pH 0.4 is located at 277 nm. These changes reflect the protonation of the unsubstituted ring nitrogen atom and their analysis affords a value of 2.0 for the corresponding pK_a. The pH dependence of I_l^I in this region, which is shared by other nitro compounds [5,10,15] would appear to be related to this process, but the exact effect on the electrode reaction remains obscure. The data listed in Table 1 show that under these conditions I_l^I is partially kinetically controlled.

Effect of pH on $E_{1/2}$ and αn

Figure 3 shows the influence of pH on the half-wave potentials of waves I and II. For I, plotting $E_{1/2}$ against pH over the range pH 1.5–7.9 yields a straight line with a slope of −74 mV per pH unit. The change in slope at pH 1.5 is attributed to the protonation of the electroactive species. In alkaline media $(E_{1/2})^{I}$ is

TABLE 2

Values of αn for wave I produced by tinidazole (1.0×10^{-4} M) in various media

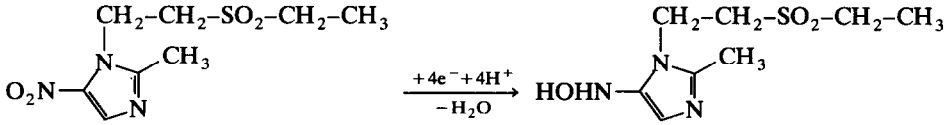
pH	αn
0.47	1.14
0.78	1.27
1.34	1.32
1.59	1.26
1.90	1.25
2.30	1.22
3.30	1.07
4.15	1.11
4.45	1.12
5.30	0.97
6.17	0.96
7.47	0.98
7.99	1.03
8.32	1.25
9.14	1.33
10.80	1.32
11.20	1.37
11.60	1.51
12.14	1.58

independent of pH. $(E_{1/2})^{II}$ is practically independent of pH over the whole range in which wave II is observed.

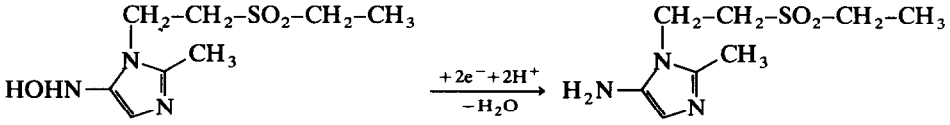
Plots of $\log[x^2(1.75 + x^2)/(1 - x)]$ against $-E$ for NPP wave I, where $x = I/I_l$, are straight lines, except at high I values when I exhibits an adsorption maximum. The slopes of the lines afford the values of $(\alpha n)^I$ listed in Table 2 [28].

Mechanism of the electrode processes

The above findings show that at pH 2.5–4.8 wave I is due to the reduction of unprotonated tinidazole to the corresponding hydroxylamine,



and wave II to the reduction of this hydroxylamine to the corresponding amine,



According to the currently accepted mechanism for the electroreduction of aromatic and heteroaromatic nitro compounds [26] the electrode reaction for I in this pH range is therefore as follows:

$$R\text{-}NO_2 + e^- \rightleftharpoons R\text{-}NO_2^{\cdot -}(ads) \quad (1a)$$

$$R\text{-}NO_2^{\cdot -}(ads) + H^+ \xrightarrow{slow} R\text{-}NO_2^{\cdot}H \quad (2a)$$

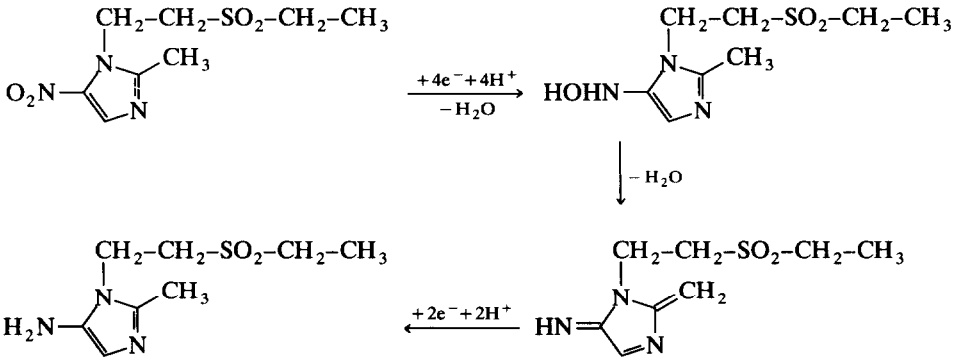
$$R\text{-}NO_2^{\cdot}H + e^- \longrightarrow R\text{-}NO_2H^- \quad (3a)$$

$$R\text{-}NO_2H^- + H^+ \longrightarrow R\text{-}NO + H_2O \quad (4a)$$

$$R\text{-}NO + 2e^- + 2H^+ \rightleftharpoons R\text{-}NHOH \quad (5a)$$

This scheme, with step (2a) as the rate-controlling step, satisfactorily explains the experimental values of $(\alpha n)^I$ and $d(E_{1/2})^I/d(pH)$. The involvement of the adsorbed anion radical as an intermediate in the electrode reaction also explains the change in electrochemical behaviour observed when DMF or sodium lauryl sulphate is included in the medium (see below).

The polarographic behaviour at pH > 4.8, where increasing pH leads eventually to a single wave corresponding to a six-electron process, shows that as pH rises, the hydroxylamine is increasingly subject to a chemical reaction whose product is reduced to the amine at the potential of wave I. The direct reduction of nitro compounds to the corresponding amine has been observed only when the hydroxylamine can adopt an quinonoid structure [24,29–32]. The electrode reaction for I at pH > 4.8 is thus



Since in the more alkaline media $(E_{1/2})^I$ is independent of pH, $(\alpha n)^I$ has a value close to 1.5 and I exhibits an adsorption maximum, the following electrode mechanism is proposed for this pH range:

$$R\text{-}NO_2 + e^- \rightleftharpoons R\text{—}NO_2^{\cdot -}\ (ads) \rightleftharpoons R\text{-}NO_2(ads) + e^- \quad (1b)$$

$$R\text{-}NO_2^{\cdot -}(ads) + e^- \xrightarrow{slow} R\text{-}NO_2^{2-} \quad (2b)$$

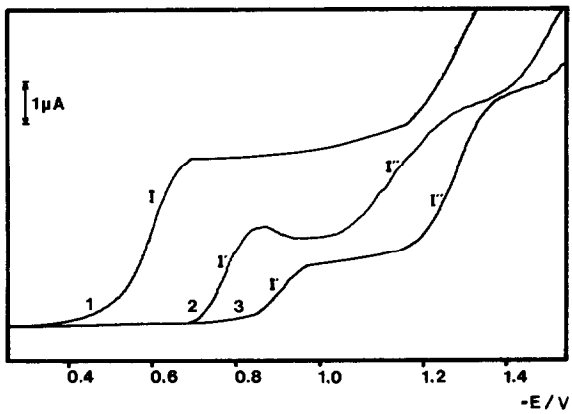


Fig. 4. NP polarograms of tinidazole (1×10^{-4} M) in 40:60 DMF+water of pH 5.64 (1), 8.66 (2) and 11.40 (3).

$$R\text{-}NO_2^{2-} + H_2O \longrightarrow R\text{-}NO + 2OH^- \quad (3b)$$

$$R\text{-}NO + 2e^- + 2H^+ \rightleftharpoons R\text{-}NHOH \quad (4b)$$

$$R\text{-}NHOH \longrightarrow R'{=}NH + H_2O \quad (5b)$$

$$R'{=}NH + 2e^- + 2H^+ \longrightarrow R\text{-}NH_2 \quad (6b)$$

Electrochemical reduction of tinidazole in aqueous media containing sodium lauryl sulphate and in DMF + water mixtures

In 40:60 DMF + water mixtures of pH < 7 wave I is observed under NPP; the viscosity of the medium results in I_l being 30% less than in aqueous media of pH 2.5–4.8 (Fig. 4). At pH > 7 wave I splits into two waves, I′ and I″, increasing pH over the range pH 7.0–9.5 reduces $I_l^{I'}$ and increases $I_l^{I''}$, while their sum remains constant, and for pH > 9.5 both limiting currents are independent of pH, with $I_l^{I'}$ equal to one-quarter of their sum.

Wave I also splits at pH 11.4–12.4 in aqueous media containing sodium lauryl sulphate, producing wave I′ and two further partially overlapping waves (Fig. 5). With a surfactant concentration of 2×10^{-3} M splitting is total, i.e. $I_l^{I'}$ is independent of pH over the pH range mentioned above and its value is one-sixth of that of I_l^{I} in alkaline media with no surfactant.

Similar splitting has been reported for the waves produced by nitrobenzene and some of its derivatives in basic solutions containing ethanol or inhibitory surfactants [26,33]. It has been attributed to the anion radical being more stable in solution than when adsorbed on the electrode surface, with the result that anion radicals displaced from the electrode surface by the surfactant or organic cosolvent are no longer involved in protonation and charge transfer at the potential of wave I.

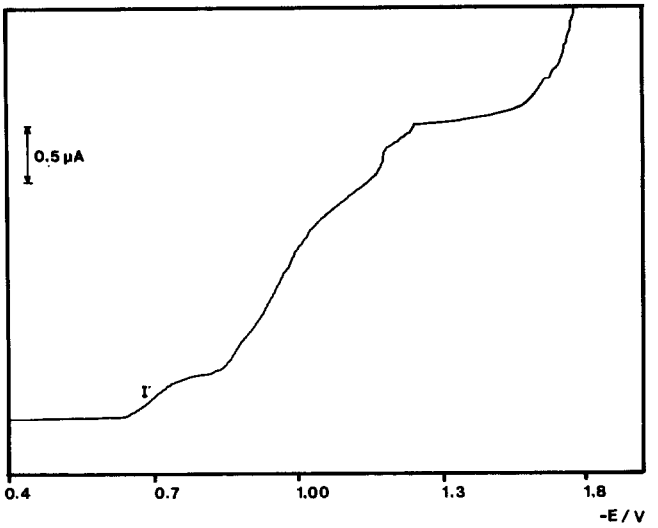
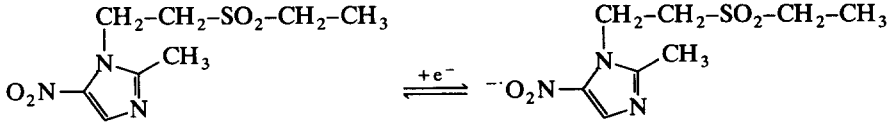


Fig. 5. NP polarogram of tinidazole (1×10^{-4} M) in 2×10^{-3} M sodium lauryl sulphate solution of pH 11.58.

We found that when the splitting of I in media with DMF or surfactant was complete, RP polarograms recorded with a base potential corresponding to the plateau of I′ exhibited a wave with both cathodic and anodic contributions and a total limiting current equal to that of I′ under NPP, showing that the electrode reaction corresponding to I′ is reversible [22]. Plotting $\log[I/(I_l - I)]$ against $-E$ for NPP data for I′ produced straight lines with reciprocal slopes of 59 mV, implying that I′ corresponds to a one-electron reaction [28]. Since the half-wave potential of I′ is independent of pH, hydrogen ions are not involved in its electrode reaction; and since $(E_{1/2})^{I'}$ is also independent of ionic strength (controlled with sodium nitrate), the anion radical produced by the one-electron transfer does not form ionic pairs with the cation of the electrolyte.

To sum up, when the splitting of I is complete, wave I′ corresponds simply to the formation of the unadsorbed anion radical at the electrode:



The half-wave potentials of I′ in the various media are therefore practically equal to the corresponding formal potentials of the redox system involved in this reaction. The value measured in aqueous media containing sodium lauryl sulphate is −720 mV with respect to a saturated calomel electrode, i.e. −460 mV with respect to a normal hydrogen electrode, which, when Davies' equation is used to calculate the activity coefficient of the anion radical in these media, gives a

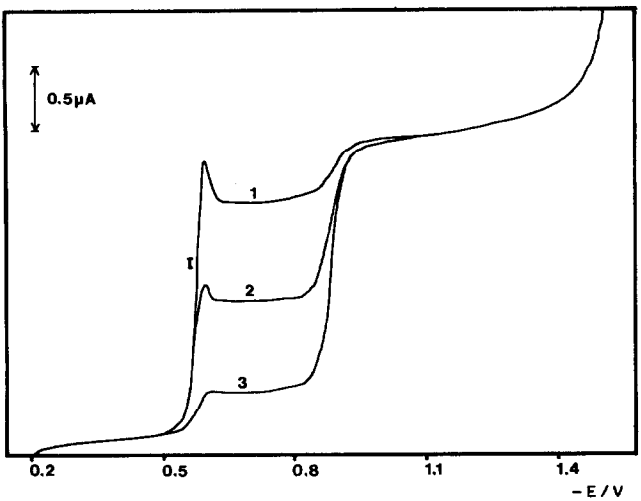


Fig. 6. NP polarograms of tinidazole (1×10^{-4} M) at pH 12.95, 621 s (1), 2708 s (2) and 7484 s (3) after preparation of the tinidazole solution.

standard potential of −468 mV in good agreement with the value of −464 mV determined by pulse radiolysis [21].

Degradation of tinidazole in alkaline media

As was mentioned above, the limiting current of wave I for aqueous tinidazole solutions of pH > 12 falls as the time elapsed since the preparation of the solutions increases, until eventually the wave is virtually suppressed. This process, which is accompanied by the appearance of a new wave at more negative potentials (Fig. 6), reflects the degradation of the tinidazole and becomes more marked as pH is increased. Once the absence of wave I in a strongly alkaline medium has shown the total degradation of the tinidazole to have occurred, polarograms recorded after bringing the pH down to pH 8.5 (at which value the degradation reaction does not occur) still exhibit no wave I, showing that the degradation reaction is irreversible.

The products of the degradation reaction were identified by ^{1}H NMR spectroscopy as described in the Experimental section. The spectrum of the chloroform extract run in $CDCl_3$ showed the presence of ethyl vinylsulphone, with signals for an ethyl group slightly upfield of the tinidazole Et (the methyl triplet being centred at 1.32 ppm and the methylene quadruplet at 2.99 ppm) and for a well-defined vinyl system (doublets at 6.18 and 6.41 ppm and a doublet of doublets at 6.60 ppm, each integrating for 1 H and corresponding to the protons respectively trans, cis and geminal to the sulphone group; $J = 9.70$ Hz for cis protons, 16.62 Hz for trans protons, no coupling for geminal protons) [34]. The spectrum of the ethyl acetate extract of the neutralized aqueous layer run in DMSO showed signals for

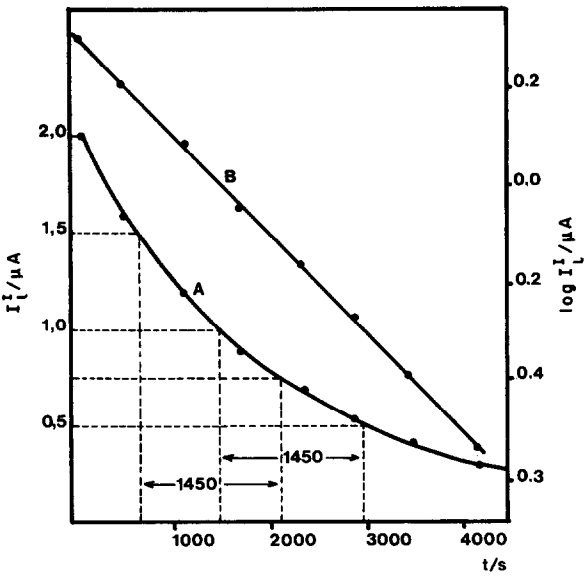
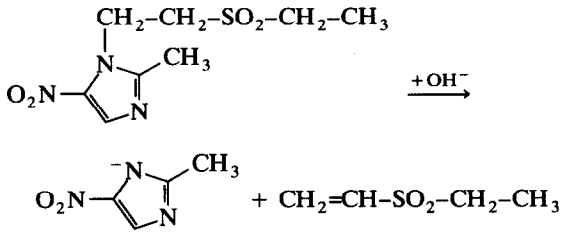


Fig. 7. Plots of I_l^I(A) and log I_l^I (B) against time for 1×10^{-4} M tinidazole at pH 13.16.

2-methyl-5-nitroimidazole, the aromatic proton on C4 appearing at 8.25 ppm as a singlet integrating to 1H and the Me group as a 3H singlet at 2.35 ppm. Accordingly, and in keeping with the known ability of sulphones to generate 2-carbanions in alkaline media [35] the degradation reaction is



The kinetics of the above reaction were studied by NPP by addition of 1×10^{-4} M tinidazole to deoxygenated 0.5 M $NaNO_3$ + NaOH solutions of pH 12.96, 13.16 and 13.26. Plots of I_l^I and log I_l^I against time in the pH 13.16 medium are shown in Fig. 7. In all three media the half-life of tinidazole was independent of its initial concentration, showing a reaction of order one with respect to tinidazole. The experimental rate constants $k_{exp} = k[OH^-]^\beta$ were 1.23×10^{-4} s^{-1} at pH 12.96, 2.04×10^{-4} s^{-1} at pH 13.16 and 2.81×10^{-4} s^{-1} at pH 13.26. Fitting a line by least squares to plots of $\log k_{exp}$ against $\log[OH^-]$ yielded a slope of 1.07 (showing the reaction to be of order one with respect to OH^-) and a value of 8.7×10^{-2} dm^3 mol^{-1} s^{-1} for the second-order rate constant k at 25°C.

Analytical application

In view of the above, the determination of tinidazole by DPP should be carried out in aqueous media of pH 8–11; in these media the peak for tinidazole (corresponding to NPP wave I) corresponds to a six-electron process and the degradation process observed at higher pH does not occur. Under these conditions tinidazole can be determined at concentrations very much lower than those that have hitherto been susceptible to polarographic measurement [5]. The calibration line obtained for determination in the range 8×10^{-7}–1.0×10^{-4} M by undamped DPP at pH 8.6 was

$$(I_{max})^{I}/\mu A = -0.05 + 4.1 \times 10^{4}\, c/M \qquad (r = 0.9992)$$

For concentrations in the range 5×10^{-8}–8×10^{-7} M determined using a damping setting of unity the calibration line was

$$(I_{max})^{I}/\mu A = 0.001 + 3.1 \times 10^{4}\, c/M \qquad (r = 0.9961)$$

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