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ELECTROCHEMICAL CHARACTERISTICS OF NITRO-HETEROCYCLIC COMPOUNDS OF BIOLOGICAL INTEREST
I. The Influence of Solvent

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The electrochemical properties of three nitroimidazoles, a nitropyrazole, a nitrofuran and three nitrobenzenoid compounds have been extensively investigated in a range of solvents. The reduction pathway for the nitro group is independent of the cyclic function to which it is attached, but is strongly influenced by the nature of the solvent. In aqueous media, generally, a single, irreversible 4-electron reduction occurs to give the hydroxylamine. In aprotic media (dimethylformamide, methylene chloride or dimethylsulphoxide), a reversible one-electron reduction takes place to form a stable nitro radical anion. At more negative values. a further 3-electron reduction occurs, irreversibly to give the hydroxylamine. In mixed aqueous-organic systems, intermediate behaviour is found, with the reversibility of the $RNO_2/RNO_2^{\cdot-}$ couple increasing with addition of organic medium. The control of the reduction pathway, by changing the electrolytic medium is discussed in relation to the biological activities of the drugs and identification of the short-lived reduction intermediate responsible for DNA damage.

KEY WORDS: Reduction, nitro-heterocyles, reversible, aqueous, aprotic.

INTRODUCTION

The use of nitroheterocyclic drugs as antibacterial, antiprotozoal and anticancer agents is well established[1,2] and newer derivatives have attracted much attention for the therapy of hypoxic tumours. The cytotoxic activity of such drugs depends upon reduction of the nitro group, usually at low redox potentials which are normally unattainable in well-oxygenated cells. The relative reduction rates in hypoxia or anoxia and under oxic conditions is the basis for their selective toxicity and therapeutic differential.

Although there is direct evidence that free-radical reduction intermediates (detected by electron spin resonance or pulse radiolysis techniques)[3] are involved in the action of these drugs,[4] and it is well established that their redox potential and biological activity are interdependent,[5] nevertheless, details of the precise mechanism of the drug-target interaction remains obscure. Whereas the redox properties of these drugs control the rate of electron and/or proton transfer to other acceptors the prototropic properties of the environment influence the rate of radical stability and decay,[3,5,6]

It is therefore essential to obtain the maximum amount of information concerning the initial charge-transfer step. By using a range of electrochemical techniques, details can be obtained not only on the reduction mechanism and the chemical stability of

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the reduction products, but also on the influence of the environmental (solvent) conditions, on kinetics of associated chemical reactions and the identity of the reduction intermediate(s) responsible for DNA damage. Such information will be germane in the design of new drugs with improved performance.

With this in mind, the electrochemical behaviour of a range of nitro compounds has been examined and compared under a variety of conditions. The drugs chosen are the 2-nitroimidazole misonidazole, the 5-nitroimidazoles metronidazole and ornidazole, a nitropyrazole, a nitrofuran and three nitrobenzenoid compounds which provide a variation in structure and range of reduction potentials. We report on their electrochemical characteristics in aqueous media, in aprotic media and in mixed aqueous organic systems.

MATERIALS AND METHODS

All compounds were supplied as follows, and used without further purification. Metronidazole and M. & B. 4998 from May and Baker Ltd., misonidazole from Roche Products Ltd., nitrofurazone from Smith Kline and French Laboratories Ltd., chloramphenicol from Sigma Chemical Co., *p*-nitroacetophenone from Aldrich Chemical Co. Ltd., and nitrobenzene from Fisons Scientific.

Voltammetric studies employed a PAR 264A polarographic analyzer, interfaced with a PAR 303E cell stand, with 3-electrode cell configuration and a Bausch and Lomb RE0088 x-y recorder. An aqueous Ag/AgCl reference electrode and a platinum wire counter electrode were used in a glass cell. The polarographic dropping mercury electrode (dme) functioned at electronically controlled drop-times (t_d normally 1 sec.). Mercury was generally used as the working electrode, but in a few instances, principally aprotic media, cyclic voltammograms employed a platinum wire or a glassy carbon disc. Routine scan rates were $100\,mVs^{-1}$ in cyclic voltammetry and $5\,mVs^{-1}$ in other modes.

Cell solutions, normally $2 \times 10^{-4}\,mol^{-1}$ in complex, were thoroughly degassed with solvent-saturated N_2 before measurements.

Distilled water employed either KCl (Hopkin and Williams, Analar grade) NaH_2PO_4, Na_2HPO_4 or trisodium citrate as supporting electrolyte. Non-aqueous solvents were Analar or better and used without further purification, where $0.1\,mol^{-1}$ $(C_3H_7)_4NClO_4$ (recrystallized twice from methanol) was used as the supporting electrolyte.

RESULTS

In Aqueous Media

Three aqueous systems, pH7, were examined. The supporting electrolytes were KCl ($1\,mol^{-1}$) and Na_2HPO_4, NaH_2PO_4 ($0.2\,mol^{-1}$) and trisodium citrate ($0.1\,mol^{-1}$, ssc) buffers. The results obtained are listed in Table I.

Previous studies in this laboratory using d.c. polarography in $0.2\,mol^{-1}$ phosphate buffer have shown that the reduction potential is influenced by the pH of the medium, so that as the pH is increased, the reduction potential moves to more negative

TABLE I
Reduction potentials[a] for a number of nitro compounds in aqueous media, pH7

Compounds	Supporting Electrolyte	Electrochemical technique: Cyclic voltammetry Ep_c (v)	differential pulse Ep (v)	d.c. polarography $E_{1/2}$ (v)
Metronidazole	0.2 mol^{-1} phosphate	−0.45	−0.417	−0.378
	0.1 mol^{-1} ssc	−0.652	−0.641	−0.611
	1 mol^{-1} KCl	−0.535	−0.507	−0.492
Ornidazole	0.2 mol^{-1} phosphate	−0.410	−0.390	−0.350
	0.1 mol^{-1} ssc	−0.637	−0.611	−0.581
	1 mol^{-1} KCl	−0.515	−0.502	−0.495
Misonidazole	0.2 mol^{-1} phsophate	−0.308	−0.282	−0.242
	0.1 mol^{-1} ssc	−0.542	−0.538	−0.508
	1 mol^{-1} KCl	−0.345	−0.322	−0.287
M & B 4998	0.2 mol^{-1} phosphate	−0.605	−0.595	−0.570
	0.1 mol^{-1} ssc	−0.742	−0.721	−0.701
	1 mol^{-1} KCl	−0.560	−0.580	−0.565
Nitrofurazone	0.2 mol^{-1} phosphate	−0.236	−0.207	−0.182
		−1.217	−1.185	−1.080
	0.1 mol^{-1} ssc	−0.412	−0.419	−0.415
	1 mol^{-1} KCl	−0.428	−0.417	−0.412
Chloramphenicol	0.2 mol^{-1} phosphate	−0.450	−0.412	−0.355
	0.1 mol^{-1} ssc	−0.615	−0.604	−0.534
	1 mol^{-1} KCl	−0.468	−0.447	−0.397
p-nitroacetophenone	0.2 mol^{-1} phosphate	−0.309	−0.277	−0.262
		−1.195	−1.232	−1.228
	0.1 mol^{-1} ssc	−0.482	−0.481	−0.456
Nitrobenzene	0.2 mol^{-1} phosphate	−0.470	−0.494	−0.459
	0.1 mol^{-1} ssc	−0.735	−0.728	−0.715

[a] Reduction potentials in all modes are not affected by changes in supporting electrolyte concentration.

values,[7,8]. This relationship can be quantified by an expression of the type,

$$E_{1/2} = x - ypH$$

between pH2 and 8.5. However, we have found that the supporting electrolyte also influences the reduction potential, with the values becoming increasingly negative as the electrolyte changes from phosphate to KCl to ssc.

In the present study we have extended our electrochemical techniques to include differential pulse polarography and cyclic voltammetry.

The mechanism of the electrochemical reduction of nitrobenzene has received considerable attention over the past few years.[9,10,11] At all pH values, nitrobenzene is reduced in a single irreversible 4-electron step. The electrochemical behaviour of nitrobenzene has been recorded with our equipment and used for comparison. All the drugs examined in this study display analogous behaviour to that found for nitrobenzene.

Generally, a single reduction process is observed, the potential of which is dependent on the technique employed, as can be seen from Table I. This in itself is indicative of an irreversible charge-transfer process, and indeed, detailed examination of the electrode response for each compound analyzes as an irreversible reduction step.

Using cyclic voltammetry, the cathodic peak potential, Ep_c, shifts to more negative values as the scan rate, v, is increased, and there is no corresponding current response on the reverse scan, irrespective of scan rate or switching potential. By differential pulse polarography, the current response is greatly influenced by the direction of scan. For example, for misonidazole in $0.2\,mol^{-1}$ phosphate buffer, scanning to more negative potentials, shows $Ep = -0.282\,V$, $ip = 13.6\,\mu A$ and a width at half-height of 100 mV. However, if the scan direction is reversed, then $E_p = -0.277\,V$, $i_p = 4.62\,\mu A$ with a width at half-height of 150 mV.

The d.c. polarogram exhibits diffusion currents directly proportional to concentration. An irreversible d.c. polarographic wave is described by the equation,

$$E = E_{1/2} - \frac{RT}{\alpha nF} \ln \frac{i}{i_d - i}$$

where

E = potential at point with current i
$\varepsilon_{1/2}$ = half-wave potential
i_d = diffusion limiting current
n = number of electrons in charge-transfer step
α = transfer coefficient

The mean value of αn for the majority of compounds is close to one.

Application of the Ilkovic equation suggests a 4-electron transfer step corresponding to the reduction of the nitro group to the hydroxylamine, and thus analogous to nitrobenzene.

$$R\text{–}NO_2 + 4H^+ + 4e^- \rightarrow R\text{–}NHOH + H_2O \quad (1)$$

For nitrofurazone, the second reduction step is assigned to the two electron reduction of the hydroxylammonium cation to the amine:

$$R\text{–}NHOH + H^+ \rightarrow R\text{–}N^+H_2OH \xrightarrow{2H^+ + 2e^-} R\text{–}N^+H_3 + H_2O \quad (2)$$

It is therefore clear that the reduction mechanism in aqueous media is the same for a variety of structures and is dominated by reduction of the nitro group. This can occur in two stages, initially by an irreversible 4-electron step to the hydroxylamine (eq. 1.). The identity of the group to which the nitro-function is attached, whether phenyl derivative, imidazole, pyrazole or furan, has no apparent influence on the reduction pathway.

In Aprotic Media

The reduction mechanism for nitrobenzene alters considerably on changing to a non-aqueous medium.[11–13] Two reversible one-electron reductions occur to give the radical anion and the dianion successively (equations 3 and 4):

$$Ph\text{–}NO_2 \underset{E_1}{\overset{+e^-}{\rightleftharpoons}} Ph\text{–}NO_2^{\cdot -} \quad (3)$$

$$Ph\text{–}NO_2^{\cdot -} \overset{+e^-}{\rightleftharpoons} Ph\text{–}NO_2^{2-} \quad (4)$$

The dianion, however, reacts rapidly with solvent, electrolyte or trace impurities and is therefore only chemically stable under rigorously controlled conditions (for example in liquid ammonia).[12]

If a proton donor is present then phenylhydroxylamine is formed, but in a two stage process. First a reversible one-electron reduction to form a stable nitrobenzene radical anion as in equation 3 above, but in this instance being followed by an irreversible 3-electron addition plus protonation to yield the phenylhydroxylamine:

$$Ph\text{–}NO_2^{\cdot-} + 4H^+ \xrightarrow[E_2]{+3e^-} Ph\text{–}NHOH + H_2O \qquad (5)$$

Gattavecchia *et al.* briefly reported that misonidazole in dimethylformamide also showed a reversible one-electron reduction by cyclic voltammetry.[14] Details however, were rather brief, as the electrochemical experiments were used as a confirmation for radiolysis studies.

We have made a brief examination of several nitro compounds in a number of aprotic solvents, and have found, not surprisingly, that their reduction behaviour is analogous to that of nitrobenzene. Table II lists the reduction potentials found by cyclic voltammetry. In all instances the electrode response analyzes as a diffusion-controlled, reversible one-electron reduction. Typically in the scan-rate range 10 to 500 mVs^{-1}, $\Delta Ep = 60$ mV, $E_{1/2}$ is independent of scan rate, Ip (return)/Ip (forward) = unity, and linear Ip *vs* $v^{1/2}$.

A second, irreversible reduction is found in dimethylformamide at potentials between 750 and 1200 mV more negative of the first reduction. A more limited cathodic range in other solvents may prevent its observation against the solvent background. The current response is two to three times greater than the first reduction step, and can therefore be assigned to a 3-electron addition to form the hydroxylamine. Inclusion of the second reduction process in the potential scan results in a decrease in the current response on the return scan for the reversible $RNO_2/RNO_2^{\cdot-}$ couple, so that $Ip(r)/Ip(f) < 1$.

In all examples, therefore the nitro radical anion formed upon one-electron reduction is perfectly stable on the time-scale of the experiments. There appears no reason why this behaviour should not extend to nitro-compounds in general outside our representative sample.

Mixed Aqueous-Organic Systems

The long-term stability of the electrochemically generated nitro radical anion in aprotic media presents the opportunity of studying any chemical following reactions

TABLE II
Reduction potentials for the $RNO_2/RNO_2^{\cdot-}$ couple in a number of non-aqueous solvents[a]

Compound	Solvent	$E_{1/2}$ (Volts)
Misonidazole	dimethylformamide	−1.065
	dimethylsulphoxide	−0.994
	methylene chloride	−0.812
Nitrofurazone	dimethylformamide	−0.871
	methylene chloride	−0.992
Chloramphenicol	dimethylformamide	−1.147
M & B 4998	dimethylformamide	−1.392

[a] All measurements recorded by the cyclic voltammetric mode using platinum or glassy carbon working electrodes.

with DNA, and hence whether the nitro radical anion is responsible, in whole or in part for the cytotoxic behaviour of the nitro compounds. However, is it feasible to compare drug action in a non-aqueous environment to its biological activity?

In search for a compromise, we have investigated the electrochemical behaviour of misonidazole, metronidazole and chloramphenicol in water-dimethylformamide mixtures of varying compositions.

The effect on the electrochemistry of adding 1 ml aliquots of dimethylformamide to 0.1 mol^{-1} ssc aqueous buffer were immediate and dramatic. The general trend found was the same for all three drugs. In place of a single reduction step, now two are observed. As the quantity of added dimethylformamide was increased, both reduction steps moved progressively to more negative potentials, with the second being influenced to a greater extent than the first. Thus, as the amount of dimethylformamide in the system increased, the voltage separation between the two reduction processes also increased. More importantly, the chemical reversibility of the first reduction step, as determined by the $Ip(r)/Ip(f)$ ratio in the cyclic voltammetric mode, increased with the addition of dimethylformamide. The second remains irreversible under all conditions.

The changes observed in the current response on going from a purely aqueous environment to a mixed aqueous-organic system are also interesting. Considering the first reduction step, a decline in current is observed in line with the change from a four-electron to a one-electron transfer, even after addition of only 1 ml of dimethylformamide. The second reduction is usually two to three times greater than the first.

These observations are in line with the change in reduction mechanism on going from a purely aqueous to an aprotic media. In other words, on addition of dimethylformamide, a one-electron reduced, nitro radical anion is formed, which is subsequently further reduced, but at *more negative potentials* to the hydroxylamine. With the increasing amount of dimethylformamide in the system, as already stated, the chemical stability of the nitro radical anion increases.

The voltammetric details recorded using a 1:1 water-dimethylformamide ratio are recorded in Table III. The electrode response for the first reduction analyzes as a reversible one-electron reduction, the product of which is prone to undergo a chemical following reaction (as evident by the increase in $Ip(r)/Ip(f)$ with v).

Comparing the data for misonidazole and metronidazole under analogous conditions, the lifetime of the misonidazole radical anion is longer than that of metronidazole (greater $Ip(r)/Ip(f)$ value). This is the first instance where such measurements have been made by an electrochemically generated nitro radical anion. Further work in this area to determine half-lives of various nitro radical anions, under different water-dimethylformamide ratios, and a comparison with their known biological activity is progressing.

TABLE III

The cyclic voltammetric behaviour of three nitro-compounds measured in a 1:1 mixture of ssc-dimethylformamide

Compound	$E_{1/2}$ (v)	$Ip(r)/Ip(f)$	ΔE (mv)	Ep_c (v) (2nd reduction)
Misonidazole	−0.732	0.919	85	−1.012
Chloramphenicol	−0.757	0.899	75	−1.395
Metronidazole	−0.802	0.764	80	−1.542

DISCUSSION

The reduction mechanism of the nitro function for a variety of compounds appears to parallel that for nitrobenzene. In aqueous solvents, reduction to the hydroxylamine occurs in a single irreversible four-electron step (as in eq. 1). In aprotic solvents, but in the presence of a proton donor (e.g. trace amounts of water), the process occurs in two stages: a reversible one-electron reduction to form the nitro radical anion, followed by a further three-electron addition plus protonation. The intermediate behaviour found with mixed aqueous-dimethylformamide systems supports the proposal put forward for nitrofurans,[15] that in aqueous systems, the first one-electron transfer is reversible, but at reduction potentials more negative than the following three-electron transfer to give the hydroxylamine i.e. $E_1 < E_2$ in eqs. 3 and 5. Thus, upon formation the nitro radical anion immediately undergoes further reduction steps to the hydroxylamine, and no intermediate reduction products are detected. The addition of dimethylformamide produces two effects. E_2 shifts to more negative values, so that $E_1 > E_2$. An independent reduction process to form the nitro radical anion is then clearly in evidence. This one-electron transfer step is fast and diffusion controlled, but the resulting nitro radical anion has a tendency to undergo a chemical following reaction (as evident from the characteristic cyclic voltammetry behaviour). However, with the addition of dimethylformamide the chemical stability of the nitro radical anion increases and the $Ip(r)/Ip(f)$ ratio from the cyclic voltammetry approaches unity at successively slower scan rates.

Using mixed solvent systems, therefore, we have the opportunity for studying further the behaviour of the nitro radical anion in a situation which will be of more biological relevance than a purely aprotic medium. This will enable a number of areas to undergo detailed investigation. By studying the stability of the nitro radical anion as a function of the aqueous-organic solvent composition, quantitative information can be obtained regarding the lifetimes of the $RNO_2^{\cdot-}$ moiety and its decomposition pathways.

The correlation of the half-lives of the nitro radical anions with the known biological activity of the corresponding drug, together with the reaction of $RNO_2^{\cdot-}$ with DNA and the kinetic studies of the process will be of particular interest in determining whether the one-electron reduction of nitro hetero- and homocyclic drugs results in the species responsible for DNA damage or if further reduction steps are necessary. Investigations are proceeding along these lines, and will be the subject of a separate communication.

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