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ELECTROCHEMICAL STUDIES OF NITROHETEROCYCLIC COMPOUNDS OF BIOLOGICAL INTEREST. VII. EFFECT OF ELECTRODE MATERIAL

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The electrochemical behaviour of three nitrofuran compounds, nitrofurazone, nitrofurantoin and furazolidone, has been studied in three solvent types; aprotic, aqueous and mixed, and at four working electrodes. Particular attention has focused on the 1-electron RNO_2/RNO_2^- couple as measured by the cyclic voltammetric mode. Using Hg in aqueous buffer, reduction of the NO_2 group proceeds directly to the hydroxylamine with no intermediate stages being identified. Addition of an aprotic solvent gave a 2-stage reduction, initially forming the RNO_2^- species. At all solid electrodes, however, the RNO_2/RNO_2^- couple was identified under simple aqueous conditions. The switch to a mixed aqueous/aprotic solvent medium produced only minor changes in the response compared with the situation on Hg. This presents the opportunity of using nitrofuran complexes as model systems for the redox behaviour of nitro aromatic compounds in general at solid electrode surfaces where the latters' more negative reduction potentials makes direct study difficult. The conditions have been defined whereby we can examine pH effects and RNO_2^--biological target interactions in simple aqueous media to allow the further refinement of the electrolytic model system for studying bio-reducible drug action.

KEY WORDS: Nitrofurans, electrode material, redox mechanism, nitro radical anions, radical stability.

INTRODUCTION

As part of a comprehensive study of the electrochemical properties of nitroheterocyclic compounds and the interaction between their reduction products and DNA we initially focused our attention on the nitroimidazoles,[1–5] used as radiation sensitizers and cytotoxins for hypoxic cells in cancer therapy and as antibacterial agents. An important group, receiving only cursory attention, to date is the nitrofurans. Since their discovery in 1940 they have achieved great importance medically, being used as antibacterial and antischistosomal agents and in the treatment of urinary tract infections and Chagas disease.[6,7] In addition they are used as additives to livestock feed and as wine stabilizers. Although the nitrofurans have been extensively employed for a number of years, they remain an area of active research interest,[8] with their exact mode of action being uncertain. In common with other nitroheterocycles it is assumed that reductive activation of the nitro group is a prerequisite for their biological action.

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The reduction potentials of the nitrofurans was examined in the late 1960's using dc polarography.[9] Due to the continued interest in the nitrofurans a re-evaluation of the redox characteristics of this important class of compound would therefore seem to be in order, using the range of electrochemical techniques now available, primarily cyclic voltammetry. This will also allow more detailed comparison with the other nitroheterocyclic drugs we have studied.

In developing an electrolytic model system for the bioreductive nitroheterocyclic compounds, the majority of electrochemical studies have employed a mercury working electrode. This is hardly surprising as most have been conducted in water-based systems, making Hg the material of choice for investigating reduction chemistry, given its high overpotential for hydrogen production. A typical potential range would be from approximately 0.0 to -2.0 volts (V) with a simple alkali halide as the supporting electrolyte. A Pt electrode, however, although being superior for the study of oxidation processes (in an aqueous solvent, typical anodic potential range to $+1.0$ V) yields only a very limited cathodic range, generally not exceeding -0.50 V[10].

We have already determined that changes to the electrochemical solvent alters the redox mechanism of nitroheterocycles. In a purely aqueous media, reduction progresses in a single irreversible 4-electron addition to yield the hydroxylamine derivative.[11] However, in an aprotic or mixed aqueous/aprotic medium (dimethylformamide, dimethylsulphoxide, acetone or acetonitrile) reduction takes place in two distinct stages. A reversible 1-electron addition, to give the nitro radical anion, followed by an irreversible 3-electron step, giving the hydroxylamine. As the quantity of the aprotic solvent in the mixed medium is increased, the stability of the nitro radical anion also increases.[3,4] There also appears to be a minimum quantity of aprotic solvent, dependent on the identity of the heterocyclic ring, which has to be added to the system before the two-stage redox mechanism is observed.[12] It would seem likely that the addition of the aprotic solvent influences the protonation of the nitro group.

From pulse radiolysis studies it has been shown that as the pH of the medium was lowered, the radical anion showed an increase in the rate of its decomposition by a 2nd order disproportionation pathway.[13,14] The lifetime of the radical anion was greatly extended in strongly alkaline or purely aprotic solvents. A mixed aqueous/aprotic electrolytic solvent may create a preferential environment round the nitro compound preventing protonation, thereby resulting in a more stable radical anion. From a study on the pH effects on a wide range of nitrofuran compounds it was postulated that the nitro group was protonated prior to the occurrence of the charge-transfer step. This was caused by the specific conditions existing at the mercury electrode surface.[9]

If the working electrode indeed plays a significant role in the reduction process, and does not merely function as an electron source, altering the electrode material might also influence the redox mechanism. We have investigated this by examining the cyclic voltammetric behaviour of three nitrofuran compounds, nitrofurazone, 5-nitro-2-furaldehyde semicarbazone, an antibacterial agent, furazolidone, 3-[(5-nitro-2-furanyl) methylene] amino-2-oxazolidinone and nitrofurantoin, N-(5-nitro-2-furfurylidine)-1-ammoylantoin, both used in the treatment of urinary tract infections, and the 2-nitroimidazole, misonidazole, 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol, in aqueous and aqueous/aprotic (dimethylformamide, dimethylsulphoxide and acetonitrile) systems, comparing the effects on Pt, Au, glassy carbon and Hg working electrodes. The effect of the supporting electrolyte has also been examined. Because of the relatively positive reduction potentials of the nitrofurans interference

from the limited solvent potential range in Pt-aqueous systems is of less importance. We therefore have the opportunity not only of re-examining the nitrofurans using the benefits of modern electrochemical techniques, but using their charge-transfer properties at solid electrode surfaces as a model for nitroheterocycles in general, where their more negative reduction potentials makes study in such conditions difficult.

Our attention was focused on the properties of the $RNO_2^{\cdot-}$ species, being a strong candidate as the electron addition product responsible for the biological activity of the nitro range of drugs.

MATERIALS AND METHODS

Nitrofurazone, furazolidone and nitrofurantoin were supplied from Smith Kline and French Laboratories, and misonidazole from Roche Products Ltd. All drugs were used as received without purification. Aprotic solvents (dimethylformamide, methylene chloride, acetonitrile and dimethylsulphoxide) were Analar grade or better.

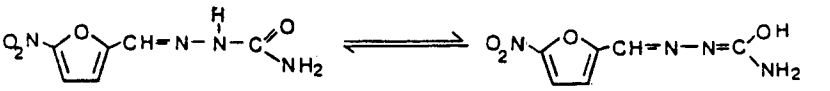
Cyclic voltammetric electrochemical experiments employed a 3-electrode configuration with a Pt wire counter electrode. Voltammetric experiments using a solid working electrode surface, Pt, Au or glassy carbon (GC), used a Metrohm E506 potentiostat interfaced with a Metrohm E612 VA Scanner in conjunction with a Hewlett Packard 7035B x-y recorder. For experiments using a hanging drop mercury electrode (PAR 303E) a PAR 264A polarographic analyzer was employed, with a Bausch and Lamb RE0088 x-y recorder. Measurements in water-based solvents used an aqueous saturated calomel electrode (SCE) whereas in aprotic solvents a non-aqueous Ag/AgCl reference electrode, against which ferrocene was oxidized at +0.60 volts was used. A range of supporting electrolytes was used, KCl (1.0 mol dm^{-3}), NaH_2PO_4, Na_2HPO_4 (0.5 mol dm^{-3}) and 1.5×10^{-2} mol dm^{-3} NaCl, 1.5×10^{-3} mol dm^{-3} trisodium citrate (0.1 SSC) buffers in aqueous media, and tetra-n-butylammonium tetrafluoroborate ($TBABF_4$) in aprotic systems. All cell solutions were thoroughly degassed with solvent-saturated N_2 prior to measurement. Drug concentrations ranged from 1×10^{-2} to 2×10^{-4} mol dm^{-3}, being typically 2×10^{-4} mol dm^{-3}. The routine scan rate was 100 mV s^{-1}, but the sweep rate was generally varied between 10 and 500 mV s^{-1}.

RESULTS

As we were primarily concerned with the measurement and analysis of the $RNO_2/RNO_2^{\cdot-}$ couple, we have confined our investigations to the cyclic voltammetric mode. With all the solid electrodes used, Pt, Au and glassy carbon (GC), adsorption onto the electrode surface was encountered, most severely for Au. Care was taken, therefore, to polish the electrode surface before each measurement. This was not a problem when using Hg, as the electrochemical system automatically generated a fresh hanging drop for each scan initiated.

Reduction potentials for the three compounds were in the typical ranges for nitrofuran complexes, being between −0.7 and −0.9 V in aprotic media and −0.2 to −0.4 V in aqueous systems, depending on the exact conditions.[9,11] In this study the actual values for the reductions were of secondary importance. Instead the character of the redox response was analyzed.

In a completely aprotic media, e.g. CH_2Cl_2, a reversible or quasi-reversible 1-electron transfer giving the nitro radical anion could be identified, irrespective of the electrode material. The return-to-forward peak current ratio, ip_r/ip_f, increased as the scan rate, v, was increased, indicative of the $RNO_2^{\cdot-}$ species undergoing a chemical following reaction, presumably disproportionation of $2RNO_2^{\cdot-} \rightarrow RNO_2 + RNO$, on the time-scale of the voltammetric experiment. Direct comparison of the ip_r/ip_f ratios would seem to suggest that nitrofurazone gave the least stable $RNO_2^{\cdot-}$ with the smallest ip_r/ip_f values. Closer inspection, however, showed that the electrochemistry of nitrofurazone was complicated by a tautomeric equilibrium which was pH dependent (equation 1).



pale yellow — blood red

acid — alkali

(1)

This was revealed as an anodic shoulder on the forward wave of the $RNO_2/RNO_2^{\cdot-}$ couple, with an accompanying colour change noted in the immediate vicinity of the working electrode. This was further verified by the addition of NaOH to an aqueous solution of the parent drug, resulting in a blood red colouration. This change was completely reversible with the addition of HCl, immediately restoring the original yellow.

The most interesting aspect was when comparing the electrode material when using aqueous and mixed aqueous/aprotic solvent systems. Using a mercury electrode in an aqueous buffer system, all three nitrofurans showed a single irreversible reduction step, corresponding to the 4-electron nitro/hydroxylamine ($RNO_2/RNHOH$) conversion. Upon the addition of an aprotic solvent, the response separated into a two-stage process, yielding the $RNO_2/RNO_2^{\cdot-}$ couple at less negative potentials, with the second reduction step being assigned to the irreversible 3-electron $RNO_2^{\cdot-}/RNHOH$ step. A well defined return wave for the 1-electron transfer was observed on the reverse scan. The ip_r/ip_f ratio increased markedly with addition of dimethylformamide, DMF, and there was an increased separation between the first and second reduction steps. Table I contains data for furazolidone which were typical of the trends observed.

Changing to a solid working electrode presented a very different situation. Figure 1 illustrates the voltammetric behaviour found for nitrofurazone on Hg and GC electrode surfaces. The 1-electron $RNO_2/RNO_2^{\cdot-}$ couple, with distinct return wave

TABLE I

Effect of DMF addition to Furazolidone in aqueous buffer at a Hg working electrode

%DMF[a]	ip_r/ip_f[b]	ΔE (mV)[c]
0	–	–
9.1	0.20	150
16.6	0.56	250
23.0	0.60	340

[a] % v:v of the DMF content

[b] ip_r/ip_f for the $RNO_2/RNO_2^{\cdot-}$ couple

[c] separation (mV) of the first and second reduction steps

could be easily identified with the GC system. This was comparable with the response on Hg but only after addition of DMF. Similar behaviour was found to that on GC when using Pt, but using Au the return wave was distended and difficult to identify accurately. This change in redox mechanism was not restricted to nitrofuran heterocycles. The same behaviour could be identified for misonidazole, a 2-nitroimidazole, using Pt and an aqueous buffer system, although due to the more negative reduction potential, the chemically reversible $RNO_2/RNO_2^{\cdot-}$ couple was largely masked by the close proximity of the solvent limit.

Addition of an aprotic solvent to the voltammetric cell incorporating a solid working electrode did not produce the dramatic changes observed with Hg. A slight increase in ip_r/ip_f ratio was found, and an increased separation of first and second reduction steps. Table II compares the electrochemical data with the addition of DMF, acetonitrile and dimethylsulphoxide, DMSO, on both Pt and GC electrode surfaces. Data for Au has not been included as the return wave for the $RNO_2/RNO_2^{\cdot-}$ couple was generally ill-defined. For comparable concentrations of aprotic solvent content in the electrolytic medium, acetonitrile would appear to be the most effective at stabilizing the $RNO_2^{\cdot-}$ by giving the greatest ip_r/ip_f ratio on both Pt and GC.

Using a mixed H_2O/DMF system, the effect of supporting electrolyte was examined. A phosphate buffer system was of little use, further limiting an already restricted potential 'window'. The use of SSC appeared to give a more stable $RNO_2^{\cdot-}$ than KCl. This might be due to the increased separation between the first and second reduction steps making it easier to chose E_λ so that the $RNO_2/RNO_2^{\cdot-}$ couple was subject to the minimum of interference from the irreversible 3-electron $RNO_2^{\cdot-}$/RNHOH step.

As a reversible 1-electron couple on both Pt and GC could be identified in an aqueous environment, the effect of pH on the stability of the nitro radical anion was examined by the addition of NaOH. The ip_r/ip_f ratio was found to increase (indicating an increase in stability) as the pH was raised. For example, nitrofurazone on GC showed an increase in ip_r/ip_f from 0.26 to 0.55 with a corresponding increase in pH from 7 to 12.

DISCUSSION

The studies reported here showed quite clearly the significant role played by electrochemical conditions in the redox behaviour of nitrofuran compounds, including the nature of the working electrode. The electrochemical behaviour in aprotic solvents of the three nitrofuran compounds examined correlated with a range of nitroaromatics in yielding a reversible or quasi-reversible $RNO_2/RNO_2^{\cdot-}$ couple followed by an irreversible 3-electron addition to give the hydroxylamine.[11] In aqueous systems at a hanging drop mercury electrode, a single 4-electron step was observed to give the hydroxylamine directly. The most important feature was in evidence only when we utilized the relatively positive reduction potentials of the nitrofurans to make use of the limited negative voltage range in water-based solvents to study their behaviour on the solid electrode surfaces of Pt, Au and GC. Despite some adsorption problems, particularly on Au, the redox character of the compounds was well resolved. Under these conditions, the $RNO_2/RNO_2^{\cdot-}$ couple could be seen *directly* (Figure 1). The addition of an aprotic solvent had little effect on the ip_r/ip_f ratio and therefore the stability of $RNO_2^{\cdot-}$ (Table II). This was entirely different from the situation found on Hg where the addition of an aprotic solvent resulted in the initial separation of the

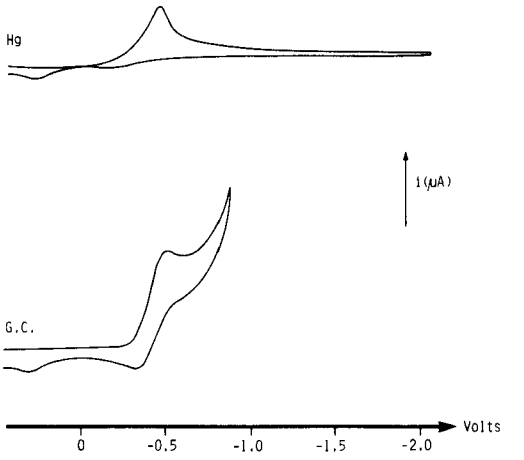


FIGURE 1 Comparison of the cyclic voltammetric response for Nitrofurazone at mercury and glassy carbon electrode surfaces.

TABLE II
The effect of aprotic solvent on the electrochemistry of Nitrofurazone at solid electrode surfaces

		Pt		GC	
% aprotic solvent		ip_r/ip_f	ΔE (mV)[a]	ip_r/ip_f	ΔE (mV)[a]
dimethylformamide	0	0.30	160	0.21	140
	16.0	0.27	190	0.22	180
	28.5	0.34	240	0.32	250
	44.0	0.31	270	0.33	290
acetonitrile	0	0.20	220	0.22	220
	13.0	0.38	220	0.32	220
	33.3	0.38	220	0.36	220
dimethylsulphoxide	0	0.20	150	0.20	160
	16.0	0.27	180	0.25	180
	44.0	0.29	230	0.35	230

[a] separation (mV) of the first and second reduction steps

reduction into a two-step process, and a marked increase in the stability of the $RNO_2^{\cdot-}$ species. This behaviour on solid electrodes was not restricted to furan heterocycles. Misonidazole behaved likewise, but because of the comparatively negative reduction potential, analysis was severely hampered by the proximity of the solvent front.

These observations were in agreement with the proposal that the irreversible 4-electron character of the nitro reduction seen on Hg was due to the specific conditions existing at the working electrode surface, in which the behaviour was caused by the pre-protonation of the NO_2 group in the diffusion layer, preceding the electron transfer step.[9] This did not occur with solid working electrodes. The role of the aprotic solvent in mixed solvent systems would therefore appear to be prevention of NO_2 protonation prior to reduction by altering the conditions at the working electrode rather than by direct reaction with the drug in the bulk solution. This would

be in line with the relatively minor influence aprotic solvent addition had on the electrode response when using Pt or GC.

We have previously established that changing the supporting electrolyte resulted in a shift in reduction potential.[11] The present work also showed that the nature of the electrolyte also influenced the separation between the first and second reduction steps and the ip_r/ip_f value. (Note, however, that the concentration of the electrolyte was of less importance.) It was also clear that various aprotic solvents could stabilize the radical anion to different degrees, with acetonitrile in the present work being the most effective. Similar observations have also been made in the study of other nitroaromatics[5] and is currently under more detailed investigation. Further insight into the nature of the changes occurring at the drug-electrode interface should arise from these studies.

The change in the redox mechanism with working electrode is important for the opportunities presented for further study. As the electrochemical character of the nitrofurans is comparable to nitroaromatics in general, the nitrofurans can be used as model compounds for the behaviour of the more difficult to reduce nitro compounds at solid electrode surfaces. Thus the electrolytically generated radical anion can be studied without the need to add an aprotic solvent to give the required redox mechanism. This will prove very useful in the study of $RNO_2^{\cdot-}$ with potential biological targets, where it is essential to keep the experimental conditions as simple (and biologically relevant) as possible. The importance of the one-electron reduction product to the biological action of the nitro drugs has been frequently proposed. The greater amount of DNA damage resulting from the electrolytic reduction of the less electron affinic drugs was assigned to differences in the relative stability of $RNO_2^{\cdot-}$[15]. More recently, this was highlighted in work by Guissani *et al.*[16] who established correlations between pharmacological properties and the rate of the $RNO_2^{\cdot-}$ disproportionation reaction, indicating that the $RNO_2/RNO_2^{\cdot-}$ couple was a key reduction step which could be finely modulated in cells. The need, therefore, for further information on the properties of the radical anion are established.

The opportunity also presents itself to examine the influence of pH on the radical lifetimes. Both electrochemical and pulse radiolysis kinetic analyses have shown a 2nd order disproportionation as the general decomposition pathway for a range of $RNO_2^{\cdot-}$ species, including the nitrofurans.[3,4,13,14] Studies on the pH-dependence by pulse radiolysis have shown that the lifetime of $RNO_2^{\cdot-}$ was greatly extended under alkaline conditions.[13,14] This has also been illustrated in the present experiments by the increase in ip_r/ip_f with addition of NaOH to the electrolytic medium. The determination of radical stability in mixed solvents as a function of pH is of limited value due to the non-reliability of pH measurements under these conditions.[17]

Acknowledgements

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