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ELECTROCHEMICAL CHARACTERISTICS OF NITROHETEROCYCLIC COMPOUNDS OF BIOLOGICAL INTEREST. VIII STABILITY OF NITRO RADICAL ANIONS FROM CYCLIC VOLTAMMETRIC STUDIES

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The stability of the one electron addition product of four biologically important nitroheterocyclic compounds has been examined electrochemically. Using cyclic voltammetry the tendency of the nitro radical anion to undergo disproportionation was studied by two methods of analysis. The first was based on determining the voltammetric time-constant required for half of the reduction product, $RNO_2^{\cdot-}$, to react further. The second concerned the minimum volume of dimethylformamide which had to be added to the aqueous electrolytic medium to give a specific cyclic voltammetric response. Both methods were found to compare well with the results obtained for $RNO_2^{\cdot-}$ stabilities using a theoretically derived procedure for a second order reaction following a charge-transfer step. The use of these alternative approaches for quantifying the reactivity of reduction products is discussed. The time-constant method in particular may be useful in studying complex reaction pathways.

KEY WORDS: Nitro radical anions, lifetimes, cyclic voltammetry.

INTRODUCTION

We are at present involved in a detailed examination of the electrochemical characteristics of a series of biologically active nitroheterocyclic compounds. These drugs are extensively used clinically, in the treatment of anaerobic infections[1] and in cancer therapy as hypoxic cell cytotoxins and radiation sensitizers.[2] Their activity is dependent on reduction of the nitro group, which may accept up to 6 electrons to give the amine. The biological target molecule is DNA, but the exact nature of the drug-DNA interaction has yet to be established. The identity of the nitro group reduction product responsible for causing DNA damage is unknown, but clearly the 1-electron reduction product, the nitro radical anion, $RNO_2^{\cdot-}$, must play an important role, whether as the damaging species itself or as an obligate intermediate in subsequent reactions or electron addition steps.

In either capacity, the lifetime of the radical anion is clearly of primary importance. We have previously shown that by using a mixed solvent system (dimethylformamide/H_2O) we can selectively generate $RNO_2^{\cdot-}$ electrochemically.[3] Using cyclic voltammetry

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(CV) as the investigation mode we can analyze the return-to-forward peak current ratio ip_r/ip_f (representing the concentration of reduced product available for re-oxidation to the original material) as a function of DMF content and the change in the potential scan rate.[4,5] This has confirmed the second order nature of RNO_2^- decomposition, in line with pulse radiolysis experiments[6-9] involving disproportionation to the nitroso derivative and the original nitro compound. Taking into account the differences between the pulse radiolysis experiments and the voltammetric measurements good agreement has been obtained for the metronidazole radical anion.[4]

There have been difficulties, however, with the CV method employed. A full analysis of the ip_r/ip_f ratio to obtain kinetic information is dependent on application of a theoretically derived working curve to derive the necessary parameters. For a second order kinetic reaction, the range of ip_r/ip_f ratios encompassed by the working curve are from 1.0 to 0.60. Experimentally, the values obtained for the RNO_2/RNO_2^- couple, particularly at low %DMF concentrations, frequently lie in the 0.60 to 0.50 region. To make full use of the available experimental data we have examined two futher procedures for quantifying the stability of nitro radical anions, also based on the variation of ip_r/ip_f as a function of DMF content of the medium.

The first is based on the determination of the voltammetric time-scale required to give an ip_r/ip_f value of 0.50. The second method is based on determining the quantity of DMF required to give a specified CV response. The merits of each are discussed and compared with the results obtained from a full kinetic analysis. In addition, the possible applications of each method are addressed with respect to situations where either the kinetics of the following chemical reaction is unknown or lies outside the limitations of the theory for first and second order reactions.

MATERIALS AND METHODS

The drugs were supplied as follows and used as received without further purification. Metronidazole and M&B 4998 from Rhone-Poulenc Ltd., nitrofurazone from Smith-Kline Beecham, and chloramphenicol from Sigma Chemical Co. Dimethylformamide, spectroscopic grade, was purchased from the Aldrich Chemical Co.

Electrochemical studies used the cyclic voltammetric, CV, mode exclusively, employing a PAR 264A polarographic analyzer interfaced with a PAR 303 cell stand with 3 electrode configuration. A hanging drop mercury electrode was used as the working surface, with a platinum wire as the counter electrode. All potentials were measured against an aqueous Ag/AgCl reference electrode. The supporting electrolyte used was 1.5×10^{-2} mol/dm^3 NaCl, 1.5×10^{-3} mol/dm^3 trisodium citrate buffer (0.1 SSC). The pH was 8.4 to 8.6, but it should be noted that there are difficulties with such measurements in mixed aqueous/aprotic solvents.[5,10] The drug concentration of 2×10^{-4} mol/dm^3 was maintained throughout.

The return-to-forward peak current ratio, ip_r/ip_f, for the reversible one-electron couple, RNO_2/RNO_2^-, was measured, varying the scan rate v from 10 to 500 mVs^{-1}. To allow the RNO_2/RNO_2^- couple to be examined with the minimum of interference from the second reduction step, $RNO_2^-/RNHOH$, the switching potential, $E\lambda$, was chosen to be 100 mV negative of the forward reduction wave peak potential.

For the determination of the time-constant at $ip_r/ip_f = 0.50$ various proportions of DMF/H_2O were used as the electrochemical solvent, expressed as % v : v of the DMF content. Initially 500 μl aliquots of DMF were added to 4 ml 0.1 SSC, up to a 50%

DMF content. It was then decided whether additional measurements were required, using 100 or 200 μl DMF additions.

To determine the minimum DMF content required to give a predetermined CV response, 100 μl aliquot additions were employed.

RESULTS

The ip_r/rp_f ratio for all four drugs was found to increase towards unity as either the DMF content or the scan rate was increased. This behaviour is in line with an irreversible chemical reaction (in this case the disproportionation of $RNO_2^{\cdot-}$) following the charge transfer step. In all cases the ip_r/ip_f data used were the average of at least three independent measurements. A variation in ip_r/ip_f of $\pm$ 5% was considered to be the maximum acceptable, otherwise the complete data set was rejected. For all linear relationships, correlation coefficients of 0.98 or better were found.

The procedures used to determine the tendency of $RNO_2^{\cdot-}$ to participate in further reactions are described below. Full details of the kinetic analysis of the CV response have been published previously[4,5] and the results are only shown here for the purposes of comparison. The half-life values are those determined at a radical anion concentration of 1×10^{-6} mol/dm^3, which is a reasonable level at physiological conditions.

Determination of Half-Life Time Constant

This method is based on the correlation between voltammetric current response and concentration of redox active species. The ip_r/ip_f ratio from the CV is therefore a direct measure of the charge-transfer product remaining for oxidation back to the original material. When there is no tendency for the reduction product, $RNO_2^{\cdot-}$, to participate in any chemical following reaction on the time-scale of the voltammetric experiment, an ip_r/ip_f value of unity results. An $ip_r/ip_f = 0.50$, therefore, represents a point where half the reduction product remains for oxidation (or where half of the product has undergone further reaction). The time-constant for the voltage sweep necessary to give an $ip_r/ip_f = 0.50$ can be viewed as being indicative of the half-life of the reduction product. In place of changing the scan rate until $ip_r/ip_f = 0.50$, (which is technically impossible with the set scan rates available) we have found the following procedure to be highly satisfactory. The time-constant was the elapsed time, t, in seconds between the appearance of the reduction (forward) peak, Ep_f, and the oxidation (return) peak, Ep_r, measured from the relationship

$$t = \Delta V/v \tag{1}$$

where ΔV is the total magnitude (irrespective of sign) of the potential change between the forward and return peaks

$$\Delta V = (Ep_f - E_\lambda) + (Ep_r - E_\lambda) \tag{2}$$

At each %DMF value, a plot of ip_r/ip_f *vs* log t was found to give a linear relationship from which the time constant for $ip_r/ip_f = 0.50$, $t_{1/2}$, could be determined graphically.

Collating the data for the complete mixed solvent range examined, a plot of %DMF *vs* log $t_{1/2}$ gave a straight line from which it was possible to extrapolate to zero

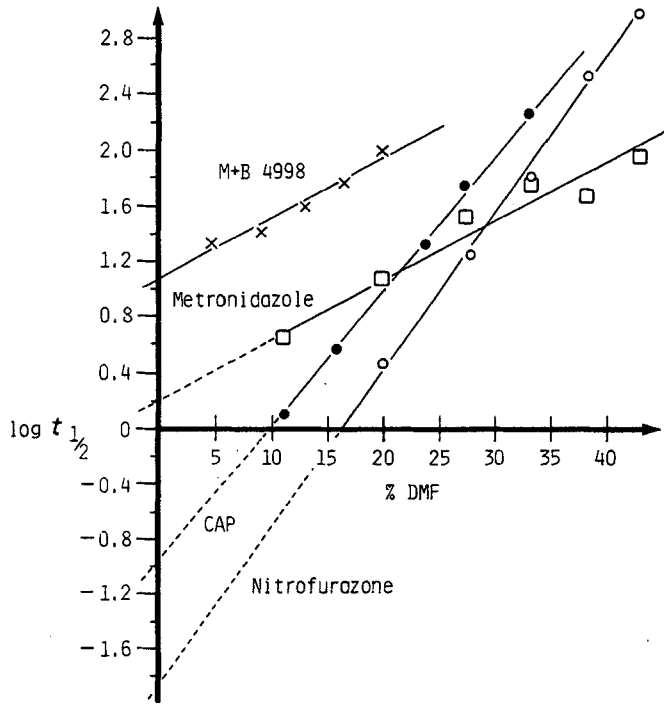

FIGURE 1 Extrapolation to determine $T_{1/2}$ at zero %DMF. Plot of log $t_{1/2}$ *vs* %DMF.

%DMF to obtain the half-life time-constant, $T_{1/2}$, of the nitro radical anion in purely aqueous media (see Figure 1). This procedure has been followed for all four drugs, with good linearity found throughout for both $t_{1/2}$ at all DMF concentrations and the half-life time-constant, $T_{1/2}$, determinations. The $T_{1/2}$ values so established are listed in Table I.

TABLE I

Comparison of the stabilities of various nitro radical anions (pH 8.4) measured by different voltammetric procedures

Drug	half-life[a] seconds	$T_{1/2}$[b] seconds	DMF[c] μl	$E_{1/2}$[d] Volts
nitrofurazone	0.0089	0.014	1,470	−0.465
chloramphenicol	2.66	0.11	950	−0.675
metronidazole	11.9	1.60	645	−0.755
M&B 4998	98	11.75	190	−0.810

[a]The half-life determined from kinetic measurements (reference 5) assuming a nitro radical anion concentration of 1×10^{-6} mol/dm^3.

[b]The time constant to give $ip_r/ip_f = 0.50$.

[c]The minimum volume of DMF required to give $ip_r/ip_f = 0.60$.

[d]The reduction potential at 20% DMF where the $E_{1/2}$ for the reversible RNO_2/RNO_2^- can be clearly distinguished.

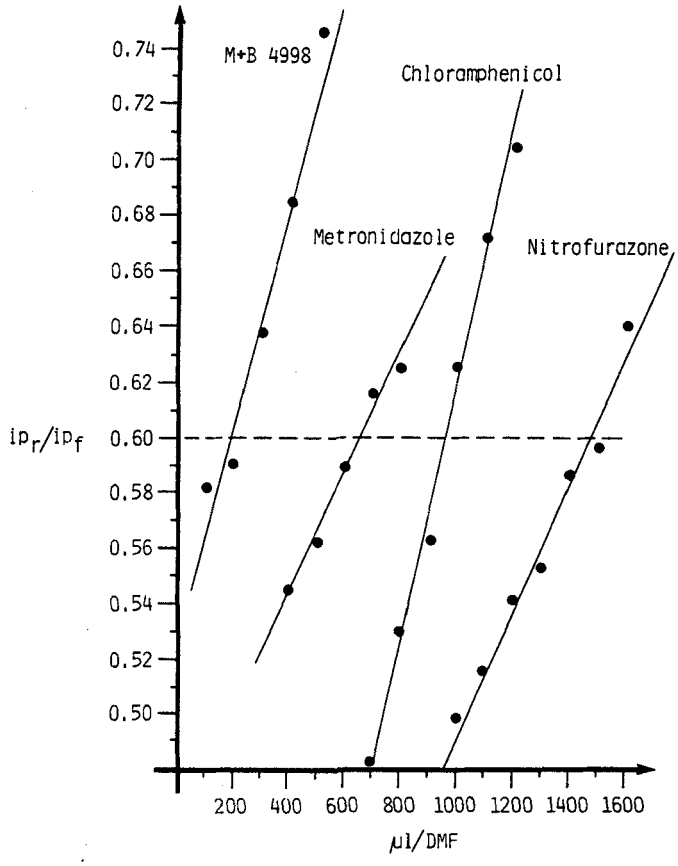

FIGURE 2 Determination of DMF required to give $ip_r/ip_f = 0.60$. Plot of ip_r/ip_f *vs* volume of DMF added (μl).

Minimum Solvent Method

As the DMF content of the electrochemical medium was increased, the tendency of $RNO_2^{\cdot-}$ to react further diminished, as shown by an increase in the ip_r/ip_f ratio towards unity. It would not seem unreasonable to hypothesize that the minimum quantity of DMF required to give a specified voltammetric response may be taken as a measure of the stability of the nitro radical anion. The smaller the quantity of DMF required, the more stable the $RNO_2^{\cdot-}$ species produced.

The ip_r/ip_f ratio chosen was 0.60, with CV parameters of E_i 100 mV more negative than Ep_f and $v = 100\,mVs^{-1}$. The ip_r/ip_f was measured between an approximate range of 0.50 and 0.65, with 100 μl additions of DMF. For all four drugs studied, a plot of ip_r/ip_f *vs* μl DMF resulted in a linear relationship from which it was possible to graphically determine the volume of DMF required for $ip_r/ip_f = 0.60$ (see Figure 2). The values obtained from this procedure are listed in Table I.

DISCUSSION

We have previously[5] shown from a full second order kinetic analysis of the CV response of the $RNO_2/RNO_2^{\cdot-}$ couple that the more electron affinic the drug (i.e. reduced at less negative potentials) the shorter the lifetime of $RNO_2^{\cdot-}$. From an examination of Table I it is clear that using the kinetic analysis and the time-constant approach described above the same order of activity is found. The second order kinetic analysis is the only procedure which has been derived from a full theoretical treatment.[11] The major drawback, however, is that only ip_r/ip_f ratios between 1.00 and 0.60 can be examined directly. As already stated, at low %DMF the ip_r/ip_f is frequently between 0.6 and 0.5. The time-constant approach makes use of the full range of ip_r/ip_f values determined experimentally. In addition, the time-constant technique retains the information regarding the sensitivity of $RNO_2^{\cdot-}$ to the aprotic content of the electrochemical medium as expressed by the slope of the $t_{1/2}$ *vs* %DMF relationship. As can be seen from Figure 1 nitrofurazone is the most sensitive, followed by chloramphenicol, M&B 4998 with the least sensitive being metronidazole. The possible significance of this behavior regarding the biological properties of $RNO_2^{\cdot-}$ is unknown. Although lacking a theoretical foundation, this method is conceptually very simple and has been successfully applied to other systems.[12] Most importantly perhaps it offers the opportunity of comparing the stabilities of charge-transfer products in at least a semi-quantitative manner where the decomposition pathway is unknown or is of sufficient complexity that a theoretical analysis is unavailable. (Only simple first and second order reactions following charge transfer have been examined fully.)

The minimum volume of DMF required to give a specific CV response is by far the quickest approach experimentally. We observe the same ordering of $RNO_2^{\cdot-}$ activity as found with a full kinetic analysis (Table I). The more electron affinic the drug, the less stable the $RNO_2^{\cdot-}$ species, and the greater volume of DMF required to produce a given CV response. It must be pointed out that focusing attention on only a small portion of the data might yield misleading results, particularly where compounds show a widely different sensitivity to DMF content.

In conclusion, to study the chemical reactions of charge-transfer products, in this case nitro radical anions, it is obviously preferable to employ a theoretically derived method. Where this is not possible, a reasonable alternative is to establish the time-constant which gives an ip_r/ip_f ratio of 0.50. This approach has the advantage of simplicity and is applicable to complex reaction pathways, possibly in comparing the natural lifetime of a reduction product with that in the presence of a likely biological target. The minimum DMF content is a rapid and easy method of approach for an initial examination of the stability of nitro radical anions.

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