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TEMPERATURE EFFECTS ON THE ONE-ELECTRON REDUCTION POTENTIALS OF NITROARYL COMPOUNDS

PETER WARDMAN* and ERIC D. CLARKE+

Cancer Research Campaign, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR, U.K.

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Pulse radiolysis was used to establish one-electron transfer equilibria between radical cations of methyl or benzyl viologens (V^{2+}) and nitroaryl compounds ($ArNO_2$): a nitroimidazole (misonidazole or metronidazole), 4-nitrobenzoate or nitrofurazone. The equilibrium constants in water at pH 8 were estimated over the temperature range ~ 5 to 75°C. The difference ΔE in mid-point one-electron reduction potentials between the nitro compounds and the viologens varied with temperature T; increasing temperature made the nitro compounds apparently less electron-affinic compared to the effects of temperature on the viologen potential. Values of $\partial(\Delta E)/\partial T$ were in the range -0.7 to $-1.1\,mV\,K^{-1}$ at 25°C. If $\partial[E(V^{2+}/V^{\cdot+})]/\partial T = -0.9\,mV\,K^{-1}$ for methyl viologen then $\partial[E(ArNO_2/ArNO_2^{\cdot-})]/\partial T$ is about $-2\,mV\,K^{-1}$ for these compounds.

KEY WORDS: Reduction potentials, viologens, nitroimidazoles, nitrobenzoate, nitrofurazone, pulse radiolysis.

INTRODUCTION

Nitroaryl compounds ($ArNO_2$) are widely used in medicine as chemotherapeutic agents with selective toxicity towards anaerobic organisms, and they have potential value in cancer therapy either because of such selective toxicity or as hypoxic cell radiosensitizers. The reduction potential E of the one-electron couple $ArNO_2/ArNO_2^{\cdot-}$ (or more accurately, the mid-point potential at pH ~ 7) controls the radiosensitization efficiency[1] and both the aerobic[2] and hypoxic[3] cell cytotoxicities of these compounds. However, measurements of the mid-point potentials are generally made at room temperature,[4,5] whilst cytotoxic responses frequently involve measurements at 37°C.[2,3] Thermodynamically-reversible one-electron reduction potentials of nitroaryl compounds in water have been obtained to date only by measurement of the equilibrium constants of electron-transfer reactions with a redox indicator using pulse radiolysis or flash photolysis,[5] and temperature effects upon such equilibria will reflect the behaviour of the redox indicator as well as that of the nitroaryl compound.

Viologens (V^{2+}), especially 1,1′-methyl- (MV^{2+}, 'paraquat') and benzyl- (BV^{2+}) 4,4′-bipyridinium compounds, have been extensively used as redox indicators for nitroaryl compounds, as illustrated in our earlier work,[4] in the accompanying paper,[6] and in an extensive compilation.[5] We report here measurements made of the effects

*To whom correspondence should be addressed.
+Present address: ICI Agrochemicals, Jealott's Hill Research Station, Bracknell, Berks., RG12 6EY.

of temperature on redox equilibria of these viologens with four representative nitroaryl compounds.

MATERIALS AND METHODS

Viologens (BDH) were re-precipitated three times from methanol by the addition of acetone. Nitrofurazone (Sigma) and 4-nitrobenzoic acid, sodium salt (BDH) was used as received; sources of all other chemicals have been described.[4] The structures of the nitro compounds are shown in Figure 1. Pulse radiolysis utilized 0.2 μs, $\sim$ 1.8 MeV electrons from a linear accelerator[7] but the spectrophotometric detection system had a short optical path[8] comprising a Philips xenon arc lamp, Hilger-Watts monochromator, and Hammamatsu R777 photomultiplier. Preliminary experiments utilized photographic recording but most measurements used a Datalab DL 905 digitizer and a PDP11/34 computer. The method of selectively generating viologen ($V^{\cdot+}$) or nitro ($ArNO_2^{\cdot-}$) radicals by radiolysis, and general experimental techniques, have been described.[4,5,6,8] Temperatures were varied with a thermostatted cell (2 cm pathlength) with Peltier-effect modulation, pre-equilibrating the solution reservoir with a thermostatted jacket.

RESULTS

Production of radicals: redox equilibria

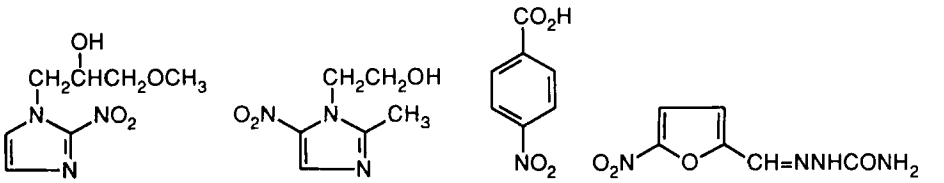
All solutions were deaerated by nitrogen-bubbling and contained 0.2 mol dm^{-3} 2-propanol to convert most of the radiolytically-produced $^{\cdot}OH$ and $H^{\cdot}$ radicals to the reductant $(CH_3)_2\,{}^{\cdot}COH$,[4,5,6,8] together with phosphate buffer (4 mmol dm^{-3}) to give pH $\sim$ 8.5. The redox equilibrium (1) was measured as described earlier:[4,6]

$$V^{\cdot+} + ArNO_2 \rightleftharpoons V^{2+} + ArNO_2^{\cdot-} \qquad (1)$$

by measurement of the absorbances at equilibria at 600 nm (where the absorptions of $ArNO_2^{\cdot-}$ are very small), calculating K_1 and hence $\Delta E_1 (= (RT/F) \ln K_1)$:

$$\Delta E_1 = E(ArNO_2/ArNO_2^{\cdot-}) - E(V^{2+}/V^{\cdot+}) \qquad (2)$$

The mid-point potentials E are similar to the standard potentials E° of the couples at this pH, since the pK of the conjugate acid of $ArNO_2^{\cdot-}$ is $\ll 8$.[9] Since K_1 will vary significantly with ionic strength,[5] corrections were made for such effects as described



(a) (b) (c) (d)

FIGURE 1 Structure of the nitroaryl compounds studied: (a) misonidazole; (b) metronidazole; (c) 4-nitrobenzoic acid; (d) nitrofurazone.

previously.[4] Doses of ~1.1–1.3 Gy (0.2 μs pulse) produced a total concentration of radicals, ($[V^{\cdot+}] + [ArNO_2^{\cdot-}]$) ~0.8 μmol dm^{-3}. Redox equilibria were established in a few microseconds as previously observed[4] and the absorbances at 600 nm at equilibrium analysed.[4,5]

Effects of temperature on solution stability, radical yield and extinction coefficient

Preliminary experiments were performed to determine whether there was any significant thermal degradation of the solutes (0.05–0.4 mmol dm^{-3} V^{2+} or $ArNO_2$ in 0.2 mol 2-propanol/4 mmol dm^{-3} phosphate in air or N_2) on holding for 1 h at 50°C or 0.5 h at 75°C. No change in the absorption spectra could be detected. The effect of temperature on the product of the yield G (μmol J^{-1}) and extinction coefficient at 600 nm, ε_{600} (m^2 mol^{-1}) of scavengeable, radiolytically-produced radicals was studied in solutions containing methyl viologen (0.5 mmol dm^{-3}). The product $G\varepsilon_{600}$ did not vary over the temperature range 5–75°C by more than ~ ±3% of the value at 20°C. Similar experiments with benzyl viologen showed only a ~4% decrease in $G\varepsilon_{600}$ over the range 5 to 75°C.

Misonidazole/viologen equilibria

The equilibration constant K_1 was measured using solutions of either MV^{2+} (0.1–0.5 mmol dm^{-3}) or BV^{2+} (0.02–0.1 mmol dm^{-3}) with misonidazole (0.05–0.5 mmol dm^{-3}). Figure 2 shows values of ΔE_1 calculated from these measurements. The curves through the data were obtained by fit of a second-order polynomial, *i.e.* the quadratic function:

$$\Delta E = aT^2 + bT + c \qquad (3)$$

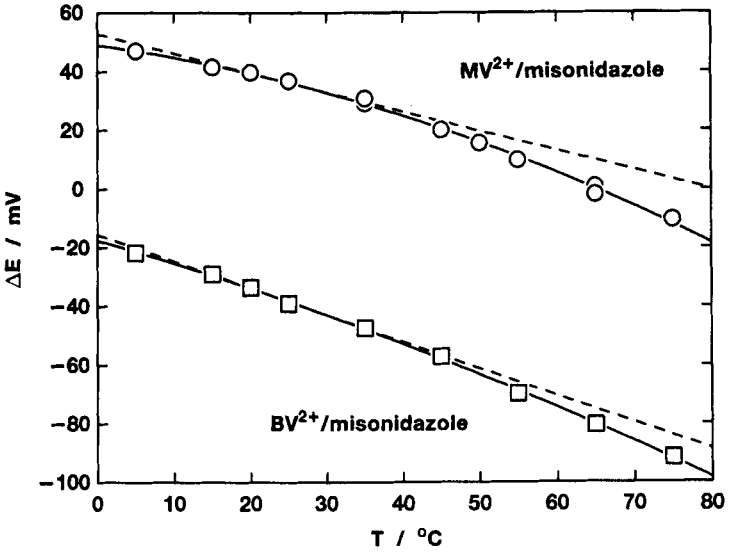


FIGURE 2 Variation of ΔE_1 with temperature for misonidazole and MV^{2+} (○), or BV^{2+} (□).

TABLE I
Effects of temperature on one-electron transfer equilibria

V^{2+}	$ArNO_2$	ΔE_1 (mV)[a]	$\partial(\Delta E_1)/\partial T$ ($mV\,K^{-1}$)[a,b]	$\partial[E(ArNO_2/ArNO_2^{\cdot-})]/\partial T$ ($mV\,K^{-1}$)[a,c]
MV^{2+}	misonidazole	36	−0.66	−1.6
BV^{2+}	misonidazole	−38	−0.92	−1.4
MV^{2+}	metronidazole	−60	−0.77	−1.7
BV^{2+}	4-nitrobenzoate	−84	−1.14	−1.6
BV^{2+}	nitrofurazone	92	−0.92	−1.4

[a] At 25°C
[b] Probable uncertainty ~ 10%
[c] If $V^{2+}/V^{\cdot+}$ couples have temperature-dependences given in text

and the slopes $\partial(\Delta E)/\partial T$ estimated for 25°C from the value of $(2aT + b)$. These are listed in Table I; the tangents at 25°C so obtained are shown in Figure 1 as dashed lines.

Metronidazole/methyl viologen equilibrium

Solutions contained MV^{2+} (0.05–0.1 mmol dm^{-3}) and metronidazole (1–2 mmol dm^{-3}). The data are given in Figure 3 and Table I.

4-Nitrobenzoate/benzyl viologen equilibrium

Solutions contained BV^{2+} (10–20 μmol dm^{-3}) and 4-nitrobenzoate (0.1–0.6 mmol dm^{-3}). Small corrections (2–4% in K_1) were made to allow for the conversion of

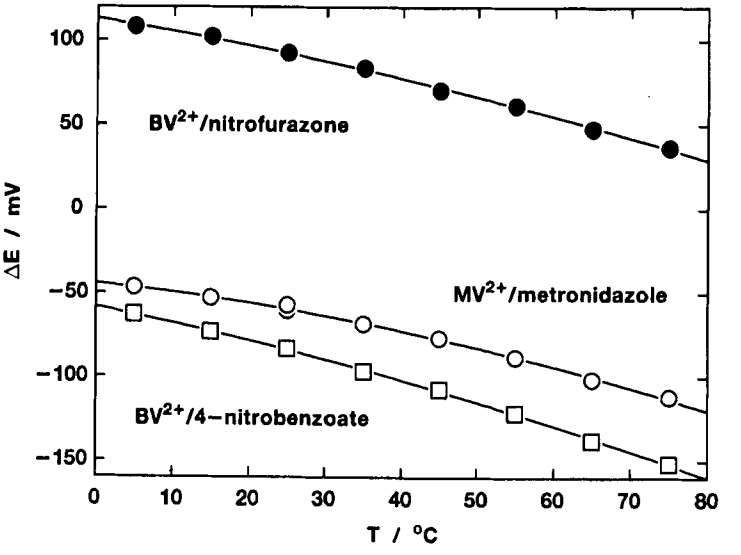


FIGURE 3 Variation with ΔE_1 with temperature for MV^{2+} and metronidazole (○), and BV^{2+} with 4-nitrobenzoate (□) or nitrofurazone (●).

0.2–0.4 μmol BV^{2+} to $BV^{\cdot+}$ at equilibrium. The data are given in Figure 3 and Table I.

Nitrofurazone/benzyl viologen equilibrium

Solutions contained BV^{2+} (0.5 mmol dm^{-3}) and nitrofurazone (25–100 μmol dm^{-3}). As above, small corrections were made to allow for the conversion of 0.4–0.7 μmol dm^{-3} nitrofurazone to its radical at equilibrium. With this nitro compound, the radical had a small absorption at 600 nm ($\varepsilon \sim 1.6\%$ that of $BV^{\cdot+}$). The data are given in Figure 3 and Table I.

DISCUSSION

Methyl viologen is an excellent redox indicator for this type of study, since there is only a small decrease in ε_{600} between 20 and 100°C (about 3%).[10] The scavengeable radical yield in N_2-saturated solutions will increase with temperature by about 10% over this temperature range, at an $^{\cdot}OH$ scavenging capacity corresponding to 0.2 mol dm^{-3} 2-propanol.[11] Thus these two variables have small and opposing temperature effects, and the product $G\varepsilon$ is sufficiently constant in this application for us to ignore its variation in calculating K_1 providing sufficient $ArNO_2$ is added to reduce the equilibrium absorption of $MV^{\cdot+}$ to (say) < 60% of that observed in the absence of $ArNO_2$. (Note an error in K_1 of 10% corresponds to an error in ΔE_1 of ~2.4 mV.)

The data in Table I shows the difference in potentials, ΔE_1 decreases with increasing temperature by ~0.7 to 1.1 mV K^{-1} at around 25°C, depending on both nitro compound and viologen. Variations of ΔE in such experiments of around 5 mV could easily occur merely from variations in ambient temperature over a (say) 5°C range, corresponding to variations in K of ~20%. Hence work of this type should at least record the experimental temperatures and recognise an appropriate contribution of temperature fluctuations to the overall uncertainty in estimates of reduction potentials. In the accompanying study,[6] redox equilibria of misonidazole with *either* benzyl *or* methyl viologens involved temperature differences of only ~0.2°C, so that the difference between BV^{2+} and MV^{2+} would not have been influenced significantly by this variable.

This study utilized 2-propanol rather than formate as $^{\cdot}OH$ scavenger because of the need to allow for ionic strength effects. The corrections necessary[4,5] vary with the particular equilibrium and ionic strength but are about 3–6 mV greater at 75°C that at 5°C. However, this is not a major contributor to the uncertainty in $\partial(\Delta E)/\partial T$ at 25°C. If only the latter parameter is of interest, it would arguably be satisfactory, or even preferable, to make this type of study using (say) 0.1 mol dm^{-3} formate as $^{\cdot}OH$ scavenger and making careful measurements of K_1 over (say) only 10–40°C, ignoring ionic strength effects.

Interpolating values of ΔE_1 for misonidazole and either MV^{2+} or BV^{2+} at 25°C (see Table I) gives a value of $E(BV^{2+}/BV^{\cdot+}) - E(MV^{2+}/MV^{\cdot+}) = 75$ mV, in excellent agreement with the value of 76 ± 3 mV derived from the studies described in the accompanying paper. These data provide estimates of $E(ArNO_2/ArNO_2^{\cdot-}) = -414$ and −412 mV for misonidazole at 25°C using reference potentials of −450 mV (MV^{2+}) and −374 mV (BV^{2+}) as now recommended,[6] and −509 mV for metronidazole. The latter is in excellent agreement with that suggested from an overview

of all the data available,[6] although a slightly higher value for misonidazole (−409 mV) was suggested. Whilst a difference of ~5 mV is very probably within the uncertainties involved (particularly since ambient temperatures probably average slightly less than 25°C), it raises the question of the variation in $E(ArNO_2/ArNO_2^{\cdot -})$ with temperature, as opposed to the variation in the measured value of ΔE with the viologen indicators.

The only study known to the authors of the thermochemical characterization of viologen/radical redox couples in water is that of Watt and Burns.[12] If we make minor corrections to their data to utilize the values of $E(V^{2+}/V^{\cdot +})$ indicated above, we arrive at slightly modified values for the standard entropy change $\Delta S_4'$ of −88 and −44 J $mol^{-1} K^{-1}$ for MV^{2+} and BV^{2+} respectively:

$$V^{2+} + 1/2\ H_2 \rightleftharpoons V^{\cdot +} + H^+ \qquad (4)$$

These changes are referred to the pH 7 hydrogen electrode, for which the authors noted $\Delta S'$ for the reaction: $1/2\ H_2 = H^+ + e^-$ is defined to be zero. Since $\partial E/\partial T = \Delta S/F$, the values correspond to $\partial(E(V^{2+}/V^{\cdot +}))/\partial T = -0.91$ and -0.46 mV K^{-1} for MV^{2+} and BV^{2+} respectively at 25°C. If we use these values, estimates for the temperature-dependence of $E(ArNO_2/ArNO_2^{\cdot -})$ are obtained as shown in Table I. In view of the problems of dimerization of $BV^{\cdot +}$ and $MV^{\cdot +}$ discussed elsewhere,[5,6] these final derivations must be viewed with considerable caution. High concentrations of viologen radicals were involved in the study of Watt and Burns,[12] and an extinction coefficient of $MV^{\cdot +}$ at 600 nm assumed which is only ~60% of that now generally accepted. A careful electrochemical determination of the temperature effect upon $E(MV^{2+}/MV^{\cdot +})$ would be of considerable value. With these reservations, the data suggest a value of $\Delta S^\circ \sim -150$ J $mol^{-1} K^{-1}$ for addition of an electron to a nitroaryl compound.

The nitro compounds studied were either neutral or essentially fully ionized at pH 7–8. Compounds with *e.g.* side chains with basic functions with $pK > 7$ are of some interest. Thus the misonidazole analogue, pimonidazole has the side-chain methoxy replaced by N-piperidino, with $pK \sim 8.8$ in the ground state. The mid-point one-electron reduction potentials of such compounds vary with pH in a well-understood manner.[5,14] Hence if the temperature coefficient of the appropriate pK (or pK's) can be estimated, the likely effects on mid-point potential from changes in prototropic equilibria can be allowed for. In general, for a monoacidic nitrogeneous base near 25°C:[13]

$$-\partial(pK/\partial T) \sim (pK - 0.9)/T(K) \qquad (5)$$

For pimonidazole, $\partial(pK)/\partial T$ would thus be expected to be $\sim -0.027\ K^{-1}$, an expectation which has been confirmed experimentally (data not shown). With slightly more complex systems such as nitroacridines it has been shown that the effect of substituents on the acridinyl pK in the ground state can predict shifts in mid-point potential:[14]

$$\Delta E_{m7} \sim \Delta E^\circ + 59.1\ \Delta pK \qquad (6)$$

and since $\partial(pK/\partial T) \sim -0.018\ K^{-1}$, $\partial(\Delta E_{m7})/\partial T$ ould be expected to have a contribution of about -1 mV K^{-1} from prototropic effects alone. A series of analogues would have quite similar values, so again such variables are not likely to be important in most biological applications of this type of data.

CONCLUSIONS

Redox equilibria of one-electron transfer reactions between viologens and typical nitroaryl compounds vary with temperature by $\sim 1\,mV\,K^{-1}$, with increasing temperature making the nitro compounds apparently less electron-affinic compared to the effects of temperature on the viologen potential. Variations in such effects with different nitro compounds over a relatively small temperature range – say from 25 to 37°C – are likely to be insignificant when potentials measured at 25°C are used in structure-activity relationships with most biological response data for 37°C, although it might be worthwhile to make the predictable corrections for the effects on reduction potential of the temperature coefficients of prototropic equilibria.

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