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THE REDUCTION POTENTIAL OF BENZYL VIOLOGEN: AN IMPORTANT REFERENCE COMPOUND FOR OXIDANT/RADICAL REDOX COUPLES

PETER WARDMAN

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

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Bipyridinium compounds such as the viologens (V^{2+}) have been used extensively as redox indicators, but radical dimerization leads to reduction potentials of the oxidant/radical one-electron couple, $E(V^{2+}/V^{\cdot+})$ varying with radical concentration: a particular problem with benzyl viologen. Pulse radiolysis has been used to characterize the one-electron transfer equilibria between methyl (MV^{2+}) or benzyl (BV^{2+}) viologen and a 2-nitroimidazole (misonidazole), and between methyl and benzyl viologens directly. Total radical concentrations were typically $\sim 0.4\,\mu mol\ dm^{-3}$ to minimize dimerization. Based on $E(MV^{2+}/MV^{\cdot+}) = -450\,mV$, a value of $E(BV^{2+}/BV^{\cdot+}) = -374\,mV$ is now recommended. The implications for other 'reference' redox couples are discussed.

KEY WORDS: Reduction potentials, benzyl viologen, methyl viologen, nitroimidazoles, misonidazole, pulse radiolysis.

INTRODUCTION

Many reactions of free radicals reflect the redox properties of the radical or its parent molecule ('ground state'). Thus for oxidants the one-electron reduction potential of the oxidant/radical couple is of interest: typical examples of relevance to biology and medicine include the mid-point potentials at pH 7 of quinone/semiquinone and nitroaryl/nitro radical oxidant/radical couples. A large number of these reduction potentials have been determined by measuring equilibrium constants of one-electron transfer reactions with redox indicators.[1] Probably the most useful of these have been bipyridinium coumpounds, the most well-known of which is 1,1′-dimethyl-4,4′-bipyridinium ('paraquat' or methyl viologen, MV^{2+}). This compound (although not its analogue benzyl viologen (BV^{2+})) and 2,2′-bipyridinium diquaternary salts are of great importance as herbicides[2] and free-radical mechanisms have long been implicated in paraquat toxicity[3] as well as in the herbicidal function.

The viologen radicals were some of the earliest to be characterized because of their stability in the absence of oxygen,[4] stability which facilitated the application of conventional electrochemical techniques to measure the one-electron reduction potentials of numerous viologen/radical couples.[1,5] Wardman and Clarke[6] used benzyl viologen not only to measure the reduction potentials of nitroimidazole/radical-anion one-electron couples, but also to provide independent estimates of potentials for quinone/semiquinone couples for duroquinone and 9,10-anthraquinone-2-sulphonate. These and other quinones have also been widely used as redox indicators for oxidant/radical couples,[1] and hence the values of many such reduction potentials rely ultimately on the value assumed for benzyl viologen, $E(BV^{2+}/BV^{\cdot +})$. It was subsequently recognised[1] that the value previously taken for this reference couple could be in error by *ca.* 20–30 mV because electrochemical measurements may utilize conditions such that the radical dimerization equilibrium (1) has a significant influence on the measured mid-point potential:

$$(BV^{\cdot +})_2 \rightleftharpoons BV^{\cdot +} + BV^{\cdot +} \qquad (1)$$

The colour changes associated with equilibrium (1) were noted by Michaelis[1] and an estimate of $K_1 = 20 \pm 5\,\mu mol\,dm^{-3}$ in 0.1 mol dm^{-3} phosphate, pH 7.5 has been reported.[7] It is possible to avoid the effects of dimerization by measuring redox equilibria where concentrations of $BV^{\cdot +}$ are typically 0.2 μmol dm^{-3}, by generating the radicals by pulse radiolysis and measurement by kinetic spectrophotometry.[1] The experiments described below utilize this approach to provide an estimate of $E(BV^{2+}/BV^{\cdot +})$ by reference to the more reliable value of the couple $E(MV^{2+}/MV^{\cdot +})$; the dimer of $MV^{\cdot +}$ has a dissociation constant *ca.* 100-fold higher than K_1, so dimerization is much less of a problem with methyl viologen.[1,8] (Most experimental values of E described in this paper are mid-point potentials, usually close to pH 7, rather than standard potentials, E° which refer to conditions of unit activity of both V^{2+} and $V^{\cdot +}$.[1] For simplicity we use E here mostly without annotation.)

MATERIALS AND METHODS

Methyl viologen (Sigma) was either used as received as the dichloride trihydrate salt or purified three times by dissolving in methanol and precipitation with acetone, then drying the anhydrous salt (which was hygroscopic) *in vacuo* over silica gel at room temperature. Benzyl viologen dichloride (Sigma) was recrystallized three times from ethanol: 2-propanol (1:1 v/v) and dried as above. Methyl viologen concentrations were corrected (up to *ca.* 3%) for variable hydration using an extinction coefficient at 258 nm, $\varepsilon_{258} = 2.07 \times 10^3\ m^2\,mol^{-1}$ ($2.07 \times 10^4\,dm^3\,mol^{-1}\,cm^{-1}$). The purified benzyl viologen gave $\varepsilon_{260} = 2.25 \times 10^3\ m^2\,mol^{-1}$. Misondidazole (compound **4** in the earlier study,[6] 2.3.35 in the compilation[1]) was a generous gift from Roche Products Ltd. Sodium formate (Merck, zur Analyse), phosphate buffers (BDH, Aristar) and potasium thiocyanate (BDH, Analar) were used as received. Gases were obtained from BOC: 'oxygen free' nitrogen or 'zero grade' nitrous oxide. Water was purified by double-pass reverse osmosis and a 'Milli-Q' unit (Millipore), and solutions for pulse radiolysis handled using a syringe system.[9]

Pulse radiolysis utilized 3.5 MeV electron pulses from a Van de Graaff generator. The spectrophotometric detection system[10] used a xenon arc analysing source with synchronized electronic shutter and appropriate high-bandpass filters before the 2 cm pathlength cell, a Spex 'Minimate' monochromator (single-pass) and a Hammamatsu R777 photomultiplier with signal acquisition using a Tektronix 7612 digitizer. Typically, the signals from 8–10 pulses, each pulse using a fresh solution, were averaged before analysis using a MicroVAX computer. Pulses were monitored using a toroidal dose monitor[11] calibrated with N_2O-saturated 10 mmol dm^{-3} KSCN using the product of yield G (mol J^{-1}) and extinction coefficient ε_{472} (m^{2}mol^{-1}) = 4.81×10^{-4} m^2 J^{-1} (= 4.64×10^4 molec (100 eV)$^{-1}$ dm^3 mol^{-1} cm^{-1}),[12] assuming all solutions had unit density. Conventional absorption spectra were recorded using a Hewlett-Packard HP8452A diode-array spectrophotometer.

RESULTS

Production of Radicals

All solutions (except the dosimeter) were deaerated by N_2-bubbling and contained 0.1 mol dm^{-3} sodium formate and 10 mmol dm^{-3} phosphate (KH_2PO_4/Na_2HPO_4). Formate converts the radiolytically-produced $^{\cdot}OH$ and $H^{\cdot}$ radicals in $< 0.2\,\mu s$ to the reductant $CO_2^{\cdot -}$, which together with e_{aq}^- reduces the viologens (V^{2+}) or nitroimidazole $ArNO_2$ (if present) to the corresponding radicals $V^{\cdot +}$ or $ArNO_2^{\cdot -}$. Under the conditions used, production of the viologen or nitro radicals is complete in *ca.* 1 μs.[13,14] The yield[15] of radicals in N_2-saturated solutions of 0.1–1 mmol dm^{-3} V^{2+} or $ArNO_2$ in 0.1 mol dm^{-3} formate will be *ca.* 0.68 μmol J^{-1} so with the dose used in this work (typically 0.6 Gy = 0.6 J kg^{-1}), *ca.* 0.4 μmol dm^{-3} total radicals was produced. In the absence of oxygen $V^{\cdot +}$ is stable (radiolytic H_2O_2, ~ 40 nmol dm^{-3}, will not react with $V^{\cdot +}$ in the ~ 100 μs observation period[16]), and at pH 7.7 the misonidazole radical anion has a half-life of ~ 3 s.[17]

Redox Equilibria

In mixtures of two viologens, or of a viologen with a nitroimidazole, the initial radical distribution will reflect kinetic competition and depend on the relative concentrations of the solutes and reactivity towards $e_{aq}^-/CO_2^{\cdot -}$. However, with mixtures of two oxidants (e.g. A and B) there may then be rapid thermodynamic control and adjustment to an equilibrium:

$$A^{\cdot -} + B \rightleftharpoons A + B^{\cdot -} \qquad (2)$$

In the case of BV^{2+}/misonidazole, redox equilibration was known to take only a few microseconds under the conditions used,[6] and equilibration betweeen MV^{2+} and BV^{2+} was expected to be similarly rapid.[18] Hence K_2 can be calculated from absorbances or optical densities, D (at a constant dose, or $G\varepsilon$) at a convenient wavelength of the individual radicals, D_A, D_B and with mixtures at equilibrium, D_{eq}:

$$K_2 = ([A]/[B])\,[(D_A - D_{eq})/(D_{eq} - D_B)] \qquad (3)$$

In earlier work,[6] K_2 was calculated using eqn. (3) and conditions such that D_{eq} was typically 20–60% of $(D_A - D_B)$. This places undue weight on individual values D_A, D_B when several solutions yielding a range of values of D_{eq} are used. In the present work, K_2 was calculated by re-arranging eqn. (3) to:

$$D_{eq} = [D_A + K_2 D_B([B]/[A])]/[1 + K_2([B]/[A])] \qquad (4)$$

and fitting the data to eqn. (4) by non-linear least-squares as implemented by RS/1 software (BBN Software Products Corp.), with K_2, D_A and D_B as adjustable parameters. The rate of approach to equilibrium, k_{obs} was analysed (where possible) using:

$$k_{obs}/[A] = k_2([B]/[A]) + k_{-2} \qquad (5)$$

and calculating $K_2 = k_2/k_{-2}$.[1,6]

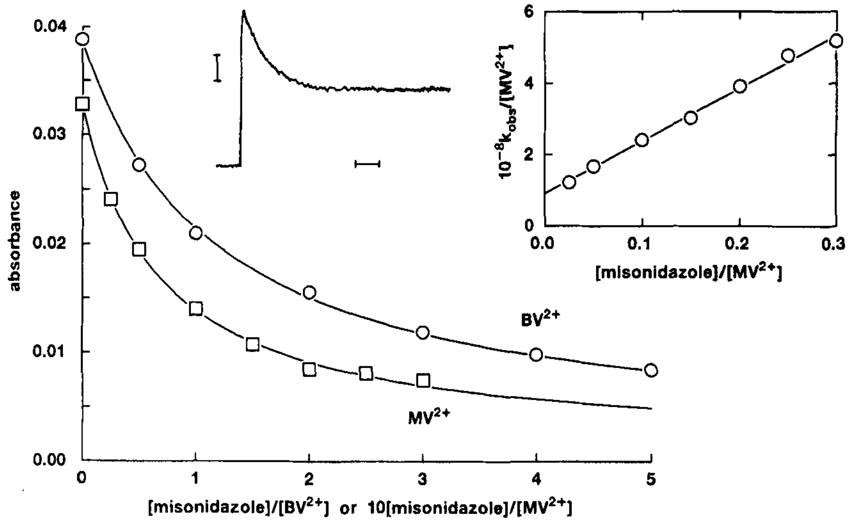

FIGURE 1 Redox equilibria between misonidazole and either MV^{2+} or BV^{2+}. Absorbances (D_{eq}) at 400 nm ~ 50 μs after a 0.6 gy pulse. Curves fitted using eqn. (4). Inset: rate of approach to equilibrium k_{obs} analysed according to eqn. (5). Typical transient: 0.005 absorbance/division, 4 μs/division (marked intervals) after pulse radiolysis of MV^{2+} (1 mmol dm^{-3}) and misonidazole (0.1 mmol dm^{-3}).

Viologen/Misonidazole Equilibria

Redox equilibria between V^{2+} and misonidazole (ArNO$_2$) were studied:

$$V^{\cdot+} + ArNO_2 \rightleftharpoons V^{2+} + ArNO_2^{\cdot-} \qquad (6)$$

Solutions at 23°C, pH 7.7 contained formate/phosphate (see above) and *either* BV^{2+} (0 or 0.1 mmol dm^{-3}) with ArNO$_2$ (0 or 0.05–0.5 mmol dm^{-3}) *or* MV^{2+} (0 or 1 mmol dm^{-3}) with ArNO$_2$ (0 or 0.025–0.3 mmol dm^{-3}). Absorbances D_{eq} at 400 nm (bandwith, $\Delta\lambda$ ~ 7 nm) were measured ~ 50 μs after a 4 ns, ~ 0.6 Gy pulse and are shown in Figure 1. (For illustation, (as for calculation) absorbances are normalised to a constant dose; the dose variation over such experiments was typically ± 3%.) Figure 1 also includes a typical transient recording and the kinetic analysis in the MV^{2+}/ArNO$_2$ system according to eqn. (5).

Applying eqn. (4) a value of $K_6 = 0.85 \pm 0.03$ was obtained for BV^{2+}/misonidazole and $K_6 = 14.8 \pm 0.7$ estimated for MV^{2+}/misonidazole. The ratio of these values gives an estimate of $K_7 = 17.4 \pm 0.9$ for the redox equilibrium between MV^{2+} and BV^{2+}:

$$MV^{\cdot+} + BV^{2+} \rightleftharpoons MV^{2+} + BV^{\cdot+} \qquad (7)$$

Kinetic analysis of the data for MV^{2+}/misonidazole gave $k_6 = (1.5 \pm 0.1) \times 10^9$, $k_{-6} = (9 \pm 1) \times 10^7\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$ ($K_6 = 16 \pm 2$), in agreement with the equilibrium absorbance analysis. With BV^{2+}/misonidazole, the absorbance displacement from kinetic to thermodynamic control was too small for reliable kinetic analysis, although kinetic data at low ionic strength were obtained earlier.[6]

It should be stressed that all the above measurements relate to high ionic strength (I) (~ 0.12), and that k_{-6} and K_6 will increase and decrease, respectively, as the ionic strength is decreased. Thus we measured earlier $K_6 = 0.35 \pm 0.01$ for BV^{2+}/misonidazole at $I = 0.004$,[6] monitoring at 600 nm and using doses of ~ 2.6 Gy.

Methyl/benzyl Viologen Equilibria

Direct measurement of K_7 proved possible because of small differences detected in the adsorption spectra of $MV^{\cdot+}$ and $BV^{\cdot+}$. The latter is, of course, apparently concentration-dependent because of dimerization, eqn. (1). Spectra of both were recorded on a diode-array spectrophotometer (bandwith, $\Delta\lambda = 2$ nm, which approximates to the practical lower limit of pulse radiolysis detection at high sensitivity). Formate/phosphate solutions containing either MV^{2+} or BV^{2+} (0.5 mmol dm^{-3}) were irradiated after N_2-saturating, using a ^{60}Co γ-radiation source at a dose rate of ~ 1 Gy min^{-1} and a cell with 10 cm pathlength. Adequate absorbances were recorded after ~ 1 Gy dose, i.e. $[V^{\cdot+}] \sim 0.7\ \mu mol\ dm^{-3}$.

Normalization of the spectra of MV^{2+} and BV^{2+} recorded under these conditions, to equal absorbances of 602 nm, demonstrated negligible difference in the shapes of the spectra between 450 and 800 nm. However, separate pulse radiolysis experiments (4 ns, 0.56 Gy) of similar solutions (0.25 mmol dm^{-3} V^{2+}) with $\Delta\lambda \sim 5$ nm showed the value of ε_{602} was 11 ± 3% higher for $BV^{\cdot+}$ compared to $MV^{\cdot+}$. Making this correction, the normalized spectra, and the difference between those of $MV^{\cdot+}$ and $BV^{\cdot+}$, were calculated and are shown in Figure 2 for the 370–420 nm region. Such differences will be very sensitive to bandwith and monochromator calibration, and after preliminary experiments, 404 nm/$\Delta\lambda \sim 3$ nm was selected for pulse radiolysis measurements. Solutions at 24°C, pH 7 containing formate/phosphate and MV^{2+} (0 or 1 mmol dm^{-3}) with BV^{2+} (0 or 0.015–0.105 mmol dm^{-3}) were irradiated (4 ns, 0.58 Gy). Results from absorbance measurements are shown in Figure 3 for the set of experiments using methyl viologen as received. The experiments were repeated using re-precipitated methyl viologen (see Materials and Methods). Similar results were obtained: $K_7 = 20.2 \pm 1.2$ and 20.7 ± 1.8 (from absorbance, untreated and precipitated vio-

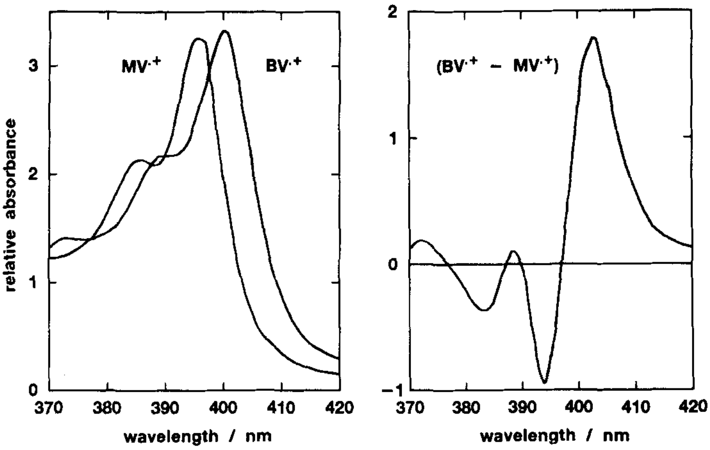

FIGURE 2 Relative extinction coefficients of $MV^{\cdot+}$, $BV^{\cdot+}$ and ($BV^{\cdot+} - MV^{\cdot+}$), relative to $\varepsilon_{602}(MV^{\cdot+}) = 1$.

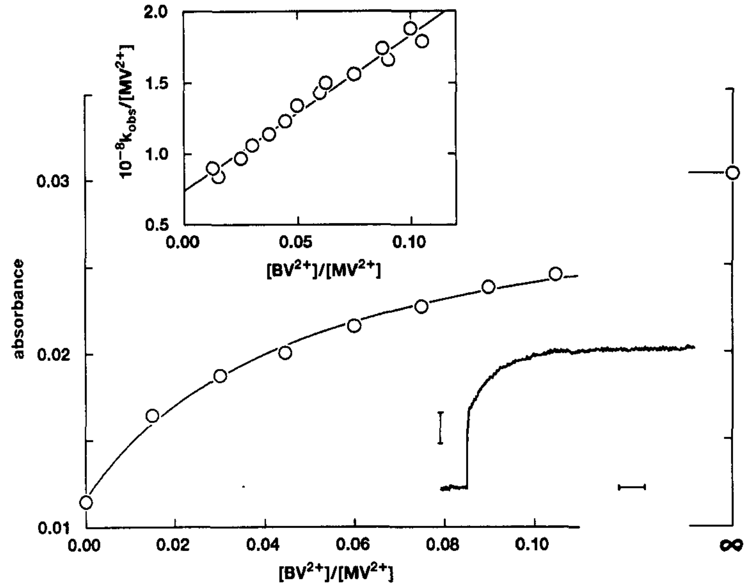

FIGURE 3 Redox equilibria between MV^{2+} and BV^{2+}. Absorbances, (D_{eq}) at 404 nm, about 50 μs after a 0.6 Gy pulse, and rate of approach to equilibrium, k_{obs}. Curve and line fitted using eqns. (4) and (5). Typical transient: 0.005 absorbance/division, 6 μs/division (marked intervals) after pulse radiolysis of MV^{2+} (1 mmol dm^{-3}) and BV^{2+} (0.075 mmol dm^{-3}).

logen, respectively). The pooled data gave estimates of $k_7 = (1.09 \pm 0.05) \times 10^9, k_{-7} = (7.4 \pm 0.3) \times 10^7$ dm^3 mol^{-1} s^{-1}, $K_7 = 15 \pm 1$ from the kinetic analysis.

Timescale of Dimerization

A value of $K_1 = 20\,\mu mol\ dm^{-3}$ at high ionic strength[7] implies ~ 98% of the radicals may be in the monomer form at $[BV^{\cdot+}] \sim 0.2\,\mu mol\ dm^{-3}$, as in the typical equilibrium situations in the present work. More radicals will be dimerized at higher doses, but less at low ionic strengths. However, in addition to the equilibrium dimer concentration, of direct relevance to pulse radiolysis observations of redox equilibria is the timescale of approach to equilibrium.

A kinetic treatment of equilibrium (1) is complex for large perturbations, but to obtain approximate estimates of k_1 and k_{-1}, repeated 100 ns, ~ 11 Gy pulses were given at intervals of ~ 30 s to a formate/phosphate solution of BV^{2+} (0.5 mmol dm^{-3}) at pH 7, 25°C. Increases in absorbance at 540 nm were seen on a timescale of tens of microseconds following the initial production of $BV^{\cdot+}$ in < 1 μs, and decreases seen at 604 and 700 nm, as expected[7] and noted earlier.[6] The extinction coefficients of monomer and dimer are reported to be similar at 604 nm,[7] and at this wavelength the steady-state transmission of the solution steadily decreased with each pulse approximately as expected from the radicals introduced and the extinction coefficients, and the rate of equilibration increased on pulsing. Analysing the approach to equilibrium exponentially, the fifth pulse (i.e. pre-irradiated to ~ 43 Gy) yielded $k_{obs} \sim 7.5 \times 10^4 s^{-1}$ from the ~ 40% decrease in absorbance at 604 nm over ~ 80 μs.

If we equate this value to $k_{-1}[\mathrm{BV}^{\cdot +}] + k_1$ (the relaxation kinetics solution for small perturbations) and set $[\mathrm{BV}^{\cdot +}] \sim 30\,\mu\mathrm{mol\ dm^{-3}}$ and $K_1 \sim 20\,\mu\mathrm{mol\ dm^{-3}}$, then we obtain a *very approximate* estimate of $k_{-1} \sim 2 \times 10^9\ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$ at ionic strength ~ 0.11.

Prediction of $E(V^{2+}/V^{\cdot +})$ from Linear Free Energy Relationships

White has shown that the reduction potentials of derivatives of methyl viologen (i.e. replacing one or both CH_3 groups by CH_2R) could be correlated with the Taft's σ^* substituent constant of the group R.[19] The inductive constant $\sigma_I(\mathrm{R})$ ($\sim 0.45\,\sigma^*$ $(\mathrm{CH_2R})$) is probably a better measure of purely inductive effects,[20] and a predictive equation of the form:

$$E(\mathrm{V}^{2+}/\mathrm{V}^{\cdot +}) = E(\mathrm{MV}^{2+}/\mathrm{MV}^{\cdot +}) + \rho\ \Sigma\sigma_I(\mathrm{R}) \qquad (8)$$

was fitted to literature data[1] for 36 mono- and di-substituted derivatives of MV^{2+}.[21] If we omit from the data set values for MV^{2+} ($\mathrm{R} = \mathrm{H}$, $\sigma_I = 0$) and mono- and di-benzyl (BV^{2+}) viologens, least-squares fit to eqn. (8) yields predictions for $E(\mathrm{MV}^{2+}/\mathrm{MV}^{\cdot +}) = -444 \pm 4\,\mathrm{mV}$ and $E(\mathrm{BV}^{2+}/\mathrm{BV}^{\cdot +}) = -383 \pm 11\,\mathrm{mV}$ if $\sigma_I(C_6H_5) = 0.12 \pm 0.02$.[20]

DISCUSSION

Reduction Potential of Benzyl Viologen

The equilibrium constant K_7 was estimated both via equilibria of the two viologens with a common redox partner and by direct observation. The overall absorbance changes observed in the former route were greater than the latter, but the former approach involves two sets of experiments. When possible, kinetic determination of equilibrium constants agreed fairly well with measurements from equilibrium absorptions, although the latter are usually considered more accurate. One source of uncertainty is the use of high ionic strengths. The use of formate rather than 2-propanol was considered preferable in this work because of the formation of $\sim 15\%$ of $(^{\cdot}CH_2)(CH_3)CHOH$ from the reaction of $^{\cdot}OH$ with $(CH_3)_2CHOH$.[22] This unwanted radical is generally neglected in this type of study; the uncertainty introduced by using 2-propanol is often less than the implications of using formate, since ionic strength corrections can be quite significant.[1] However, kinetic salt effects on the measured values of K_7 would be expected to be small because of the equality of the products of charge on reactants and products, and effects of K_6 would tend to cancel out when data for both viologens are used to estimate K_7.

Taking $K_7 = 19 \pm 2$, $\Delta E_7 = E(\mathrm{BV}^{2+}/\mathrm{BV}^{\cdot +}) - E(\mathrm{MV}^{2+}/\mathrm{MV}^{\cdot +}) = (RF/F)\ln K_7 = 76 \pm 3\,\mathrm{mV}$. Numerous values of $E(\mathrm{MV}^{2+}/\mathrm{MV}^{\cdot +})$ have been reported; a selection of 30 measuresments are listed.[1] These have a mean value of $-448\,\mathrm{mV}$ (s.d. $12\,\mathrm{mV}$) but only 16 values lie with the 99% confidence limits of the mean (-441 to $-454\,\mathrm{mV}$). Taking this range we obtain a mean value of $-447\,\mathrm{mV}$; the standard error of this mean is only 1 mV, but this is obviously a false estimate of the uncertainty. There are systematic errors associated with such measurements which are not yet resolved; thus concentration-dependent midpoint potentials for *methyl* viologen using cyclic voltammetry have been reported, as well as a weak adsorption component during polarography.[23] It can be calculated[1] that if conditions are used e.g. such that the dissociation constant of the $(MV^{\cdot +})_2$ dimer is 2 mmol dm^{-3} and the sum of the reductants is only 0.1 mmol dm^{-3}, the mid-point potential will be ~ 2 mV more positive than $E°(MV^{2+}/MV^{\cdot +})$. Taking all these factors into account, the author feels justified in now recommending as 'standard' $E°(MV^{2+}/MV^{\cdot +}) = -450$ mV and hence $E°(BV^{2+}/BV^{\cdot +}) = -374 \pm 3$ mV (where the uncertainty ignores a contribution from the uncertainty in the methyl viologen value).

Both these values are consistent with the linear free energy relationship characterizing the effects of substituents on the reduction potentials of > 30 derivatives of methyl viologen. Another 'low' value (−380 mV) for benzyl viologen has been previously derived[24] relative to −446 mV for methyl viologen, but involving assumptions and using a system less well-definied that that used in the present work. In view of the widespread use of methyl viologen in diverse studies, attention is drawn to the need for careful electrochemical measurements to resolve the uncertainties noted above.

Sources of Potential Error in the use of Viologens as Redox Indicators in Pulse Radiolysis and other Studies

Radical dimerization, clearly a problem with some electrochemical techniques, is very much less with methods based on sensitive, time-resolved spectrophotometry. Ignoring dimer dissociation, since $k_{-1} \sim 2 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$ at $I \sim 0.1$, the first half-life for formation of the dimer in pulse radiolysis experiments involving production of 0.5 µmol dm^{-3} $BV^{\cdot +}$ will be ~ 500 µs. Redox equilibria of oxidants with viologens are often complete in 5–50 µs,[6] i.e. well before equilibrium (1) is established. However, other factors must be considered. Variation in the total yield of scavengeable, radiolytically-produced radicals with solute concentration is usually ignored in this type of work, but is well documented[15] and is unlikely to be a significant source of error in the present work. Temperature variations are similarly usually ignored, but as noted earlier[1] and discussed in detail elsewhere,[25] could be the source of systematic error of a few millivolts in measuring redox equilibria using the pulse radiolysis method. The analysis above of literature data for $E(MV^{2+}/MV^{\cdot +})$ ignores temperature variations, as well as errors from correcting reference electrodes to the hydrogen scale. In addition to radical dimerization, aggregation of the ground state (MV^{2+}) of methyl viologen has been reported at concentrations of ~ 10 mmol dm^{-3} in water.[26]

Interaction of MV^{2+} with anions may also be a problem.[27,28] Variability in water content of samples of $MVCl_2$ is not particularly important, since an uncertainty of ~ 5% in the MV^{2+} concentration only influences ΔE by ~ 1 mV. A more serious problem in some applications is potential association of viologens with macromolecules. Thus a high binding affinity of MV^{2+} for DNA has been measured:[29] this is reflected in an unusually high efficiency of quenching by MV^{2+} of delayed fluorescence from acridine orange bound to DNA,[30] also seen (to a lesser extent) with BV^{2+} (data of Mr. J. White, unpublished work). Such association was also demonstrated by pulse radiolysis some years ago.[31] Hence these viologens are probably unsuitable redox indicators for systems involving this type of macromolecule, unless the effects of such interactions are carefully considered. Another problem with viologens as redox indicators is the propensity towards rapid (approaching diffusion-controlled) oxidation of the radical $V^{\cdot +}$ by some other radicals present in redox equilibria, e.g. semiquinones,[6] but this is also minimized by low radical concentrations and readily detected by kinetic spectrophotometry.

Reduction Potentials of other Couples often used as References

In the earlier study,[6] mid-point potentials at pH 7 of duroquinone, $E(\mathrm{DQ/DQ^{\cdot -}})$ and 9,10-anthraquinone-2-sulphonate, $E(\mathrm{AQS^-/AQS^{\cdot 2-}})$ were estimated by equilibration with $\mathrm{BV^{2+}}$:

$$\mathrm{BV^{\cdot +} + Q \rightleftharpoons BV^{2+} + Q^{\cdot -}} \tag{9}$$

but assuming $E(\mathrm{BV^{2+}/BV^{\cdot +}}) = -354\,\mathrm{mV}$. Our estimate[6] of $\Delta E_9 = 110 \pm 4\,\mathrm{mV}$ for DQ has been closely bracketed by two subsequent measurements[1] and a value of $E(\mathrm{DQ/DQ^{\cdot -}}) = -264 \pm 5\,\mathrm{mV}$ is now recommended. For $\mathrm{AQS^-}$, we measured $\Delta E_9 = -21 \pm 5\,\mathrm{mV}$ (similarly subsequently substantiated[1]) and $\Delta E_{10} = 132.6 \pm 1.4\,\mathrm{mV}$:

$$\mathrm{AQS^{\cdot 2-} + DQ \rightleftharpoons AQS^- + DQ^{\cdot -}} \tag{10}$$

Taking these and other relevant measurements[1] into account a value of $E(\mathrm{AQS^-/AQS^{\cdot 2-}}) = -396 \pm 6\,\mathrm{mV}$ is recommended (but note the suggested uncertainties do not include any contribution from the uncertainty in $E(\mathrm{MV^{2+}/MV^{\cdot +}}) = -450\,\mathrm{mV}$, upon which value all these recommendations are based). These recommendations based on experimental determination of K_9 and K_{10} are considered to be more reliable than, but greatly strengthened by, the earlier calculations[1] from values of $E(\mathrm{Q/QH_2})$ and semiquinone formation constants, which often involve considerable potential effects of ionic strength and/or co-solvent.

The revised recommendation for $E(\mathrm{DQ/DQ^{\cdot -}}) = -264\,\mathrm{mV}$ is 20–30 mV lower than that assumed by earlier authors in using similar data for ΔE_{11} to estimate $E(\mathrm{O_2/O_2^{\cdot -}})$:[1,32,33]

$$\mathrm{DQ^{\cdot -} + O_2 \rightleftharpoons DQ + O_2^{\cdot -}} \tag{11}$$

If $\Delta E_{11} = 85 \pm 10\,\mathrm{mV}$,[32,33] then $E(\mathrm{O_2[1\,mol\,dm^{-3}]/O_2^{\cdot -}}) = -179 \pm 11\,\mathrm{mV}$ and $E^\circ(\mathrm{O_2/O_2^{\cdot -}}) = -350 \pm 11\,\mathrm{mV}$.

CONCLUSIONS

Based on $E(\mathrm{MV^{2+}/MV^{\cdot +}}) = -450\,\mathrm{mV}$, estimates are now made of the mid-point potentials at pH 7 in water at ~25°C for the one-electron couples $\mathrm{BV^{2+}/BV^{\cdot +}} = -374\,\mathrm{mV}$, $\mathrm{DQ/DQ^{\cdot -}} = -264\,\mathrm{mV}$, $\mathrm{AQS^-/AQS^{\cdot -}} = -396\,\mathrm{mV}$ and $\mathrm{O_2(1\,mol\,dm^{-3})/O_2^{\cdot -}} = -179\,\mathrm{mV}$. The corresponding recommendations for misonidazole and metronidazole are -409 and $-507\,\mathrm{mV}$ respectively. The absolute uncertainty in any of these estimates is probably at least ± 10 mV. Several linear free-energy relationships have been derived from literature data[1] to predict reduction potentials of important classes of oxidant/radical one-electron couples.[21] These are now being refined to take into account these new recommendations.

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