New insights into mechanisms of dye degradation by one-electron oxidation processes

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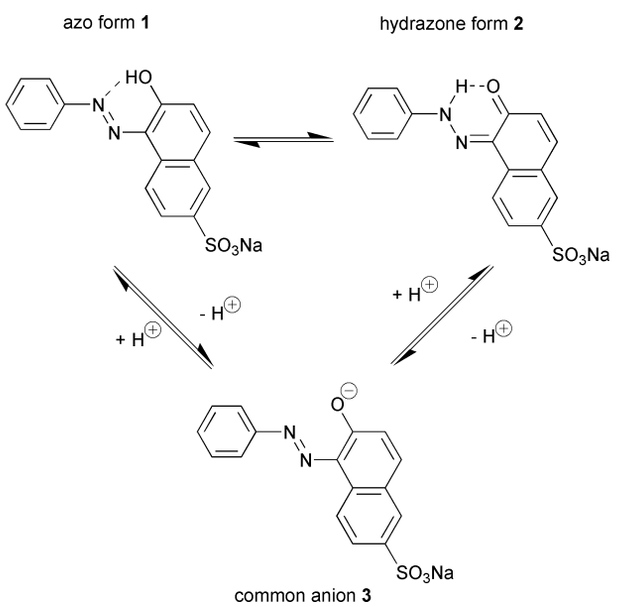
The one-electron transfer oxidation of a series of arylazonaphthol dyes has been investigated by pulse radiolysis techniques. These techniques have been used to measure dye redox potentials and to study the initial phases of dye degradation by detecting and following the fate of reaction intermediates. It is shown that bimolecular reaction of dye radicals generated by one-electron oxidation of the common anion represents a novel and generic route for bleaching of azo dyes. Dye degradation by enzymes such as peroxidase or laccase that also generate dye radicals is likely to proceed by the same route.

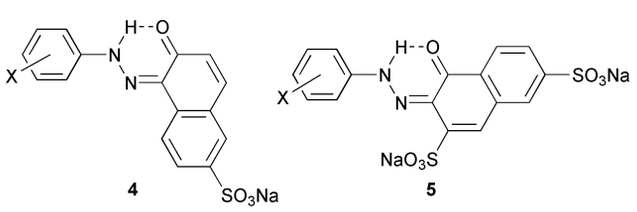
Introduction

Despite the fact that the high extinction coefficients of dyes render them amenable to scientific investigation by UV-Vis spectroscopy, key mechanistic features of the reactive chemistry of azo dyes have remained largely unexplored. Their complex structures, tendency to aggregate in aqueous solution and the presence of indigenous impurities, particularly trace metals, create technical difficulties, while the multiplicity of reaction products obtained during oxidation complicate mechanistic interpretation. Recently,[1–6] it has been shown that the fundamental physico-chemical properties of commercial azo dyes can be accurately reproduced in a series of simple model azo dyes (see **4**, **5**). These reflect the 'core structure' of commercial dyes without their poor solubility characteristics and complex structures. This has dramatically simplified kinetic studies of dye oxidation by peroxyacids, leading to valuable mechanistic insights into the oxygen-transfer reactions involved in decolourisation. It is known for example that the susceptibility of the dye to oxidation is controlled primarily by its pK_A. The reactive oxidant and dye forms are often present in deceptively low concentrations and chemical reactivity is dominated exclusively by one of the dye forms (**1**–**3**, Scheme 1) participating in the tautomeric equilibrium. The dye pK_A's are in turn controlled by the nature of the *ortho* substituent.

This paper addresses the oxidation of azo dyes through electron-transfer reactions utilising the same series of substituted arylazonaphthol dyes as previously studied.[1–6] In this work we use pulse radiolysis techniques to monitor the initial phases of the oxidation process to detect reactive intermediates involved in dye breakdown. It has been proposed that isolated reducing groups (*e.g.* NMe_2) attached to azo dye chromophores participate in electron-transfer reactions that ultimately lead to dye degradation.[7,8] In this paper we report a new, *generic* one-electron pathway for the oxidation of all azo dyes. This type of dye represents over two-thirds of the dyes in commercial use. In order to gain insights into the thermodynamics of dye oxidation by both chemical oxidants[1–6] and peroxidase enzymes[9] and to assess the widespread applicability of the new pathway, we have also determined the redox potentials of a series of substituted 1-(arylazo)-2-naphthol (**4**) and 2-(arylazo)-1-naphthol (**5**) dyes.



Scheme 1 Azo–hydrazone tautomerism exhibited by arylazo-2-naphthol dyes.



Provided there is judicious use of filters or traps, pulse radiolysis techniques provide an ultrafast method for the quantitative generation of specific oxidative radicals for studying the

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Table 1 Measured redox potentials of *ortho*- and *para*-substituted arylazonaphthol dyes (**4** and **5**) at pH 12 and room temperature

Dye	k_f/M^{-1} s^{-1}	k_r/M^{-1} s^{-1}	ΔE/V	E^a/V	pK_A
4 (X = H)	$1.11 \pm 0.14 \times 10^9$	$4.90 \pm 1.30 \times 10^8$	0.03	0.70	10.8
4 (X = *o*-COOH)	$4.68 \pm 0.25 \times 10^8$	$4.16 \pm 0.64 \times 10^7$	0.06	0.71	12.1
4 (X = *p*-COOH)	$6.39 \pm 0.41 \times 10^8$	$4.16 \pm 0.64 \times 10^7$	0.04	0.69	10.4
4 (X = *o*-Cl)	$1.43 \pm 0.07 \times 10^9$	$2.05 \pm 0.24 \times 10^8$	0.05	0.72	10.4
4 (X = *p*-Cl)	$1.39 \pm 0.14 \times 10^9$	$3.83 \pm 0.82 \times 10^8$	0.04	0.71	11.3
4 (X = *o*-OCH_3)	$1.30 \pm 1.16 \times 10^8$	$9.85 \pm 0.75 \times 10^8$	−0.04	0.64	10.8
4 (X = *p*-NO_2)[b]	$1.96 \pm 0.11 \times 10^9$	$1.23 \pm 0.53 \times 10^8$	0.09	0.76	10.7
5 (X = H)[c]	$6.69 \pm 2.41 \times 10^4$	$2.11 \pm 0.09 \times 10^8$	−0.20	0.84	11.5

[a] Values corrected for ionic strength. [b] Redox potential calculated according to ref. 16, as employed in ref. 29. [c] Using ClO_2 as standard.

initial phases of dye oxidation. In the pulse radiolysis technique, pulses of ionising radiations are used to generate radicals in the nanosecond timescale. In a recent study we have used dilute aqueous solutions of a series of dyes (<1 mM), saturated in nitrous oxide (N_2O) and containing 50 mM sodium azide (NaN_3). Radiolysis of these solutions produces the azidyl radical ($N_3^{\bullet}$) by the reaction of N_3^- with the primary generated hydroxyl radical [eqn. (1)].[10]

$$HO^{\bullet} + N_3^- \longrightarrow HO^- + N_3^{\bullet} \quad (1)$$

The azidyl radical was found to react with the dye common anion (D^-) by electron-transfer to yield the dye radical $D^{\bullet}$ [eqn. (2)].

$$N_3^{\bullet} + D^- \longrightarrow N_3^- + D^{\bullet} \quad (2)$$

Results and discussion

Formation and stability of a reactive dye radical

In this investigation, one-electron oxidation of the dye common anion (D^-)—the most reactive dye form towards oxygen-transfer reactions[1]—was studied by pulse radiolysis techniques. All experiments were conducted at pH 12.0 where the dye common anion (D^-) is the predominant form (see Table 1 for pK_A values of the dyes studied). Fig. 1 shows the dye radical spectrum resulting from a radiolytic burst of 1 Gy, corresponding to ~0.7 μM dye radical from **4** (X = H), together with the corresponding spectrum of the dye common anion. The difference spectrum from which the radical spectrum was calculated is also shown as an insert.

To our knowledge, this is the first time $D^{\bullet}$ has been generated selectively from the common anion by one-electron oxidation. The dye radical has peaks, at λ_{max} = 390 and 510 nm and a trough, at λ_{min} = 460 nm (with $\varepsilon = 2 \times 10^4$, 1.5×10^4, and 1.1×10^4 M^{-1} cm^{-1}, respectively). Compared to the native hydrazone[1] form **2**, the spectrum of $D^{\bullet}$ is shifted even more towards lower wavelengths than the common anion **3**, suggesting that it too, may exist predominantly in the azo tautomeric form with the radical being primarily oxygen centred. Indeed, a reasonable approximation is to examine the molecular orbital properties of the common ion, as the molecular orbital probability functions should approximate to those of the radical. This predicts a high probability of the electron being on the oxygen atom.

The kinetic profile for the formation and decay of the radical after a pulse of 1 Gy is shown in the inset to Fig. 2. The increase in absorbance due to the formation of the dye radical had completely decayed within a millisecond *via* second order kinetics (reciprocal concentration plots *vs.* time are linear). In contrast to some radicals *e.g.* tryptophanyl radicals,[11] the rate and extent of the reaction were unaffected by the oxygen concentration in solution.

Plots of the rate of dye radical decay (plotted as inverse lifetime, Fig. 2) against radiation dose were linear. Since the concentration of dye radical generated is proportional to the radiation dose, this confirms that the dye radical decays by a second order process[12] resulting in dye decolourisation [*e.g.*, eqn. (3) and (4)].

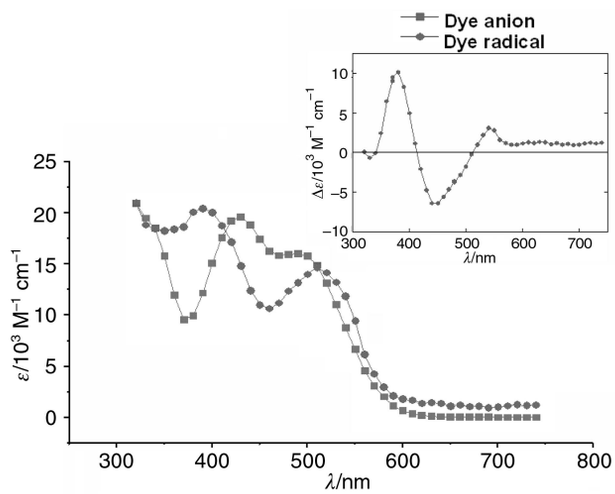


Fig. 1 UV-Vis spectrum of the common ion of dye **4** (X = H) and dye radical (squares and circles, respectively). The insert shows the original difference spectrum. The dye radical spectrum was calculated from the experimental difference spectrum using the starting dye spectrum. All spectra were obtained in 50 mM NaN_3, 10 mM NaOH, starting with 0.2 mM dye **4**. The ordinate shows ε in 10^3 M^{-1} cm^{-1} (or $\Delta\varepsilon$ in the insert).

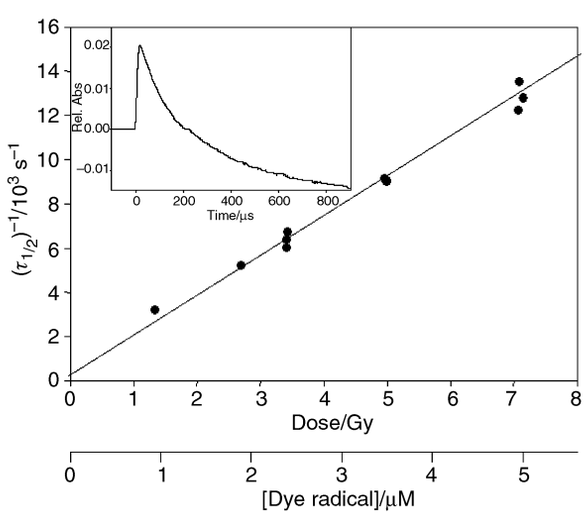


Fig. 2 Effect of dose and hence radical concentration upon the rates of dye **4** (X = H) radical decay. Conditions were as for Fig. 1. The calculated radical concentration corresponding to the radiation dose delivered is also shown on the abscissae. The decay of a transient corresponding to an initial dye radical concentration of 0.7 μM is shown as an insert.

$$2D^{\bullet} \longrightarrow D^- + D^+ \quad (3)$$

$$D^+ \longrightarrow \text{Bleached Dye} \quad (4)$$

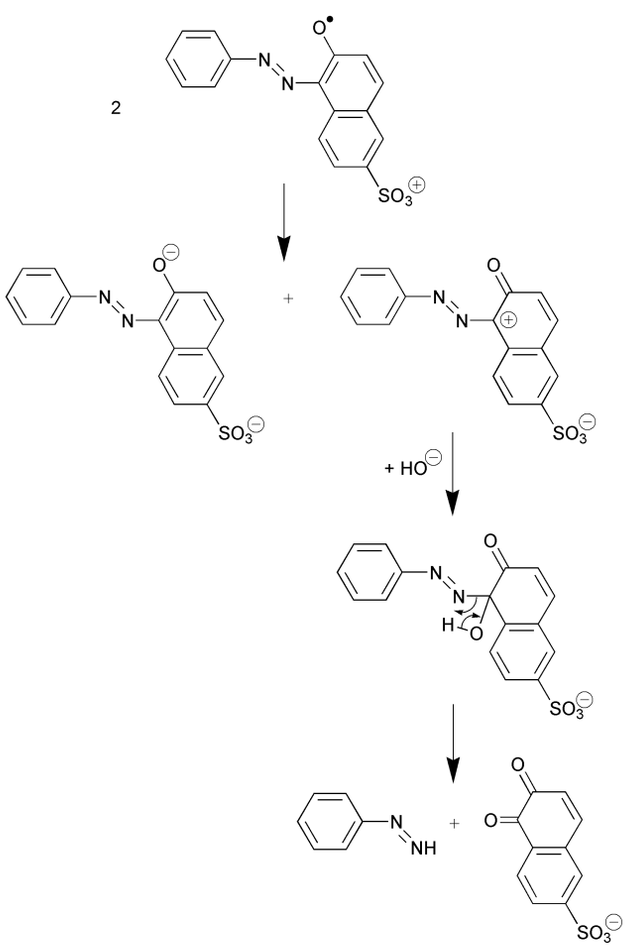
The lifetimes, $\tau_{\frac{1}{2}}$, of the other dye radicals were similar, except for **5** (X = H) which is longer lived than the corresponding radical formed from **4** (2.0 *vs.* 0.28 ms respectively at 0.7 μM dye radical concentration); the greater lifetime of the radical formed from **5** parallels the thermodynamic stability[1] of the corresponding naphthols (pK_A of 2-naphthol and 1-naphthols are 9.5 and 9.3, respectively).[1] The disproportionation or termination rate constant, k_t, of the dye **4** series was calculated using eqn. (5) and found to equal 2.5×10^9 M^{-1} s^{-1}.

$$2k_t = (\tau_{\frac{1}{2}})^{-1}/[D^\bullet] \quad (5)$$

Interestingly, whereas many unhindered phenolic radicals tend to have short lifetimes ($k_t \approx 10^{7-9}$ M^{-1} s^{-1}) due to participation in coupling reactions,[13] the corresponding dye radicals may disproportionate.‡ Evidently, the electron-withdrawing azo group induces delocalisation of the unpaired electron, thereby facilitating a further electron transfer reaction from a more reactive centre.

Mechanism of electron-transfer mediated dye bleaching

The overall mechanism of dye decolourisation may therefore involve two consecutive one-electron transfer steps (Scheme 2)



Scheme 2

with formation of a carbenium ion at position 1 of the naphthol ring. Apart from the disproportionation step, this mechanism is the same as that invoked to explain the dye degradation products produced by two consecutive one-electron transfer steps during peroxidase mediated dye oxidation, albeit at very high and untypical enzyme–dye concentrations.[15] Further dye degradation results from subsequent hydrolysis of the carbenium ion to yield the corresponding naphthoquinone and diazene species, which are subject to further oxidation.[1–4,15] Specifically, the reaction products from the oxidation of the diazene are benzene and nitrogen.

Measurement of dye redox potentials and implications for dye oxidation

Measurements of redox potentials by pulse radiolysis have been described generally[16] and more specifically for phenols and indoles.[17] We have applied the procedure to the measurement of dye redox potentials (E_{12}), at pH 12 by the addition of 2,2′-azinobis(3-ethyl[1,3]benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) **6** to the system as a known redox standard ($E = 0.648$ V)[18] (see methods section for calculations).

Earlier studies have shown that azidyl radicals react with ABTS to generate[19] the stable blue-green radical ABTS$^{+\bullet}$ [eqn. (6)] with an absorption maximum, at λ_{max} = 416 nm ($\varepsilon = 3.47 \times 10^4$ M^{-1} cm^{-1}).[18]

$$N_3^\bullet + ABTS \longrightarrow N_3^- + ABTS^{+\bullet} \quad (6)$$

The ABTS radical was found to establish a redox equilibrium with the dye [eqn. (7)].

$$ABTS^{+\bullet} + D^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ABTS + D^\bullet \quad (7)$$

The proximity of its redox potential to that of the dyes made it suitable as a redox standard, however its intense spectral features overlap those of the dye and dye radical. A second smaller absorption band centred[18] at λ_{max} = 728 nm ($\varepsilon = 1.5 \times 10^4$ M^{-1} cm^{-1}) was therefore used to monitor the formation of ABTS$^{+\bullet}$. The evidence for the establishment of a reversible equilibrium between D$^\bullet$ (dye **4**, X = H) and ABTS is depicted in Fig. 3.

In the absence of dye, a rapid increase in absorbance at λ_{max} = 750 nm (see Fig. 3) was observed due to the formation of ABTS$^{+\bullet}$. In solutions containing only the dye the increase was much smaller and was attributable to a weak contribution from the dye radical. In the presence of equimolar amounts of ABTS and dye the initial rate of increase in absorbance was intermediate, showing that both the dye and ABTS compete for available azidyl radicals. Following this very rapid step, a slower increase in absorption was observed as the dye radical reacted with ABTS to give more ABTS$^{+\bullet}$.

The final absorbance achieved was less than in the absence of dye and since radical–radical reactions are negligible on the time-scales involved at the radiation dose used in these experiments (~1 Gy), this is indicative of a reversible equilibrium [eqn. (7)]. This is supported by the observation that the absorption at λ_{max} = 750 nm due to ABTS$^{+\bullet}$ radical measured at completion, varied with the ratio of ABTS to dye, indicating competition for the azidyl radical. The rate of approach to the equilibrium (k_{obs}) determined from an exponential fit to the delayed growth of absorbance at 750 nm depended both on the dye and ABTS concentration. Plots of k_{obs}/[dye] against the concentration ratio [ABTS]/[dye] were linear (Fig. 4) with a finite intercept, from which the forward (k_f) and reverse (k_r) rate constants of equilibrium (7) could be calculated.

These values were determined for each of the dyes in Table 1 and are shown together with their redox potentials (derived from the calculated equilibrium constants, k_f/k_r and corrected for ionic strength differences). Inspection of Table 1 yields two key points: Firstly, the measured redox potentials show only modest dependence on the nature of the substituent, X, in the aryl ring. Evidently, one-electron oxidation occurs at the negatively charged oxygen atom of the common anion and the

‡ Certain hindered phenols *e.g.* Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), also decay by a disproportionation process ($k_t = 3.1 \times 10^4$ M^{-1} s^{-1}), but the radicals formed are sufficiently stable (recorded lifetime of several ms) so that their decay can be followed by EPR.[14]

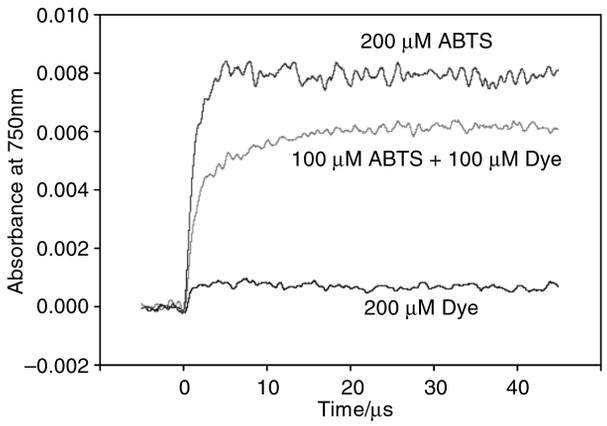


Fig. 3 Traces illustrating formation of radicals in solution by pulse radiolysis. Absorbance measurements of radicals from solutions containing i) 200 μM ABTS, ii) 200 μM dye **4** (X = H) and iii) 100 μM ABTS and 100 μM dye **4** (X = H) were conducted at λ_{max} = 750 nm. Conditions were as for Fig. 1. λ_{max} = 750 nm was used to monitor accumulation of $ABTS^{+\bullet}$ rather than λ_{max} = 728 nm as it is further from the dye peak but still covered by the broad band arising from $ABTS^{+\bullet}$.

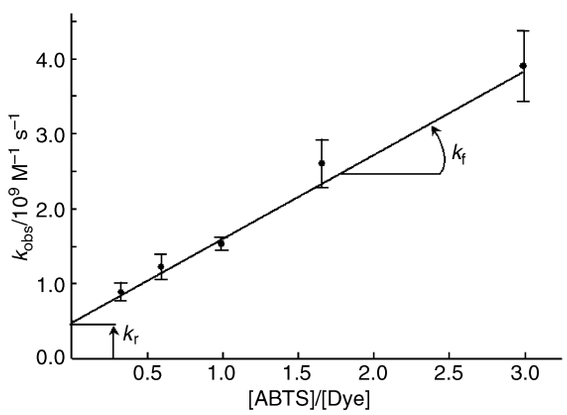
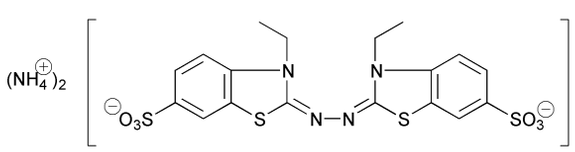


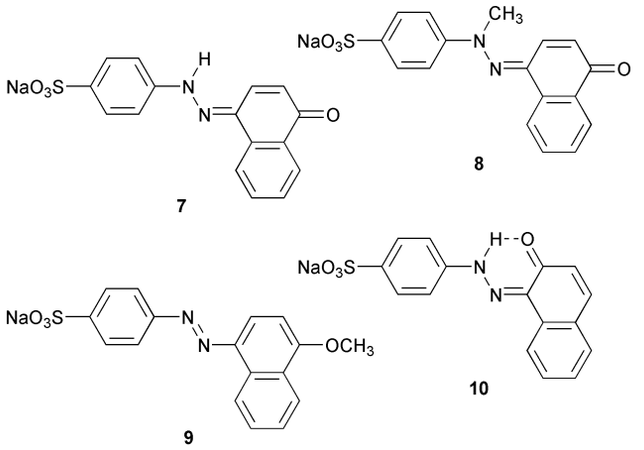
Fig. 4 Measurement of the rates of electron transfer between dye and ABTS as a function of [ABTS]/[dye]. A second order plot is illustrated from which the values k_f and k_r for reactions involving dye **4** (X = H) were determined and equilibrium constants calculated from which redox potentials were derived. Experimental conditions are given in Fig. 3, using methods described in the experimental section.

substituents are too remote to influence the electron-transfer process. Secondly, the typical redox potential for the model dyes studied was ~0.7 V. The value measured at pH 7 for the corresponding hydrazone tautomer is 0.94 V; this value is in good agreement with that calculated from data at pH 12, assuming the potential is coupled to a proton-transfer process. This value is close to those reported[20] for hydrazones of related 1-arylazo-4-naphthol dyes, **7** (E = 1.12 V) and **8** (E = 1.03 V) in acetonitrile but, as anticipated, substantially less than for the azo form, **9** (E = 1.40 V). Although beset with problems of irreproducibility, redox potentials measured by differential pulse voltammetry as a function of pH were found to exhibit a Nernstian response for a proton coupled process (data not shown). Furthermore, the pK_A of the dye radical can be estimated to be ~5, much higher than for typical phenols (−2). Consequently, although it was reported[21] earlier that one-electron oxidation of **10** at pH 6 produced the dye cation radical, we would suggest that the neutral dye radicals—formed



6

ABTS



Structures of Orange I in native form **7**, 'fixed' hydrazone form **8** and 'fixed' azo form **9**; Orange II in native form **10**

by simultaneous proton loss—is a more likely possibility. The similarity of the spectral transients to those observed in this work reinforces this view.

Peroxidase enzymes[9,22] typically perform two consecutive one-electron substrate oxidation steps[19] and presumably operate similarly with dyes.[23] Peroxidase high oxidation state intermediates typically have redox potentials of 0.8–1.0 V.[24,25] This would be insufficient to allow direct oxidation of the azo or hydrazone dye tautomers but quite sufficient for the common dye anion. The active dye species for peroxidases is therefore likely to be the dye common anion, with disproportionation of the dye radical giving rise to the bleached species. One possible exception to this general route might be lignin peroxidase which has been shown to oxidise methoxybenzenes with potentials in excess of 1.3 V.[26] Thus, dye bleaching *via* one-electron oxidation of the common dye anion to a labile radical is considered to represent a hitherto unrecognised and very significant dye degradation pathway; one which should be directly relevant to the action of peroxidase enzymes on dyes. Indeed the action of peroxidases on dyes can be potentiated by the addition of mediator molecules such as ABTS, which function as a redox shuttle in the oxidation of dyes by the above mechanism. The values in Table 1 show that these processes are generally kinetically fast and close to diffusion controlled ($k_f \approx 10^8$–10^9 M^{-1} s^{-1}).

Experimental

Materials

The synthesis, purification, characterisation, and properties of the azo dyes investigated have been described earlier.[1,4]

Pulse radiolysis measurements

Measurement of redox potentials of radicals by pulse radiolysis were performed using a 6 MeV linear accelerator that delivered 0.6 μs pulses, as previously described.[16,27] Before irradiation the solutions were saturated with zero-grade nitrous oxide (oxygen content < 10 ppm) or with nitrous oxide–oxygen mixtures ($[O_2] \approx 250$ μM) when the effect of oxygen on radical stability was studied. Doses per pulse of ~ 1 Gy were used, as determined by thiocyanate dosimetry,[28] which corresponds to a concentration of radicals of ~ 1 μM. The redox equilibration with a reference couple was determined by kinetic spectrophotometry, before any radicals could otherwise decay[16] (tens of microseconds).

Calculation of redox potentials

The equilibrium constant K_1 for reaction (7) is related to the difference ΔE between the couples [eqn. (8)] and by the Nernst

equation [eqn. (9)] where n is the number of electrons and F is the Faraday constant.

$$\Delta E = E(\text{dye}^{-}/\text{dye}^{\bullet}) - E(\text{ABTS}/\text{ABTS}^{+\bullet}) \quad (8)$$

$$\Delta G = -nF\Delta E = -RT\ln K_1 \quad (9)$$

$$k_{obs}/[\text{dye}] = k_f([\text{ABTS}]/[\text{dye}]) + k_r \quad (10)$$

$$\Delta E = \Delta E_{exp} - 0.059 f(I)(z_S^2 + z_{X\cdot}^2 - z_{S\cdot}^2 - z_X^2) \quad (11)$$

$$f(I) = -0.059\, I^{\frac{1}{2}}/(I^{\frac{1}{2}} + 1) \quad (12)$$

$$E(I) = E(I = 0) - 0.059 f(I)(z_{S\cdot}^2 - z_S^2)^{16} \quad (13)$$

Plots of the ABTS–dye ratio against the rate at which equilibrium was reached [for reaction (7)] give a straight line (see Fig. 4) according to eqn. (10).

The intercept of this plot on the ordinate is equal to the back rate k_r and the slope of the line is equal to the forward rate k_f for the second order process by which equilibrium is attained [eqn. (10)]. From the ratio k_f/k_r the value of K_1 can be obtained, from which the value of ΔG can be calculated [see eqn. (9)], and hence ΔE.

These electron-transfer equilibria involve charged species and therefore, in solutions of ionic strength $I > 0$, the value calculated by eqn. (10) deviates from the true difference in reduction potentials ΔE according to eqn. (11)[16] where z denotes the charge on the species involved in the equilibrium as indicated by the subscript: S is the reduced form of the redox standard (ABTS), S$^{\bullet}$ the oxidised form (ABTS$^{+\bullet}$); and X and X$^{\bullet}$ the species of unknown (dye) redox potential in the reduced and oxidised (radical) form, respectively. In eqn. (11) $f(I)$ is the ionic-strength correcting function; under the conditions of these experiments (I = 0.06 M), the Debye–Hückel equation [eqn. (12)] is applicable. Table 1 lists the measured ΔE values corrected in this way. On the basis of these values and the reduction potentials of the standards used, the reduction potentials of the dye radicals can be calculated. The reduction potential of ABTS$^{+\bullet}$ has been measured electrochemically: E(ABTS$^{+\bullet}$/ABTS) = 0.68 V at I = 1.5 M. The reduction potential of ABTS is dependent on the ionic strength according to eqn. (13) where $E(I)$ is the reduction potential at ionic strength I, and $E(I = 0)$ the reduction potential at zero ionic strength. Using eqn. (13), it is possible to calculate for I = 0.06 M (the conditions under which the experiments were performed), E(ABTS$^{+\bullet}$/ABTS) = 0.648 V. The reduction potentials of the dye radicals listed in Table 1 were calculated on the basis of this value, apart from dye **5** which used ClO_2 as standard. For dye **4** (X = *p*-NO_2), the redox potential was calculated according to ref. 16 as employed in ref. 29.

Acknowledgements

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