Redox Reactions of Neutral Red. A Pulse Radiolysis Study

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One-electron reduction and oxidation reactions of the phenazine dye neutral red (NR) have been studied in homogeneous aqueous medium at different pHs employing various reducing {hydrated electron, H atom, isopropyl ketyl $[(CH_3)_2\overset{\bullet}{C}OH]$} and oxidizing ($Cl_2^-$, Tl^{2+}, Br, Br_2^-, N_3) radicals. The absorption spectra of the product transient species (semireduced and semioxidized neutral red) and their kinetic parameters have been evaluated. By monitoring the absorbance changes as a function of pH pK_a values of 2.4 ± 0.2 for the semireduced species and 2.9 ± 0.2 and 6.7 ± 0.2 for the semioxidized neutral red have been determined. While all the radicals employed in this investigation react completely by electron transfer, H and OH are found to react to the extent of 42 ± 5% and 100% respectively with neutral red to bring about adduct formation. The one-electron oxidation potential of neutral red has been evaluated.
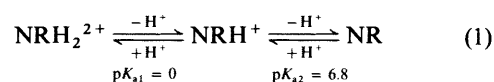
Neutral red (3-amino-6-dimethylamino-2-methylphenazine hydrochloride) belongs to the class of phenazine dyes which are used as sensitizers in photogalvanic cells for the conversion of light to electrical energy.[1,2] These dyes also find applications in other areas where their redox chemistry plays a significant role. Thus photopolymerisation of styrene sensitised by this dye involves the formation of the semireduced species.[3] Dye-sensitised electron transport in liposome systems[4] has been the subject of several investigations. For example it has been suggested that electron transport in the sodium ascorbate–neutral red–ferricyanide system in liposomes is completely controlled by the redox property of the dye. Photoreduction of phenazine dyes sensitised by chlorophyll has also evoked considerable interest[5] where the key intermediate is the semireduced species. The redox reactions of an analogous dye Safranine T have been previously studied in our laboratory.[6] There is no report in the literature on the one-electron redox chemistry of neutral red employing the pulse radiolysis technique. In a laser flash photolysis study of neutral red[7] the semireduced species has been generated by reductive quenching of the dye triplet using ascorbic acid and p-phenylenediamine as reductants. However, the decay of the semireduced species was found to be dependent on the identity of the reductant. In the presence of the latter reductant, the decay of the semireduced species was found to be much faster than in the presence of ascorbic acid. This has been ascribed to the possibility that the one-electron oxidation product of p-phenylenediamine is a better oxidant than ascorbic acid. In order to evaluate the potential usefulness of dyes in photogalvanic and biochemical redox systems a knowledge of the redox properties of dyes is essential. Pulse radiolysis is a useful technique in determining the kinetic properties and redox potentials of the intermediate radicals formed on radiolysis. It permits the generation and observation of these radicals in aqueous solutions before they can decay by disproportionation or by radical–radical re-combination. We have studied the one-electron reduction and oxidation of neutral red using specific reducing and oxidizing radicals. In the present study we have used e_{aq}^- generated by nanosecond pulse radiolysis as a specific one-electron reductant whose concomitant oxidation product is water and hence is not expected to complicate the decay kinetics. The results have been compared with those obtained using other organic reducing radicals. On the other hand Cl_2^-, Tl^{2+}, Br, Br_2^-, N_3 have been used as specific one-electron oxidizing radicals to generate semioxidized neutral red. The results of the reactions of these oxidizing radicals have been compared with those of OH

radicals. The formation and decay rate constants, acid–base equilibria and spectral parameters of semireduced and semi-oxidized neutral red have been evaluated. Knowledge of the semireduced and semioxidized species can thus throw more light on the role of these intermediates in photogalvanic and biological redox systems.

Experimental

Details of the pulse radiolysis set up have been described elsewhere.[8] Transients were recorded on an Iwatsu transient digitiser interfaced to an IBM PC for data analysis.[9] Single pulses of 7 MeV electrons of 25 ns duration were made use of, typical doses being of the order of 20 Gy as determined by monitoring the transient absorbance of $(SCN)_2^-$ formed by pulsing aerated solutions of 0.01 mol dm^{-3} KSCN ($G\varepsilon$ for $(SCN)_2^- = 2.23 \times 10^{-4}$ m^2 J^{-1} at 500 nm,[10] where G is radiation chemical yield and is defined as the number of moles formed per Joule of energy absorbed and ε is the extinction coefficient at 500 nm). Much lower doses (*ca.* 8 Gy) were employed for precise evaluation of extinction coefficients, formation rate constants and redox potential values.

Neutral red (Fluka) was purified by recrystallization from water. The pH dependence of the absorption spectrum of neutral red revealed two pK_as at 0 ± 0.1 and 6.8 ± 0.1. It should be noted that the neutral red molecule has three nitrogen atoms and is expected to have three sites of protonation. The higher pKa of 6.8 refers presumably to the ring nitrogen and the lower one to one of the two substituents *viz.* $-NH_2$ and $-N(CH_3)_2$. The third pKa was not detected in the pH range studied. The different conjugate acid-base forms are represented in eqn (1).

$$NRH_2^{2+} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} NRH^+ \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} NR \qquad (1)$$
$$pK_{a1} = 0 \qquad\qquad pK_{a2} = 6.8$$

All other chemicals were of the highest available purity. Solutions were prepared in nanopure water having a con-ductivity of 0.1 μS cm^{-1}, obtained by passing distilled water through a Barnstead Nanopure water system to remove all ionic and organic impurities. The solutions were purged with pure N_2, O_2 or N_2O ['IOLAR' grade from Indian Oxygen Ltd.] depending on the choice of reaction conditions. Appropriate pretraps were used as and when necessary to compensate for solvent losses due to evaporation. The pHs of
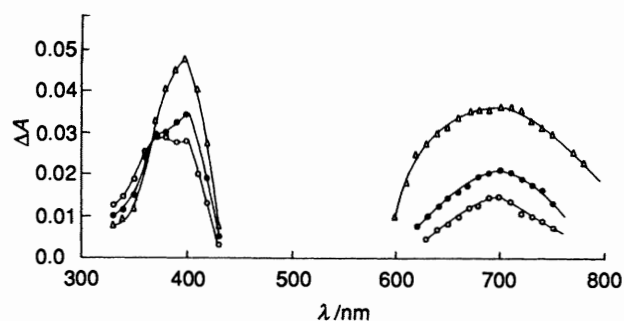
Fig. 1 Time-resolved spectra of transient semireduced species formed by reaction of e_{aq}^- in electron pulse irradiated N_2-saturated *tert*-butyl alcohol $(0.1 \text{ mol dm}^{-3})$ matrix containing $1 \times 10^{-4} \text{ mol dm}^{-3}$ neutral red at pH 5 (dose = 22.4 Gy)

the solutions were adjusted using H_2SO_4, $Na_2HPO_4 \cdot 2H_2O$ or KH_2PO_4.

When studying the reaction of the hydrated electron, *tert*-butyl alcohol $(0.1 \text{ mol dm}^{-3})$ was used for scavenging the OH radicals. N_2O-saturated matrices (above pH 3.5) were used for studying the reaction of isopropyl radicals, while below pH 3, N_2-saturated matrices were employed wherein isopropyl radicals were generated according to eqn. (2).

$$(CH_3)_2CHOH + OH(H) \longrightarrow$$
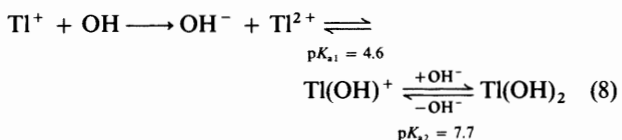$$(CH_3)_2\overset{\cdot}{C}OH + H_2O(H_2) \quad (2)$$
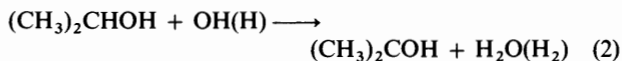
Reactions of neutral red with other organic reducing radicals such as those derived from cytosine, THF (tetrahydrofuran), glucose *etc.* were investigated using N_2O-saturated solutions in which the organic radicals were generated according to reactions (3) and (4).
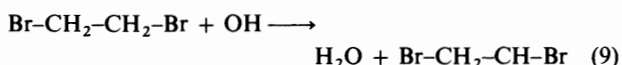
$$N_2O + e_{aq}^- \longrightarrow OH + OH^- + N_2 \quad (3)$$

$$RH + OH(H) \longrightarrow R^{\cdot} + H_2O(H_2) \quad (4)$$
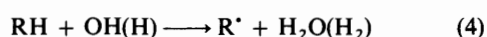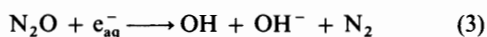
The various oxidizing radicals *viz.* $Cl_2^{\cdot-}$, $Br_2^{\cdot-}$, $(SCN)_2^{\cdot-}$, N_3 used for one-electron oxidation of neutral red were generated by reactions (5)–(8).[11,12]

$$OH + X^- \longrightarrow HOX^- \quad (5)$$

$$HOX^- \longrightarrow X^{\cdot} + OH^- \quad (6)$$

$$X^{\cdot} + X^- \longrightarrow X_2^{\cdot-} \quad (7)$$
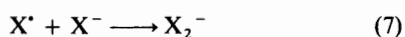
(X^- represents halide/pseudohalide ions: Cl^-, Br^-, SCN^-, *etc.*)

$$Tl^+ + OH \longrightarrow OH^- + Tl^{2+} \rightleftharpoons$$
$$\text{p}K_{a1} = 4.6$$
$$Tl(OH)^+ \underset{-OH^-}{\overset{+OH^-}{\rightleftharpoons}} Tl(OH)_2 \quad (8)$$
$$\text{p}K_{a2} = 7.7$$

The oxidizing Br atoms were generated by OH-induced degradation of 1,2-dibromoethane (DBE) in aqueous solution followed by β-bromine cleavage[13] [reactions (9) and (10)]

$$Br-CH_2-CH_2-Br + OH \longrightarrow$$
$$H_2O + Br-CH_2-\overset{\cdot}{C}H-Br \quad (9)$$

$$Br-CH_2-\overset{\cdot}{C}H-Br \longrightarrow CH_2=CH-Br + Br \quad (10)$$

These radicals can react with neutral red by electron transfer

giving rise to a one-electron oxidized form of the dye [eqn. (11),

$$NRH^+ + OX \longrightarrow NRH^{2+} + Red^- \quad (11)$$

where Ox is the oxidizing radical and Red^- represents its reduced form].

For studying the reaction of OH radicals in acidic medium H atoms were scavenged by oxygen to give HO_2 and it was shown that no transient light-absorbing species were formed by reaction of HO_2 with the dye.

Results and Discussion

One-electron Reduction

Reaction with e_{aq}^-.—The decay of the absorption band of the hydrated electron at 610 nm (at this wavelength, both parent and the product transient species have negligibly small absorbance) in an electron-beam pulsed deoxygenated 0.1 mol dm^{-3} *tert*-butyl alcohol matrix (pH 5) was found to be much faster in the presence of neutral red and followed first-order kinetics. The decay was pseudo-first-order with respect to neutral red concentrations. The bimolecular rate constant, as determined by following the pseudo-first-order decay of the hydrated electron absorbance at 610 nm, was found to be $(4.5 \pm 0.2) \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. The absorption spectrum of the reaction product monitored in the 300–800 nm region is shown in Fig. 1. Two well defined bands having λ_m at 400 and 700 nm were observed. Time-resolved spectral studies indicate that as the intensities of the two bands decrease with time, a new band (not decaying even on the ms time scale) having absorption at 370 nm is seen growing. This is possibly formed from the decay of the transient. The transient species decayed by good second-order kinetics with identical $2k$ values: $(1.70 \pm 0.3) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ at both 400 and 700 nm. These two bands can therefore be inferred to be due to a single transient species *i.e.* the semireduced species formed from the one-electron reduction of neutral red. The dye exists as NRH^+ at this pH, hence its reduction with e_{aq}^- can be represented as eqn. (12).

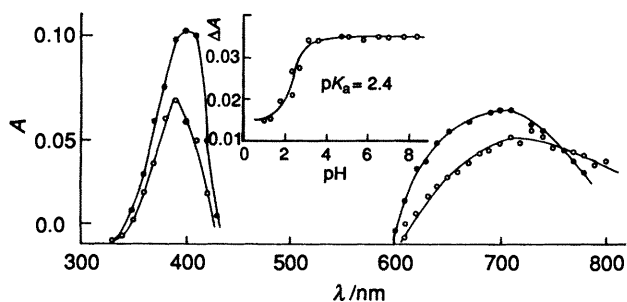$$NRH^+ + e_{aq}^- \overset{H^+}{\longrightarrow} NRH_2^+ \quad (12)$$

In eqn. (12) addition of an electron has been shown, followed by a proton. This is inferred by the results of a 'kinetic salt effect' experiment, which establishes the charge on the transient product to be unity at pH 5. Thus the transient, which decays with $2k/\varepsilon l = (2.4 \pm 0.3) \times 10^5 \text{ s}^{-1}$ in the absence of any added salt (total ionic strength, $\mu = 0.02 \text{ mol dm}^{-3}$) was found to decay faster with $2k/\varepsilon l = (3.6 \pm 0.3) \times 10^5 \text{ s}^{-1}$ in the presence of added sodium sulfate salt ($\mu = 0.08 \text{ mol dm}^{-3}$). Also, as we shall see later, the transient species has a $\text{p}K_a$ of 2.4 [equilibrium (14)] and the predominant species above this pH is shown to be NRH_2^+.

The build up of the absorbance monitored at 400 nm due to the formation of semireduced species reached a maximum at *ca.* 3.0 μs after the pulse at NRH^+ concentration of 5×10^{-5} mol dm^{-3}. The bimolecular rate constant evaluated from the build up traces is $(4.4 \pm 0.2) \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ which is close to the one obtained by following the decay of the hydrated electron absorbance at 610 nm. The extinction coefficients of the product transient species (ε_T) were evaluated at any wavelength λ employing corrections for the ground state absorption using eqn. (13) where ε_P is the extinction

$$\varepsilon_T = \varepsilon_P + \frac{[A_T][G_{(SCN)_2^-}][\varepsilon_{(SCN)_2^-}]}{G_T[A_{(SCN)_2^-}]} \quad (13)$$

Table 1 Spectral and kinetic parameters of the transient species

| Transient	pH	λ_{max}/nm	$\varepsilon/m^2\ mol^{-1}$	$2k/dm^3\ mol^{-1}\ s^{-1}$
NRH_3^{2+}	1.8	390	750 ± 90	$(1.3 \pm 0.3) \times 10^9$
		700	540 ± 65	
NRH_2^+	5–8	400	1200 ± 140	
		700	700 ± 85	$(1.7 \pm 0.3) \times 10^9$
H-adduct	1.8	380	840 ± 100	—

Fig. 2 Difference absorption spectra of transient semireduced neutral red species in electron beam pulsed isopropanol (1 mol dm^{-3}) matrices containing 1×10^{-4} mol dm^{-3} neutral red (O, pH 1.8; ●, pH 5). Dose = 14.4 Gy. Inset: pH dependence of the absorbance of the transient species at 700 nm in deoxygenated isopropanol (1 mol dm^{-3})–acetone (0.1 mol dm^{-3}) matrix containing 1×10^{-4} mol dm^{-3} neutral red (dose = 9.6 Gy).

coefficient in m^2 mol^{-1} for the parent dye molecule at wavelength λ, G_T is the G value of the product transient species and $[A_T]$ and $[A_{(SCN)_2^-}]$ are the observed absorbances of the transient dye at λ and of the (SCN)$_2^-$ radical at 500 nm respectively under isodose conditions. The extinction coefficient of the product transient species formed by the reaction of hydrated electron evaluated at low dose is shown in Table 1.

The reaction with the deprotonated form of the dye at pH 8 is an order of magnitude slower, the bimolecular rate constant being $(3.9 \pm 0.3) \times 10^9$ dm^3 mol^{-1} s^{-1} evaluated by monitoring the decay of the hydrated electron absorbance at 590 nm. The absorption spectrum and the decay of the reaction product is however similar to the one observed at pH 5.

Reaction with Isopropyl Ketyl Radicals.—The absorption spectra of the transient species produced by reaction of isopropyl radicals at both pH 5 and 8 are identical to the one obtained by the e_{aq}^- reaction at the same pHs. At pH 5 the absorbance reaches a maximum at *ca.* 20 μs after the pulse for a NRH$^+$ concentration of 1×10^{-4} mol dm^{-3}. The bimolecular rate constant for the reaction of isopropyl ketyl radicals with neutral red as evaluated from the growth of absorbance at their absorption maxima at 400 and 700 nm of the product semireduced species is $(2 \pm 0.2) \times 10^9$ dm^3 mol^{-1} s^{-1}. Isopropyl ketyl radicals react rather slowly with the deprotonated form of neutral red present at pH 8.0, the rate constant being $(6.9 \pm 0.2) \times 10^8$ dm^3 mol^{-1} s^{-1}. At both the pHs the product transient species decayed by good second-order kinetics with rate constants which closely agreed with the ones obtained in the *tert*-butyl alcohol matrix wherein e_{aq}^- is used as the reductant.

The similarity of the spectra and the closeness of the decay kinetics of the transients formed in the two matrices indicate that isopropyl ketyl radicals also bring about one-electron reduction of neutral red, although they have been reported to undergo addition reaction in the case of pyridinols[14] as well as abstraction of H atoms.[15] The spectra recorded at acid pH exhibited small shifts in the absorption maxima and also changes in the extinction coefficients (Fig. 2). This effect can be attributed to the existence of a pK_a of the semireduced species
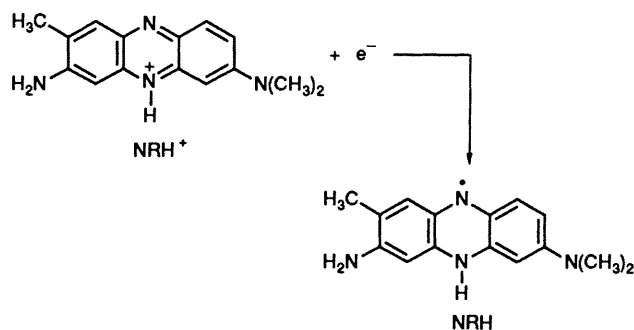
Scheme 1

rather than to different modes of isopropyl ketyl radical reaction. The spectral and kinetic parameters at different pHs are shown in Table 1. The one-electron reduction of neutral red can be represented as shown in Scheme 1, where one of the possible resonance forms of the semireduced neutral red is shown.

pK_a of Semireduced Neutral Red.—The effect of pH on the absorption maxima and the extinction coefficients of the semireduced species was made use of to determine the pK_a of the semireduced species. The pK_as were determined by pulse irradiation of a deoxygenated isopropanol (1 mol dm^{-3})–acetone (0.1 mol dm^{-3}) matrix containing 1×10^{-4} mol dm^{-3} neutral red whereby e_{aq}^-, H and OH are quantitatively converted to (CH$_3$)$_2$ĊOH radicals.[8] The normalized absorbances of the product transient species when monitored at 700 nm as a function of pH revealed a single inflexion point at pH 2.4 ± 0.2 (inset of Fig. 2), which is close to the value reported by Marks *et al.*[7] We have been able to carry out our studies only up to pH 8.75, since above this pH the parent dye is unstable and begins to precipitate out. The observed pK_a can be assigned to equilibrium (14). Equilibrium (14) is further confirmed by the results of the 'kinetic salt effect' experiment, which, as discussed before indicate the presence of a singly-charged transient species at higher pH than *ca.* 5.

$$NRH_3^{2+} \xrightleftharpoons{pK_a\ =\ 2.4} NRH_2^+ + H^+ \qquad (14)$$

In their flash photolysis study Marks *et al.*[7] have reported two pK_as at 2.6 and 7.6 for the semireduced species formed by reduction of the dye triplet using ascorbic acid and EDTA as reductants. While the lower value compares closely with the one observed by us, there is no indication of another pK_a at least up to pH 8.7 investigated by us. Inference of a second pK_a at 7.8 is somewhat ambiguous in view of the fact that the organic reductant EDTA also has a pK_a in this region. Moreover, the neutral red triplet has pK_a in the close vicinity[7] which can further complicate the interpretations of the experimental titration curve. However, it should be noted that in the case of other dyes such as safranine-O,[16] thionine[8,17] and toluidine blue[18] two pK_as for the respective semireduced species are reported and the values for the higher pK_as are respectively 9.5, 8.2 and 8.5. In the present study as stated before, we have not observed the higher pK_a for the semireduced neutral red at least up to pH 8.7 studied. Under the circumstances, it appears that the second pK_a lies somewhere above 8.7.

Decay of Semireduced Neutral Red.—The lower $2k$ value for the second-order decay of the semireduced species at pH 1.8 as compared to the value at higher pHs is understandable in view of the above acid–base equilibrium. Thus at pH 2 where the radical is present as a dication the repulsion between like
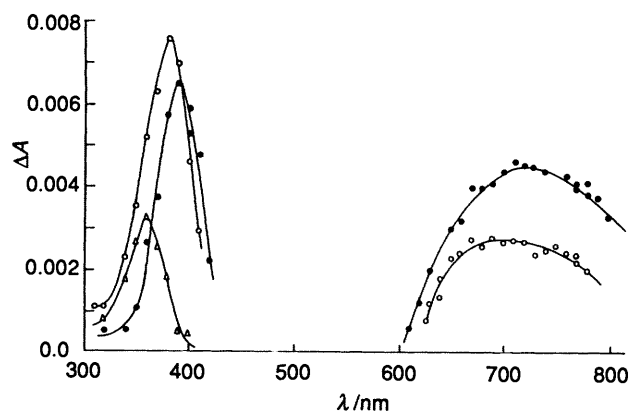
Fig. 3 Difference absorption spectra of the transient species at pH 1.8 formed by reaction of H-atoms in N_2-saturated Bu^{t}OH (1 mol dm^{-3}) matrix containing 1×10^{-4} mol dm^{-3} neutral red (O) and reaction of isopropyl ketyl radicals in deoxygenated solutions containing 1 mol dm^{-3} isopropanol and 1×10^{-4} mol dm^{-3} neutral red (●) (dose = 9 Gy, A computed for G value of 1×10^{-7} mol J^{-1})
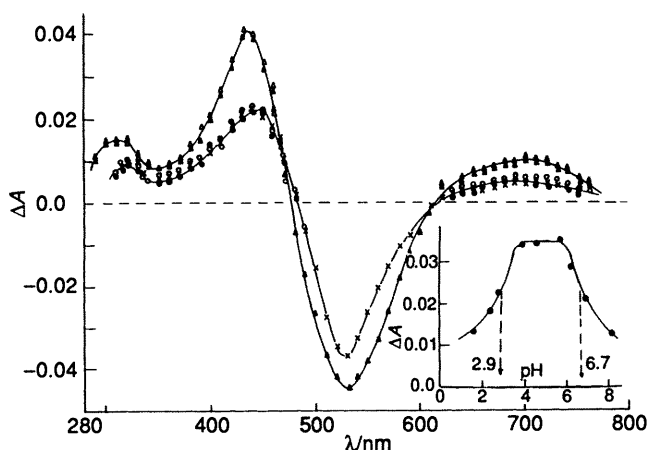

Fig. 4 Difference absorption spectra of the transient semioxidized species formed by reaction of NRH$^+$ with Cl$_2^-$ (O); Br$_2^-$ (×); Tl^{2+} (●) at pH 1.8 in O_2-saturated and N_3(▲); Br$_2^-$ (△) at pH 5 in N_2O-saturated aqueous solutions (dose = 13.5 Gy, ΔA computed for G value of 2.9 × 10^{-7} mol J^{-1}). Inset: Variation of ΔA at 440 nm with pH for the semioxidized species formed by Br$_2^-$ in an O_2-saturated matrix containing 1×10^{-4} mol dm^{-3} NRH$^+$ and 0.01 mol dm^{-3} Br$^-$ (dose = 11 Gy).

charged species is expected to be higher as compared to pH 5 where it is singly charged. It was observed that at the end of the decay the extent of absorption by NRH$^+$ (as seen by the recovery of bleaching) is about half the initial absorption thereby suggesting that the semireduced radical decays by disproportionation [eqn. (15)].

$$2NRH_2^+ \longrightarrow NRH^+ + NRH_3^+ \qquad (15)$$
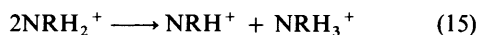
The second-order rate constant for the decay of semireduced species generated in laser flash photolysis experiments[7] was an order of magnitude slower and dependent on the reductant, being faster in the presence of p-phenylenediamine than in the presence of ascorbic acid. This was explained on the basis of the fact that the one-electron oxidation product of p-phenylenediamine is a better oxidant than ascorbic acid. The transient decay was attributed to a recombination reaction with the oxidised form of the reductant. However, in our studies we have employed e_{aq}^- and isopropanol radicals whose oxidation products are unreactive water and acetone. Hence this rules out the back electron transfer pathway and supports the disproportionation mechanism. Also in the radiolysis of neutral

red in N_2-saturated 0.2 mol dm^{-3} tert-butyl alcohol $G(-NRH^+)$ was half the value of $G(e_{aq}^-)$ indicating that the semireduced species disproportionates to give the fully reduced leuco form and the ground state molecule. The fully reduced leuco form was found to absorb in the UV region at 310 nm.

Reaction with H Atoms.—The absorption spectra of the transient species formed by reaction with H atoms are shown in Fig. 3. The spectra show absorption in the 300–800 nm region with λ_m at ca. 380 and 700 nm respectively. On comparison with the spectrum of the semireduced species generated by the reaction of isopropyl ketyl radicals at the same pH and normalized to the same dose and G value, it is observed that the nature of the 700 nm band is the same except that its intensity is lower in the case of the H atom reaction product. Also H atoms were found to react with the dye with a bimolecular rate constant of $(5 \pm 0.2) \times 10^9$ dm^3 mol^{-1} s^{-1} (as determined by following the pseudo-first-order formation of the transient species) which is much higher than that obtained for the isopropanol radical reaction product. The reducing ability of the H atoms are expected to be much higher than that of the isopropyl ketyl radicals on the basis of their one-electron reduction potentials. Hence the lowering in intensity of the 700 nm band may not be due to the inefficiency of the H atom reaction with neutral red. H atoms are known to react also by modes other than electron transfer. Thus in the case of thiazine dyes it has been inferred that some of the total H atoms generated react by addition and the remainder react by electron transfer.[19] Evidence of H-adduct formation was obtained from comparison of the shorter wavelength band in the spectra of Fig. 3. The spectra reveal a shift in the absorption maxima and an increase in the intensity of the H-atom reaction product. Point by point subtraction reveals a new band having absorption maximum at 380 nm which can be attributed to the adduct species. On the basis that isopropyl ketyl radicals bring about 100% electron transfer, H atoms were found to react by electron transfer to the extent of $58 \pm 7\%$ and the remaining $42 \pm 5\%$ was attributed to the formation of an adduct species whose extinction coefficient was evaluated to be 840 m^2 mol^{-1}.

Reaction with other Reducing Radicals.—Pulse irradiation of N_2O-saturated solutions containing 1×10^{-4} mol dm^{-3} NRH$^+$ and 0.1 mol dm^{-3} of ethanol, tetrahydrofuran (THF), cytosine, glucose etc. gave rise to the 700 nm band indicating the formation of semireduced neutral red brought about by the one-electron reduction of NRH$^+$. However, the extent of reduction in terms of the yield of semireduced neutral red species was not the same in all the cases. While the radicals derived from ethanol and THF could react with the dye to give semireduced neutral red species to the extent of 100%, the yield of semireduced species in the case of cytosine and glucose was only ca. $40 \pm 5\%$ and ca. $20 \pm 3\%$ respectively. The radicals derived from tert-butyl alcohol could not induce any measurable reduction of the dye. This trend reflects the ability of these organic radicals as reducing agents to donate an electron to the dye molecule.

One-electron Oxidation

The spectral results of the reaction of various oxidizing radicals with neutral red at different pHs are shown in Fig. 4. One-electron oxidizing radicals suitable for use in the acidic pH range are Cl$_2^-$, Br$_2^-$ and Tl^{2+}. For studying the reactions of these radicals with NRH$^+$ at pH 1.8, O_2-saturated solutions containing 1×10^{-4} mol dm^{-3} NRH$^+$ and 0.01 mol dm^{-3} Cl$^-$ or Br$^-$ or 0.002 mol dm^{-3} Tl$^+$ were employed. The spectrum of the product transient species revealed two absorption bands at

Table 2 Spectral and kinetic parameters of the reactions of Safranine T with various oxidizing radicals

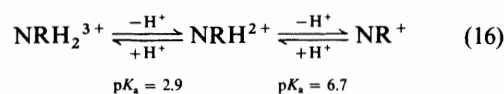| Transient	pH	λ_{max}/nm	ε/m^2 mol^{-1}	$(2k/\varepsilon l)/10^5$ s^{-1}	Oxidizing radicals	$k/10^9$ dm^3 mol^{-1} s^{-1}
NRH$_2^{3+}$	1.8	445	1000 ± 120	8.0 ± 0.2	Cl$_2^-$	7.5 ± 0.2
		700	—		Br$_2^-$	3.1 ± 0.2
					Tl^{2+}	1.7 ± 0.2
NRH^{2+}	5	435	1600 ± 190	5.7 ± 0.2	N$_3$	3.0 ± 0.2
		700	250 ± 30		Br$_2^-$	4.0 ± 0.3
NR$^+$	8	440	739 ± 85	—	N$_3$	7.0 ± 0.2
		700	—		Br$_2^-$	4.8 ± 0.3
OH-Adduct	1.9	390	—			
		680	268 ± 30	8.7 ± 0.3	OH	10.0 ± 0.3
	5	390	913 ± 110			8.8 ± 0.2
		660	540 ± 65	2.1 ± 0.3		9.0 ± 0.3

445 nm and 700 nm (Fig. 4). Time-resolved spectral studies revealed that while the intensity of the two bands decreased proportionately with time a new band having absorption in the 310–320 nm region and not decaying even on the ms timescale was observed, possibly arising from the decay of the transient formed at 445 nm and 700 nm. At both these wavelengths the transient decayed by second-order kinetics with $2k = (8 ± 0.2) \times 10^9$ dm^3 mol^{-1} s^{-1} thereby indicating the two bands to be due to a single transient species. The rates of formation of the transients monitored at their absorption maxima followed first-order kinetics. The plots of the pseudo-first-order rate constants against the dye concentration were linear. From the slope of these plots the bimolecular rate constants for the reaction of NRH$^+$ with the different oxidizing radicals were evaluated. The spectral and kinetic parameters of the transients are shown in Table 2.

Reaction of Cl$_2^-$ and Tl^{2+} cannot be studied in the neutral pH region since the reaction of Cl$^-$ with OH becomes slower as the pH of the solution increases above pH *ca.* 3 whereas Tl^{2+} is hydrolysed to Tl(OH)$^+$ and Tl(OH)$_2$ (see for discussion, ref. 6). Useful one-electron oxidants in the neutral pH region include N$_3$ and Br$_2^-$. The spectra of the transients obtained by reaction of Br$_2^-$ and N$_3$ at pH 5 on pulsing N$_2$O-saturated solutions containing 1×10^{-4} mol dm^{-3} NRH$^+$ and 0.01 mol dm^{-3} Br$^-$ or N$_3^-$ are also shown in Fig. 4. It is observed that although these spectra qualitatively resemble the one formed at acid pH, there is a shift in the absorption maxima and an increase in the extinction coefficients indicative of an acid–base equilibrium involving the transient species between pH 1.8 and 5. In order to verify that the change in extinction coefficient is genuinely due to variation of pH and not due to an artefact arising from inefficiency of electron transfer in the case of Br$_2^-$ or N$_3$ reactions, a relatively stronger oxidant, Br was considered at this pH. The transient spectrum obtained by reaction of Br at pH 5 in electron-beam pulsed N$_2$O-saturated solution containing 1×10^{-4} mol dm^{-3} NRH$^+$ and 4×10^{-3} mol dm^{-3} 1,2-dibromoethane, closely resembled those obtained by using other oxidizing radicals at this pH. We therefore conclude that involvement of an acid–base equilibrium is genuine. Extinction coefficients and other parameters of the reaction product at this pH are shown in Table 2.

Although Cl$_2^-$, Br, Br$_2^-$, Tl^{2+} and N$_3$ are all specific one-electron oxidants and can bring about one-electron oxidation of a number of molecules, there are reports that Cl$_2^-$ and Br$_2^-$ can also react by modes other than electron transfer (see for example, ref. 6). The spectra and decay kinetics of the transient species formed by the reaction of all these oxidizing radicals at different pHs are similar. Hence it can be inferred that the transient species in the present case must be the semioxidized neutral red formed from the one-electron oxidation of the dye. Safranine T, an analogous phenazinium dye also reacts with these oxidizing radicals to give a transient spectrum with

absorption maxima at 350 and 470 nm attributable to the semioxidised safranine species.[6]

pK_a *of Semioxidized Neutral Red.*—To determine the acid–base dissociation constant of the semioxidized species, O$_2$-saturated solutions containing 1×10^{-4} mol dm^{-3} NRH$^+$ and 0.01 mol dm^{-3} Br$^-$ and adjusted to the required pH were employed. Under this condition e$_{aq}^-$ and H atoms were scavenged by O$_2$ so that the resulting transient would be due to the reaction of Br$_2^-$ only. Br$_2^-$ radicals were chosen since they have no pK_a in the entire pH range 0–12 and a single matrix can be employed throughout for the study. By monitoring the absorbance as a function of pH, two inflexion points at pH = 2.9 ± 0.2 and 6.7 ± 0.2 were obtained (inset of Fig. 4). In the ground state, neutral red has two pK_as [equilibrium (1)]. At pH 5 'the dye exists as NRH$^+$ and the resulting semioxidised species NRH^{2+}, being electron deficient, should be expected to lose a proton more easily. The acid–base equilibrium of the semioxidized neutral red can therefore be represented as in eqn. (16).

$$NRH_2^{3+} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} NRH^{2+} \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} NR^+ \qquad (16)$$
$$pK_a = 2.9 \qquad\qquad pK_a = 6.7$$

The closeness of the second pK_a to that of the ground state molecule indicates that deprotonation must be taking place at the same site as in the parent molecule. In the case of the analogous dye, Safranine T only one pK_a of 4 for the semioxidized species was observed. The higher pK_a was not observable even up to pH 10 studied.[6] Safranine T has a pK of < 0 in the ground state and no other pK_a exists at higher pH unlike the case of neutral red, which has two pK_as of 0 and 6.8. Thus the two pK_a values observed for the semioxidized neutral red appear to be genuine.

It must be noted that at both acid and neutral pH the efficiency for electron abstraction from neutral red by the various oxidants is close to 100%. This is in contrast to that observed for safranine T and other phenothiazine dyes[6] where efficiency of Br$_2^-$ has been found to be lower, which could be due to the formation of an adduct wherein the unpaired p-electron in Br$_2^-$ combines with the non-bonding p-electron pair in the hetero atom.

Redox Potential for the One-electron Oxidation of Neutral Red.—Among the various standard couples with their known one-electron reduction potentials tried, (SCN)$_2^-$/2SCN$^-$ (E^0 1.25 V *vs.* NHE)[20] was found to establish an equilibrium with NRH^{+2}/NRH$^+$ [eqn. (17)]. For determining the corres-

$$NRH^+ + (SCN)_2^- \rightleftharpoons NRH^{2+} + 2SCN^- \qquad (17)$$
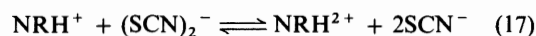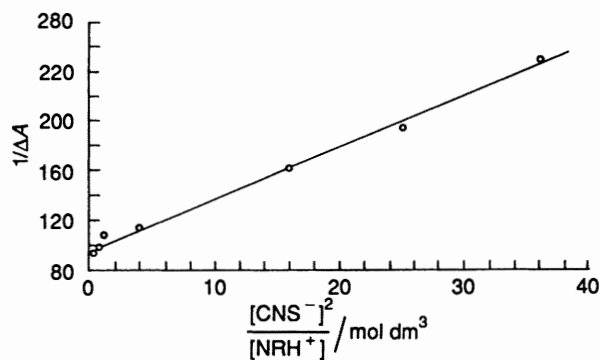
Fig. 5 Plot of $1/\Delta A$ vs. $[SCN^-]^2/[NRH^+]$ in N_2O-saturated 5×10^{-5} mol dm^{-3} NRH$^+$ and varying concentrations of $(SCN)_2^-$ at pH 5 (ionic strength was kept constant at 0.1 mol dm^{-3}), dose employed = 8 Gy)
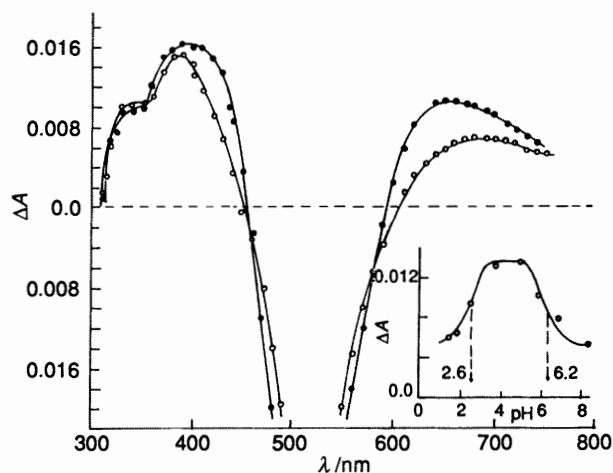

Fig. 6 Absorption spectra of the transient formed by reaction of OH with NRH$^+$ in an electron beam pulsed aqueous solution of 1×10^{-4} mol dm^{-3} NRH$^+$: N_2O-saturated, pH 5 (●); O_2-saturated, pH 2 (○). Dose = 22.4 Gy, $\triangle A$ computed for G value of 1×10^{-7} mol J^{-1}. Inset: Variation of $\triangle A$ at 660 nm with pH (dose = 10 Gy).

ponding equilibrium constant an N_2O-saturated matrix containing 1×10^{-4} mol dm^{-3} NRH$^+$ and KSCN (whose concentration was varied between 0.005 and 0.06 mol dm^{-3}) was employed. The pH of the solution was adjusted to 5 and ionic strength was kept constant at 0.1 mol dm^{-3} by the addition of sodium sulfate. Under these conditions SCN$^-$ reacts exclusively with OH to form $(SCN)_2^-$ which then reacts with the dye according to eqn. (17). The equilibrium constant, K for the above reaction is given by eqn. (18). SCN$^-$ and $(SCN)_2^-$

$$K = \frac{[NRH^{2+}][SCN^-]^2}{[NRH^+][(SCN)_2^-]} \qquad (18)$$

do not absorb at 700 nm and hence the absorbance monitored at 700 nm corresponds to that of NRH^{2+} which can be shown to obey eqn. (19). A plot of $1/A$ vs. $[SCN^-]^2/[NRH^+]$ would

$$1/A = (1/RK\varepsilon)[SCN^-]^2/[NRH^+] + 1/R\varepsilon \qquad (19)$$

be a straight line with slope = $1/R\varepsilon K$ (where R represents the total radical concentration generated in the system) and intercept = $1/R\varepsilon$ (Fig. 5). Experimental data fit a good linear plot from which K (intercept/slope) was calculated to be 21. Knowing the intercept and the total radical concentration obtained from the dose and the G value, the extinction coefficient of the semioxidized species was found to be 240 m^2 mol^{-1} which was in agreement with the value determined earlier.

The equilibrium constant can be expressed in terms of the standard one-electron potentials of the NRH^{+2}/NRH$^+$ and $(SCN)_2^-$/2SCN$^-$ couples by eqn. (20). Taking the E^0 $(SCN)_2^-$/

$$E^0(NRH^{2+}/NRH^+) = E^0[(SCN_2^-/2SCN^-] -$$

$$0.059 \log K \qquad (20)$$

2SCN$^-$ value to be 1.25 V vs. NHE[20] the one electron potential for NRH^{+2}/NRH$^+$ couple was calculated to be $+ 1.17 \pm 0.02$ V vs. NHE.

Reaction with OH *Radicals.*—The rate constants for the reaction of OH radicals with neutral red at different pHs were determined by directly following the build up of the product transient absorbances at their absorption maxima and are given in Table 2. It was observed that the rate constant at pH 8 (where the dye is in the deprotonated form) is an order of magnitude higher. This could be due to the fact that at pH 8 the nitrogen atom in the dye molecule is in the deprotonated state and hence the electrophilic character of the molecule is enhanced and its reactivity towards OH is expected to be higher as compared to pH 2 and 5 where the nitrogen atom is in the protonated state.

The absorption spectra of the product transient species is shown in Fig. 6. Two well defined bands with λ_{max} 390 and 660 nm were observed. The decay of the transient species monitored at various wavelengths of the two bands followed second-order kinetics with identical decay rates. We infer that the two bands are due to the same transient species formed by OH reaction. The different spectral and kinetic parameters are shown in Table 2. It can be seen that there is a shift in the absorption maxima and a change in the extinction coefficient as we go from acid to neutral pH. This fact was made use of to determine the pK_a of the OH reaction product. The normalized absorbances (monitored at 660 nm) when plotted as a function of pH revealed the presence of two pK_as at 2.6 ± 0.2 and close to 6.2 (inset of Fig. 6). The exact value of the second pK_a could not be established since above pH 8.7 the dye is unstable and begins to precipitate out.

The OH adducts did not hydrolyse to yield the semioxidized species (no spectra attributable to the semioxidized species could be detected). Further, if the cation had been formed from the OH adduct by elimination of OH$^-$, then a first-order decay would have been expected and not a second-order decay. Although OH is a strong oxidizing radical and is expected to react by an electron transfer process with N-containing compounds such as neutral red, it is evident that in the present case OH addition to the aromatic ring of the heterocyclic molecule is favoured over the electron transfer reaction. A similar conclusion was made in the case of thiazine and safranine[6] dyes where OH addition has been found to be favoured.

Conclusion

The reactivity of neutral red towards pulse radiolytically generated reducing radicals is very high and the rate constant is close to the diffusion controlled value. It was found to be dependent on the state of protonation of the N atom in the ground state molecule. At pH 8 where the nitrogen atom in the dye is in the deprotonated form its reactivity with e_{aq}^- and isopropanol radicals is lower by an order of magnitude. This may be because of the fact that the electrophilic character of the molecule increases with the deprotonation of the N atom and hence its reactivity towards nucleophilies is lowered. H atoms react with neutral red by both electron transfer and addition mechanisms giving rise to semireduced and H-adduct species to the extent of $58 \pm 7\%$ and $42 \pm 5\%$ respectively. Comparison

of neutral red with an analogous phenazinium dye Safranine T indicates that the reactivity of the latter with e^-_{aq} is lower. This could be due to the presence of the phenyl group in Safranine T which, being electron donating in nature, is expected to lower the reducibility of the molecule.

The dye undergoes easy one-electron oxidation by the oxidizing radicals employed and the rate constants for the reaction of these radicals are close to the diffusion controlled value. The one-electron oxidation potential of the dye is estimated to be 1.17 ± 0.02 V $vs.$ NHE. The semioxidized neutral red exists in three forms and has $pK_a = 2.9 \pm 0.2$ and 6.7 ± 0.2. Reaction of OH radicals with the dye gives rise to a transient species which exhibits spectral and kinetic features different from those of the semioxidized species, indicating that OH radicals preferentially react by modes othe than electron transfer.

Acknowledgements

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