

One-electron oxidation of toluidine blue. A pulse radiolysis study

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Abstract. The one-electron oxidation of toluidine blue by specific oxidising radicals such as Cl_2^- , Ti(II) , N_3 , Br_2^- etc. has been studied by employing the pulse radiolysis technique. The Br_2^- radical was found to be less efficient in oxidising toluidine blue as compared to Cl_2^- , Ti^{+2} and N_3 . The semioxidised species exhibited only one pK_a indicating the presence of two conjugate acid–base forms whose spectral and kinetic features were evaluated. Reaction of OH radicals with the dye gave rise to a transient species which exhibited spectral and kinetic features different from that of the semioxidised species indicating that the mode of reaction of OH is different.

Keywords. Pulse radiolysis; one-electron oxidation; toluidine blue; thiazine dyes.

1. Introduction

Methylene blue, toluidine blue and thionine belong to the class of phenothiazine dyes which have found applications in diverse areas such as sensitisers in photogalvanic cells for the conversion of light to electrical energy (Rabinowitch 1940; Kamat *et al* 1979) and in the photo-oxygenation of organic compounds (Cassarotto and Smith 1987). It is therefore interesting to compare the behaviour of the short-lived intermediates, such as excited states and radical ions, from these compounds. Such studies can be carried out using pulse radiolysis and flash photolysis techniques. Using the technique of nanosecond pulse radiolysis, a detailed study of the one-electron redox processes involving these dyes is being carried out in our laboratory in order to gain some insight into the nature of the semioxidised and semireduced species of these molecules. The results of our investigation on thionine (Kishore *et al* 1987; Guha *et al* 1987) and methylene blue (Kishore *et al* 1988) have been reported earlier. In a laser flash photolysis study of toluidine blue (Pottier *et al* 1975), the rate constants for quenching of toluidine blue triplets by oxygen were determined and the quantum yield for singlet oxygen production was estimated. However, no detailed studies on the redox reactions of toluidine blue have been reported in the literature. Earlier we reported the results of our investigation on its one-electron reduction (Mahadevan *et al* 1989). In continuation of this, we have now investigated the one-electron oxidation of this compound using the technique of pulse radiolysis and kinetic spectrophotometry. The absorption spectra of the transient species produced by reaction of various oxidising radicals such as N_3 , Cl_2^- , OH^\cdot etc. with toluidine blue and the kinetics of formation and decay of these species have been studied in detail and are reported here.

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2. Experimental

Toluidine blue (TB) used was from E Merck and was purified as described earlier (Mahadevan *et al* 1989). Other chemicals were of analytical reagent grade quality and were used as such. Gases used for purging the solutions, viz. N_2 , N_2O , and O_2 were Iolar grade from Indian Oxygen. All solutions were prepared in nanopure water obtained from Barnstead nanopure water system. Sulphuric acid, sodium hydroxide, phosphate and borate buffers were used for adjusting the pH values in the respective regions. The pulse radiolysis experiments were carried out using 25 nanosecond electron pulses from a 7 MeV linear accelerator as described earlier (Guha *et al* 1987). The absorbed dose was measured using an aerated aqueous solution of 0.05 mol dm^{-3} potassium thiocyanate taking $G\epsilon$ for the $(CNS)_2^-$ radical generated in the system as $21522 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ per 100 eV at 500 nm (Fielden 1984).

3. Results and discussion

Irradiation of water by ionising radiations such as 7 MeV electrons generates the primary species e_{aq}^- (2.7), H (0.55), OH (2.8), H_2 (0.45) and H_2O_2 (0.71) where the numbers in parentheses represent the primary G values for water radiolysis at neutral pH. Oxidising radicals, viz. Cl_2^- (only below pH 3), N_3^- , $Tl(II)$ and Br_2^- were generated by reaction of OH radicals with Cl^- , N_3^- , $Tl(I)$, and Br^- respectively as described earlier (Kishore *et al* 1987).

Reactions of Cl_2^- and Tl^{+2} with toluidine blue cannot be studied in the neutral pH region because the reaction of Cl^- with OH is very slow above pH 3 (Anbar and Thomas 1964), whereas Tl^{+2} hydrolyses to $Tl(OH)^+$ and $Tl(OH)_2$, pK_a values being 4.6 and 7.7 (Bonifacic and Asmus 1976). One-electron oxidants useful in the neutral pH region are N_3^- , Br_2^- etc. The net G values for the oxidising species is 2.8 in oxygen-saturated solutions and 5.5 in N_2O -saturated solutions. The spectra of the transient species formed on pulsing oxygen-saturated $10^{-4} \text{ mol dm}^{-3}$ toluidine blue solutions containing $0.01 \text{ mol dm}^{-3} Cl^-$ or Br^- or $0.002 \text{ mol dm}^{-3} Tl(I)$ at pH 1.7 are shown in figure 1. It can be seen that these spectra qualitatively resemble each other and reveal two absorption bands with maxima at 500 and 360 nm. Also, bleaching (negative absorbance) of toluidine blue is seen in the 540–700 nm region in all cases. This indicates that Cl_2^- , Br_2^- and Tl^{+2} give rise to the same transient species. For the same dose it was observed that the transient absorbances were equal in the case of Cl_2^- and Tl^{+2} as oxidants but were much lower in the case of Br_2^- (although the G values for these oxidising radicals are the same $=G_{OH}$). This indicates that Cl_2^- and Tl^{+2} oxidise toluidine blue to the same extent, whereas with Br_2^- the extent of oxidation is much lower. Assuming the oxidation to be 100% in the former case, the extent of oxidation by Br_2^- was estimated to be $\sim 30\%$. The oscilloscope traces of the transient signals obtained at 500 and 360 nm using Cl_2^- as oxidant are shown in figure 2. It is seen that at 360 nm the signal first rapidly rises and then remains steady even upto 1 ms, whereas at 500 nm it decays almost completely within 500–600 μs . Although Cl_2^- absorbs in the 360 nm region, its contribution in this time scale can be ruled out because decay of Cl_2^- is complete within 150–200 μs even in the absence of toluidine blue and even earlier in its presence. Hence it can be inferred that the decay of the species formed from toluidine blue by reaction with various oxidising

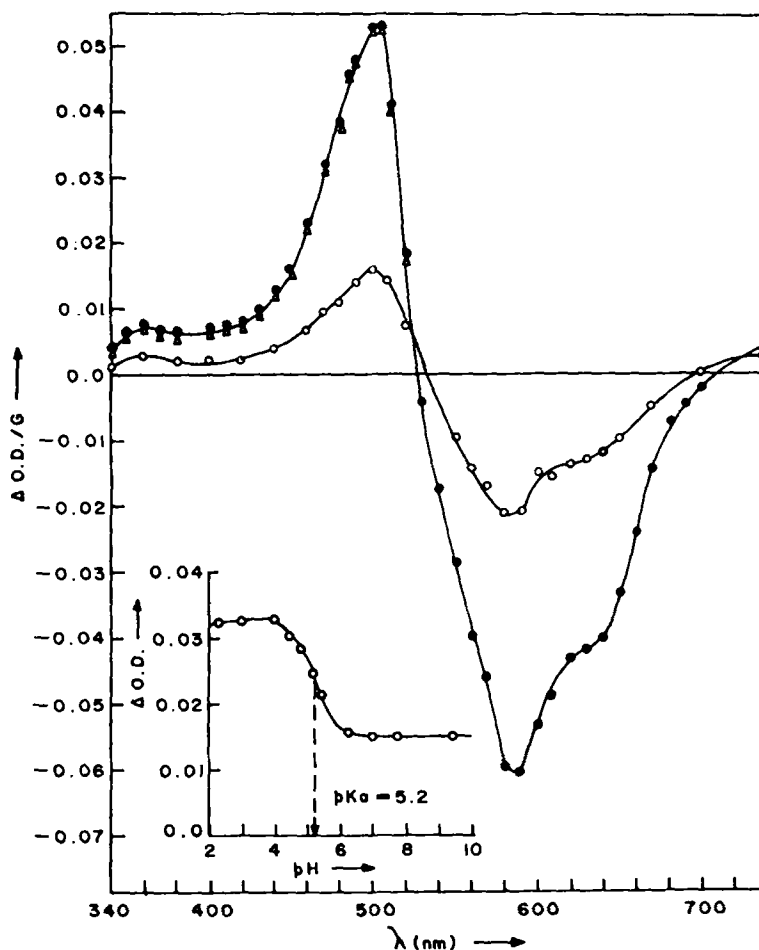


Figure 1. Absorption spectra of the transient species formed by reaction of toluidine blue with a) ● Cl_2^- △ Ti^{+2} , b) ○ Br_2^- , in e -pulse irradiated aqueous solutions containing Cl^- or Br^- (0.01 mol dm^{-3}) or Ti^+ ($0.002 \text{ mol dm}^{-3}$) at pH 1.8. Inset shows the absorbance changes with pH at 500 nm for the semioxidised species formed by Br_2^- ($10^{-4} \text{ mol dm}^{-3}$ toluidine blue solution saturated with oxygen).

radicals also gives rise to a product absorbing in the 360 nm region. The 360 nm band has also been observed using other oxidising radicals. The rate constant for the reaction of these oxidising radicals with toluidine blue was determined by following the rate of build-up of the transient absorbance at 500 nm. A representative trace showing the build-up of the 500 nm absorbance of the transient species using Cl_2^- is shown in figure 3. The formation of the transient species was found to follow very good first-order kinetics (figure 4: ln absorbance vs time) and deviated from second-order linearity (fig 4: $1/\text{absorbance}$ vs time). The first-order rate constant was found to be linearly dependent on toluidine blue concentration (figure 5). From this plot the rate constant for the reaction of Cl_2^- with toluidine blue was evaluated as $3.4 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. Similarly the rate constants for the reaction of Ti^{+2} and Br_2^- with toluidine blue were evaluated as 1.7×10^9 and $1.1 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ respectively. From the decay of absorption (figure 2) it was found that the transient

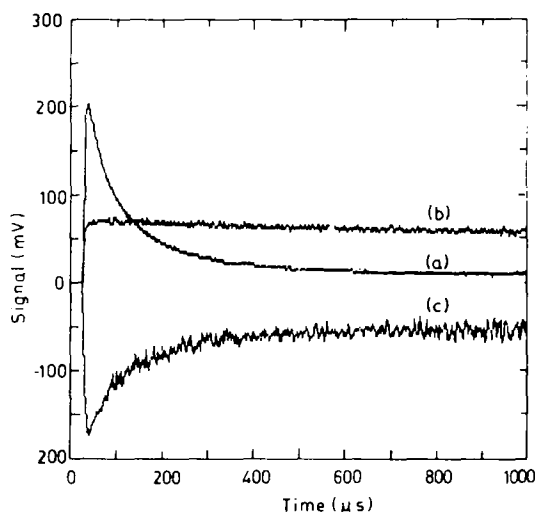


Figure 2. Oscilloscope traces showing absorbance changes with time on pulse radiolysis of oxygen saturated $10^{-4} \text{ mol dm}^{-3}$ toluidine blue solution containing 0.01 mol dm^{-3} NaCl at pH 1.8. (a) decay of semioxidised species at 500 nm, (b) growth of absorbance at 360 nm, (c) recovery of bleaching at 580 nm.

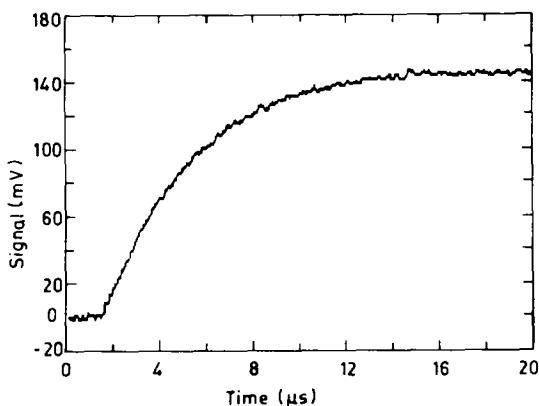


Figure 3. Oscilloscope trace showing growth of absorbance of semioxidised species at 500 nm with time on pulse radiolysis of an oxygen-saturated solution containing $10^{-4} \text{ mol dm}^{-3}$ toluidine blue and 0.01 mol dm^{-3} NaCl at pH 1.8.

species decay by good second-order kinetics with a $2k/\epsilon l$ value of $1.7 \times 10^5 \text{ s}^{-1}$ (figure 6: $1/\text{absorbance}$ vs time). The extent of bleaching recovery was $\sim 50\%$ indicating that the decay of the species is by disproportionation (figure 2). Figure 7 shows the transient spectra obtained on pulsing N_2O -saturated solutions containing $10^{-4} \text{ mol dm}^{-3}$ toluidine blue and $0.01 \text{ mol dm}^{-3} \text{N}_3^-$ at pH 6.8. Although these spectra are similar to the one obtained in acidic solutions there is a shift in the absorption maxima to 510 nm and 340 nm and a decrease in the extinction coefficient at 500 nm (see table 1) indicating the presence of a pK_a between pH 1.7 and 6.8. The nature of the spectra of the transient species formed by reaction of toluidine blue with Br_2^- and $\text{Tl}(\text{OH})^+$ was similar to the one formed by N_3^- (figure 7). By following the first-order

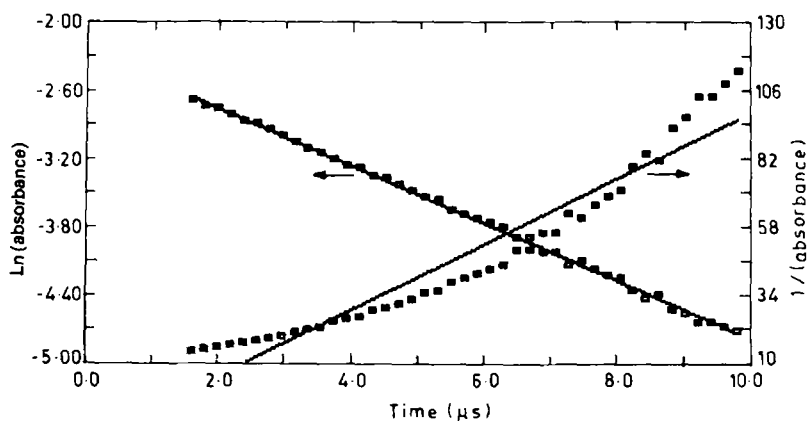


Figure 4. Kinetic analysis of the formation of the semioxidised species monitored at 500 nm in oxygen-saturated $10^{-4} \text{ mol dm}^{-3}$ toluidine blue solution containing $0.01 \text{ mol dm}^{-3} \text{ Cl}^-$ at pH 1.8.

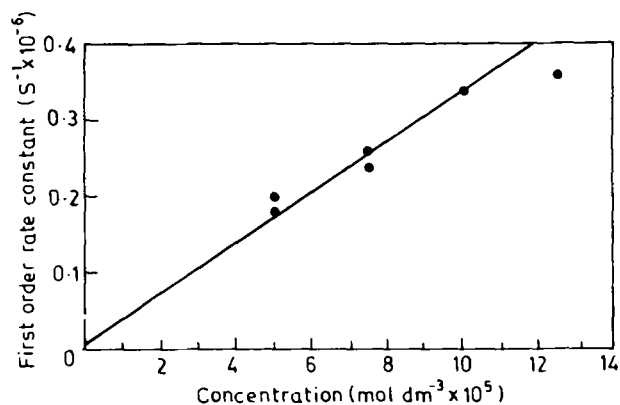


Figure 5. Plot of pseudo-first order rate constant for the reaction of toluidine blue with Cl_2^- vs toluidine blue concentration in oxygen-saturated $10^{-4} \text{ mol dm}^{-3}$ toluidine blue solution containing $0.01 \text{ mol dm}^{-3} \text{ Cl}^-$ at pH 1.8.

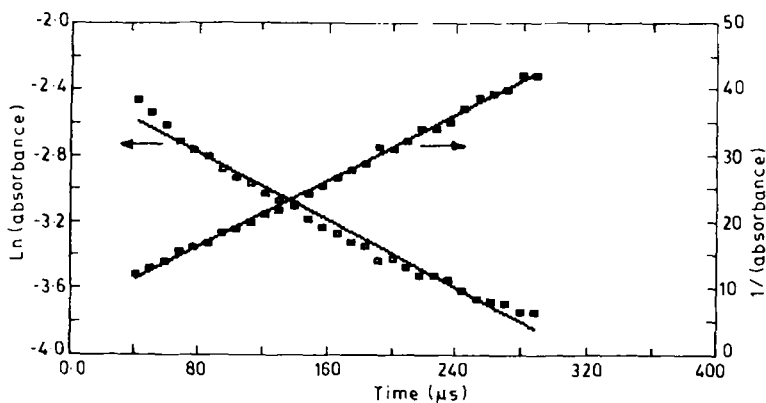


Figure 6. Kinetic analysis of the decay of the semioxidised species monitored at 500 nm in oxygen-saturated $10^{-4} \text{ mol dm}^{-3}$ toluidine blue solution containing $0.01 \text{ mol dm}^{-3} \text{ Cl}^-$ at pH 1.8.

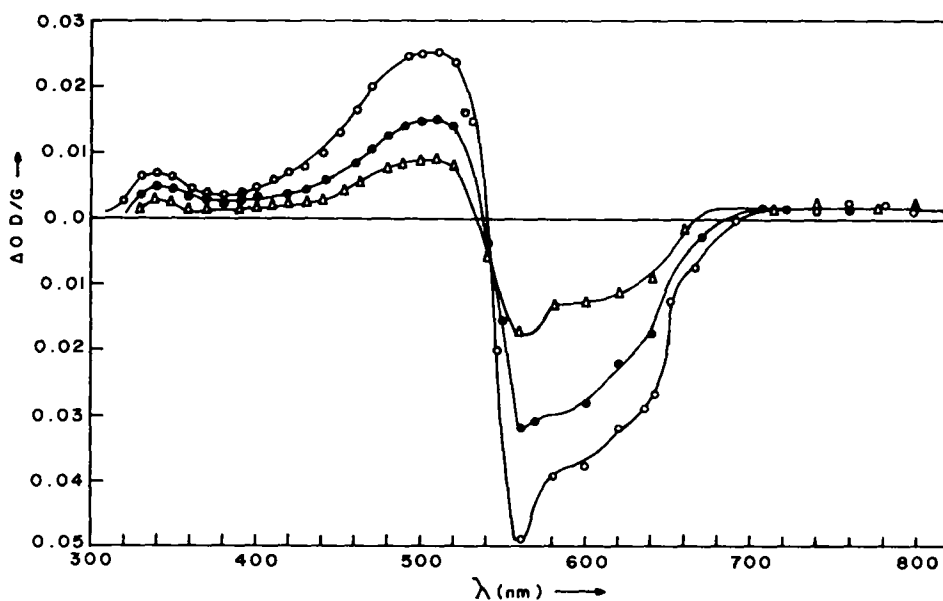


Figure 7. Absorption spectra of the transient species formed by reaction of toluidine blue with: (a) Δ Br_2^- , (b) \circ N_3^- , and (c) \bullet $\text{Ti}(\text{OH})^+$, in the pulse radiolysis of N_2O -saturated $10^{-4} \text{ mol dm}^{-3}$ toluidine blue solutions containing respectively $0.01 \text{ mol dm}^{-3} \text{ Br}^-$ or N_3^- and $0.002 \text{ mol dm}^{-3} \text{ TI}^{+2}$ at pH 6.8.

Table 1. Spectral parameters of the different forms of semioxidised toluidine blue.

Protonated form	pH	λ_{max} (nm)	ϵ ($\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)
TBH_2^+	1.7	500	29,000
		360	
TBH^{2+}	6.8	490	14,100
		360	

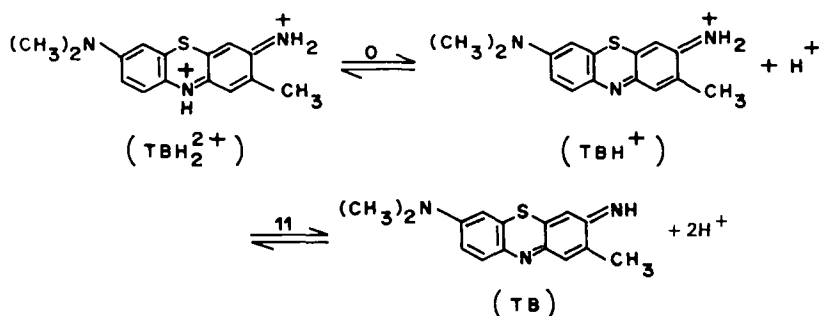
growth of absorbance at 500 nm at different concentrations of toluidine blue the bimolecular rate constants for its reaction with N_3^- , Br_2^- and $\text{Ti}(\text{OH})^+$ at pH 6.8 were determined to be 4×10^9 , 1.1×10^9 and $1.7 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ respectively. Since Br_2^- has no pK_a in the pH region 0–14, the efficiency of oxidation of toluidine blue by Br_2^- at neutral pH must be the same as at acidic pH (viz. 30%). In comparison with this the values for efficiency of N_3^- and $\text{Ti}(\text{OH})^+$ were found to be 100% and 50% respectively as evaluated by comparison with the Br_2^- system at neutral pH. The extinction coefficients of the semioxidised species were calculated from the absorbance measured under conditions such that toluidine blue oxidation is quantitative. The results are given in table 1.

Cl_2^- , $\text{Ti}(\text{II})$, Br_2^- , and N_3^- are specific oxidising radicals and bring about one-electron oxidation of the solute. The spectra as well as the decay constants of the transient species formed by these oxidising radicals at different pH values are similar. Hence the species is inferred to be semioxidised toluidine blue. A similar observation has

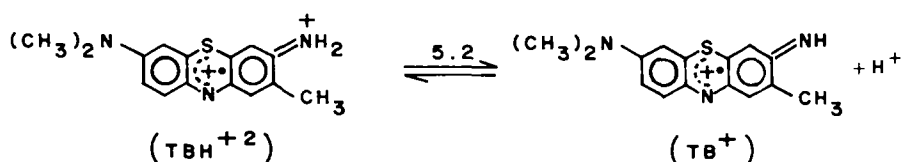
been made in the case of other phenothiazine dyes namely thionine (Kishore *et al* 1987) and methylene blue (Kishore *et al* 1988).

3.1 pK_a of semioxidised toluidine blue

The decrease in the extinction coefficients and the shift in the λ_{\max} as we go from acid to neutral pH suggest the existence of a pK_a in this region. To determine the pK_a value, the Br_2^- radical was used as the oxidising species since it has no pK_a in the pH region 0–14 and the transient absorbance changes at 500 nm were followed as a function of pH in electron beam pulsed oxygen-saturated solutions containing $10^{-4} \text{ mol dm}^{-3}$ toluidine blue and $0.02 \text{ mol dm}^{-3} \text{ Br}^-$. From the inflexion point of the ΔOD vs pH plot (figure 1:inset) the pK_a value was estimated to be 5.2. In the ground state toluidine blue has two pK_a values near 0 and 11 which correspond to the equilibria.

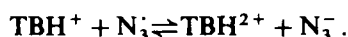


The ground state toluidine blue species present at neutral pH is therefore TBH^+ . The semioxidised species resulting from this, viz. TBH_2^{2+} , because of the additional positive charge, can be expected to lose a proton more readily than the parent TBH^+ and, hence, its acid dissociation constant must be lower. Therefore we attribute the observed pK_a of 5.2 for the semioxidised species to the equilibrium



3.2 Standard potential for the one-electron oxidation of toluidine blue

With N_3^- as the oxidant the yield of the semioxidised species was found to depend on the concentration of N_3^- . Variation of the azide ion concentration from $0.001 \text{ mol dm}^{-3}$ to 0.2 mol dm^{-3} resulted in a 40% decrease in the transient absorbance at 500 nm whereas the pseudo first-order rate constant for the reaction of toluidine blue with N_3^- correspondingly increased. This was suggestive of an equilibrium of the type



From this equilibrium it follows that

$$k_{\text{obs}} = k_f[\text{TBH}^+] + k_r[\text{N}_3^-].$$

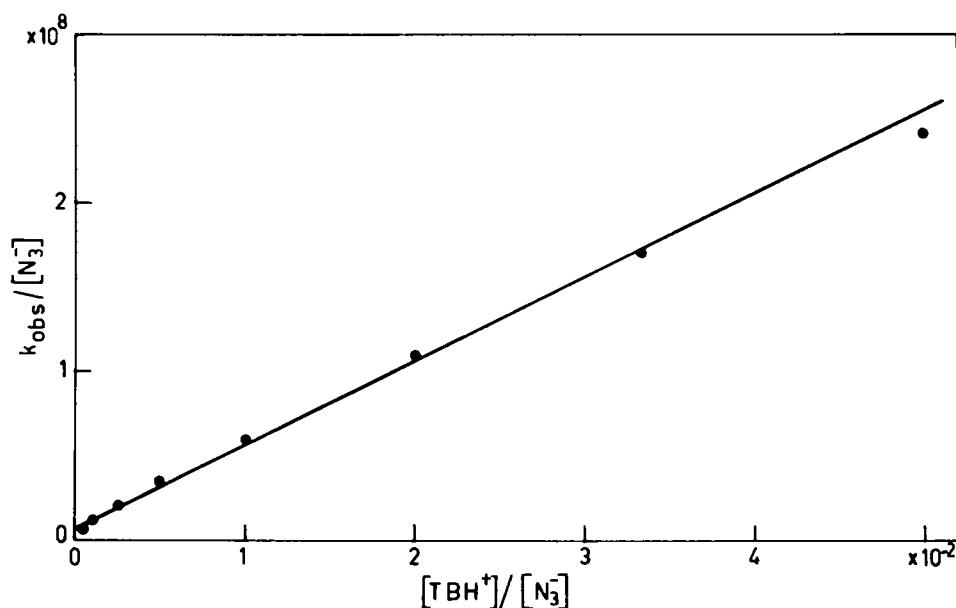


Figure 8. Plot of $k_{obs}/[N_3^-]$ vs $[TBH^+]/[N_3^-]$ for the reaction of N_3 radicals with toluidine blue in e -pulse irradiated aqueous solutions at pH 6.8.

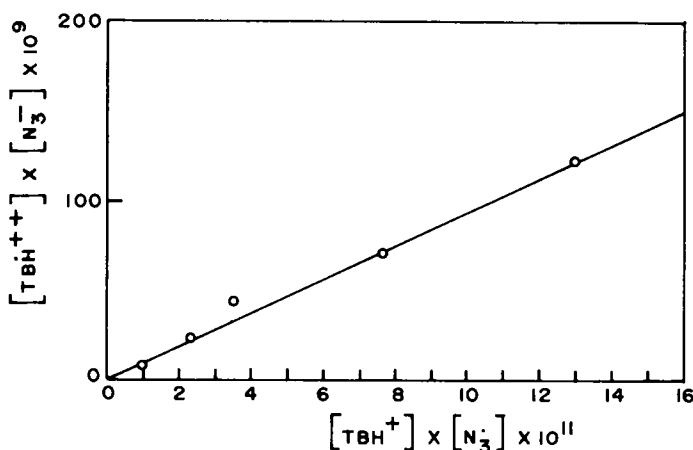


Figure 9. Plot of $[TBH^{2+}][N_3^-]$ vs $[N_3][TBH^+]$ for the reaction of N_3 radicals with toluidine blue in e -pulse irradiated aqueous solutions at pH 6.8.

Therefore a plot of $k_{obs}/[N_3^-]$ vs $[TBH^+]/[N_3^-]$ would be a straight line with slope = k_f and intercept = k_r . Experimental data fit a good linear plot (figure 8). The equilibrium constant K ($=k_f/k_r$) calculated from the slope and intercept of figure 8 is 822. K was also determined from the slope of plot of $[TBH^{2+}][N_3^-]$ vs $[N_3][TBH^+]$ (figure 9) and was found to be 922. We may therefore take an average value of 870 ± 50 for K . As the difference in the standard reduction potential of the two couples i.e. N_3/N_3^- and TBH^{2+}/TBH^+ is related to K by the expression $\Delta E = (RT/nf) \ln K$ we arrive at a value of 0.173 V for ΔE . Taking the literature value

of 1.32 V vs NHE for the standard reduction potential for the N_3/N_3^- couple (Alfassi *et al* 1987) the value for the TBH^{2+}/TBH^+ couple was evaluated as 1.16 V vs NHE. This value is close to that determined for the other phenothiazine dyes, viz. thionine (Kishore *et al* 1987) and methylene blue (Kishore *et al* 1988).

3.3 Reaction product of OH radicals with toluidine blue

Hydroxyl radicals can react with organic molecules by different modes, viz. one-electron oxidation, addition to double bonds, abstraction of H-atoms etc. Hence the absorption spectrum may not be due to a single transient species. The reaction of OH with toluidine blue was studied at pH 1.8 in an oxygen-saturated matrix containing 10^{-4} mol dm $^{-3}$ toluidine blue. Two well-defined bands having absorption maxima at 370 nm and 495 nm were observed. Under these conditions both HO_2 and OH radicals can react with toluidine blue. In order to assess the contribution of reaction by HO_2 , an O_2 -saturated *t*-butanol matrix (0.1 mol dm $^{-3}$) containing toluidine blue (10^{-4} mol dm $^{-3}$) was pulse-radiolysed. Under these conditions *t*-butanol scavenges the OH radicals and only HO_2 radicals are available for reaction with toluidine blue. No transient light-absorbing species was observed indicating that either HO_2 radicals do not react with toluidine blue or if they do, the resulting transient species have no absorption in the 300–750 nm region. The spectrum of the transient species in an N_2O -saturated neutral matrix (figure 10) revealed two broad absorption bands having absorption maxima at 470 and 370 nm and was qualitatively similar to the one

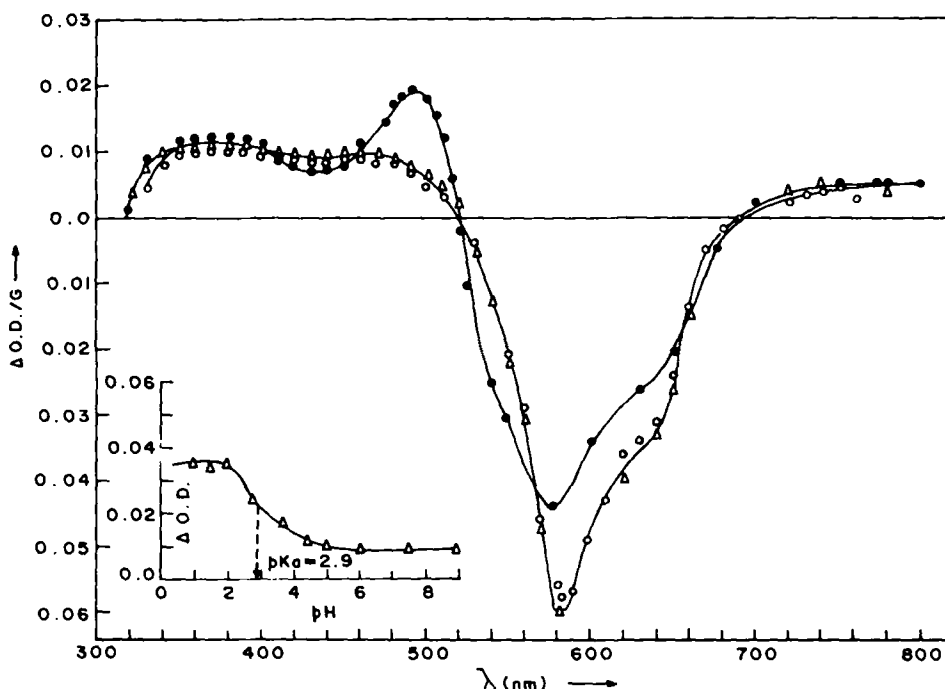


Figure 10. Absorption spectra of reaction products of OH radicals with toluidine blue ● (O_2 -saturated 10^{-4} mol dm $^{-3}$ toluidine blue solutions, pH 1.8); Δ (N_2O -saturated 10^{-4} mol dm $^{-3}$ toluidine blue solutions, pH 6.8); ○ (O_2 saturated 10^{-4} mol dm $^{-3}$ toluidine blue solutions, pH 6.8).

Table 2. One-electron reduction potentials of phenothiazine dyes and the efficiency of their oxidation by N_3^- and Br_2^- radicals.

Dye(D)	Reduction potential for oxidation (V vs NHE)	$k_{D+N_3^-}$ ($dm^3 mol^{-1} s^{-1}$)	%Rxn	$k_{D+Br_2^-}$ ($dm^3 mol^{-1} s^{-1}$)	%Rxn
TH ⁺	> +1.3	3×10^9	18	No rxn	–
MB ⁺	+1.22	4.2×10^9	100	1.6×10^9	100
TBH ⁺	+1.16	4×10^9	100	1.1×10^9	30

obtained using an oxygen-saturated matrix at the same pH indicating that this spectrum has no contribution from species arising from reaction of O_2^- with toluidine blue. The ΔOD vs pH plot obtained in oxygen-saturated $10^{-4} mol dm^{-3}$ toluidine blue matrix is shown in figure 10 (inset). This reveals an inflexion point at pH 2.9 which is different from the pK_a obtained for the semioxidised species formed by reaction of Br_2^- as described earlier. The decay of the species monitored at 470 nm was second order with $2k/εl = 5 \times 10^5 s^{-1}$ which is different from the value obtained for the semioxidised species. From these it is evident that the contribution of semioxidised toluidine blue is negligible in the transient spectrum observed on the reaction of OH radicals with this compound. Hence OH must be reacting with toluidine blue largely through modes other than electron-transfer. The bimolecular rate constant for the formation of the OH reaction product (at both acid and neutral pH) at both their absorption maxima as determined from pseudo first-order growth of absorbance at these wavelengths is $1.2 \times 10^{10} dm^3 mol^{-1} s^{-1}$.

4. Conclusions

A comparison of the three phenothiazine dyes studied indicate that all of them readily undergo one-electron oxidation to yield the semioxidised species having absorption maxima at ~ 500 nm. An additional band at 360 nm observed in all the three cases has been attributed to a permanent product possibly formed by the decay of the semioxidised species. The similarity of the spectra would suggest that the species must be similar, the site of oxidation being the phenothiazine ring. It is seen that the semioxidised species have been formed with 100% efficiency in the case of the strong oxidants Cl_2^- and TI^{+2} . Comparative data for the three compounds regarding their oxidisability are summarized in table 2. The weaker oxidants N_3^- and Br_2^- are unable to oxidise (or only poorly oxidise) thionine. This may be due to the fact that toluidine blue and methylene blue have methyl substituents which being electron-donating in character can be expected to enhance the oxidisability of the phenothiazine ring. Wherever the oxidation was observed, the rates were found to be close to the diffusion-controlled values. OH radicals, although very strongly oxidising by nature, preferentially react by modes other than electron transfer.

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