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

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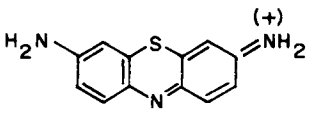
Abstract. The technique of nanosecond pulse radiolysis has been used to study the one-electron reduction of toluidine blue. Apart from H and e^-_{aq} various organic reducing radicals derived from isopropanol, tetrahydrofuran, cytosine etc. have been employed. The rate constants for the one-electron reduction was evaluated from build up traces. By monitoring the absorbance changes as a function of pH, two pK_a values were obtained suggesting the existence of the species in three conjugate acid-base forms. The spectra of the different protonated forms were monitored and their characteristic features have been presented here. In the case of H-atoms it has been observed that in addition to the electron-transfer reaction, other processes like adduct formation also appear to be taking place. Using the redox titration method, with radicals of known redox potential the one-electron reduction potential of toluidine blue was also evaluated (vs. NHE).

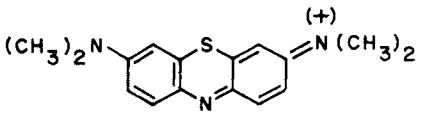
Keywords. One-electron reduction; pulse radiolysis; phenothiazine; toluidine blue.

1. Introduction

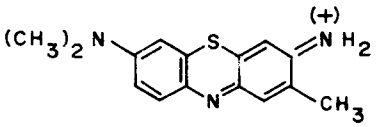
Study of the radiation chemistry of organic dyes in aqueous solutions has received widespread attention particularly because of the fact that dyes can serve as model compounds for biological redox systems. Methylene blue, thionine and toluidine blue belong to the class of thiazine dyes which are also used in photogalvanic cells (Rabinowitch 1940; Kamat *et al* 1979). The structures of these dyes are shown in scheme 1. Although considerable work has been done on redox reactions of methylene



THIONINE (TH^+)



METHYLENE BLUE (MB^+)



TOLUIDINE BLUE (TBH^+)

Scheme 1.

* For correspondence

blue and thionine employing both pulse radiolysis (Solar *et al* 1982; Keene *et al* 1965) and flash photolysis techniques (Hatchard and Parker 1961; Guha *et al* 1979; Kamat and Lichtin 1982) there are no reports on similar studies in the case of toluidine blue. We have carried out studies on the one-electron reduction of toluidine blue employing the pulse radiolysis technique. The transient species formed when the compound reacts with the reducing species generated by irradiation of water and aqueous solutions have been identified and their formation and decay rate constants have been evaluated. The results of these investigations are reported here.

2. Experimental

Toluidine blue used was from E Merck (W Germany). It was crystallized from hot water by adding one and a half volumes of alcohol to a 5% aqueous solution of the compound and chilling it in ice. Further purification was done by repeated extraction with chloroform till the pink colour of the impurities in the organic phase disappeared. The aqueous solution was then evaporated and dried to constant weight in a vacuum oven. Its purity was checked by measuring the extinction coefficient at 620 nm (ε_{620} = 40,000 $dm^3 mol^{-1} cm^{-1}$) which agreed well with the literature values (Merrill and Spencer 1948). Solutions for pulse radiolysis were prepared in triply distilled water. The pH's of the solutions were adjusted with H_2SO_4, K_2HPO_4, NaH_2PO_4, $Na_2B_4O_7 \cdot 10H_2O$ or NaOH as required for the respective pH regions. Depending on the choice of reaction the various solutions were purged with Indian Oxygen Iolar grade N_2, O_2, or N_2O. The pulse radiolysis set-up has been fully described elsewhere (Guha *et al* 1987). Unless otherwise stated, 25 ns pulses from a 7 MeV linear accelerator were used for irradiating the samples. Thiocyanate dosimetry was used for measuring the dose delivered per pulse. For this purpose an aerated solution of KCNS was used for which $G\varepsilon$ was taken to be 21,522 $dm^3 mol^{-1} s^{-1}$ per 100 ev at 500 nm (Fielden 1984). The dose normally used was $1{\cdot}5 \times 10^{17}$ ev/cm^3. However a lower dose was used for extinction coefficient measurements.

The reaction of the hydrated electron with toluidine blue at pH 6·8 was studied by using an N_2 saturated matrix containing 10^{-4} mol dm^{-3} of the compound and 0·1 mol dm^{-3} *t*-butanol. The latter was used for scavenging OH radicals. The radicals formed from *t*-butanol were found to be inert towards toluidine blue.

Similarly, the reaction of isopropyl radicals with toluidine blue at various pHs above 3·5 was studied by employing an N_2O saturated isopropanol (1 mol dm^{-3}) matrix containing 10^{-4} mol dm^{-3} toludine blue. Below pH 3·5, N_2 saturated isopropanol matrices were used.

N_2 saturated acidic solutions (pH < 3·5) containing 0·1 mol dm^{-3} *t*-butanol were employed to study the reaction of H-atoms. Under this condition the hydrated electrons are quantitatively converted to H-atoms by reaction with H_3O^+ and the OH radicals are scavenged by *t*-butanol whose concentration was not high enough to scavenge the H-atoms.

Reactions of toluidine blue with other organic radicals such as those derived from tetrahydrofuran (THF), cytosine, glucose etc. by reaction with OH radicals were also studied at pH 6·8. For this purpose N_2O saturated matrices were employed in which the organic radicals were generated according to the following equations.

$$N_2O + e^-_{aq} \rightarrow OH + OH^- + N_2, \quad (1)$$

$$RH + OH(H) \rightarrow R + H_2O(H_2). \quad (2)$$

3. Results and discussion

Irradiation of a deoxygenated *t*-butanol matrix (pH 6·8) gave rise to a transient light absorbing species having λ_{max} at 720 nm due to the formation of the hydrated electron. The hydrated electron signal monitored immediately after the pulse at 700 nm (where toluidine blue and its product transient species have negligible absorption) was found to decay much faster in the presence of toluidine blue than in its absence (figure 1A).

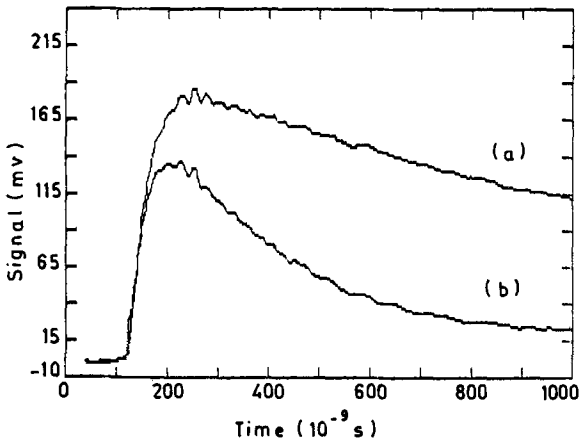


Figure 1A. Trace showing decay of the hydrated electron signal (monitored at 700 nm) in deoxygenated 0·1 mol dm^{-3} *t*-butanol matrix at pH 6·8. Curve (a): in the absence of toluidine blue, curve (b): in the presence of toluidine blue (10^{-4} mol dm^{-3}).

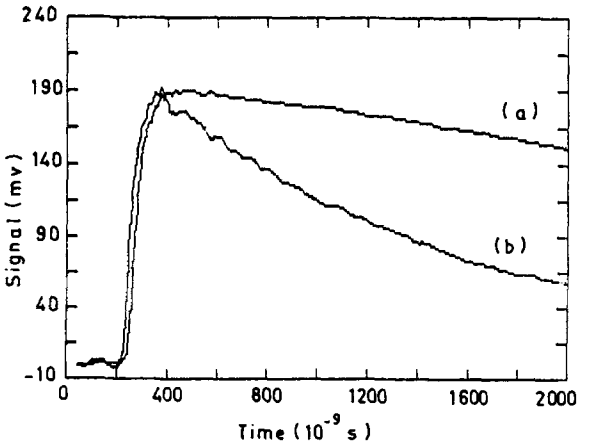


Figure 1B. Trace showing decay of the hydrated electron signal (monitored at 700 nm) in deoxygenated 0·1 mol dm^{-3} *t*-butanol matrix at pH 12·2. Curve (a): in the absence of toluidine blue, curve (b): in the presence of toluidine blue (10^{-4} mol dm^{-3}).

The decay of the hydrated electron absorbance closely followed first-order kinetics (figure 2A: ln absorbance vs. time) and derived from second-order (figure 2A: 1/absorbance vs. time). The first order rate constant was linearly dependent on toluidine blue concentration from which the bimolecular rate constant for the reaction of the hydrated electron with the solute was calculated to be $(1{\cdot}7 \pm 0{\cdot}4) \times 10^{10}$ dm^3 mol^{-1} s^{-1}. This indicates high reactivity of the hydrated electron with this compound. The behaviour was very similar at pH 12·2 also (see figures 1B and 2B). The bimolecular rate constant was about six times lower at this pH where 95% of toluidine blue is present in the deprotonated neutral form. As the compound is not stable above

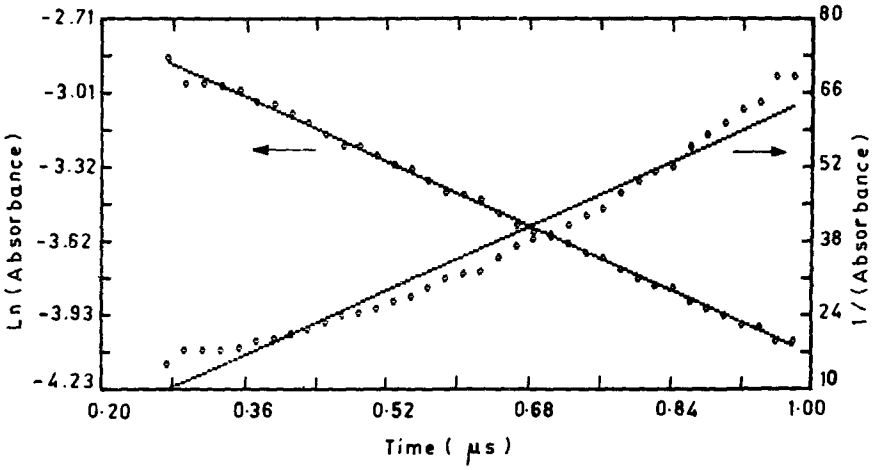


Figure 2A. Kinetic analysis of the decay of the hydrated electron absorbance at 700 nm in 0·1 mol dm^{-3} *t*-butanol and 10^{-4} mol dm^{-3} toluidine blue at pH 6·8.

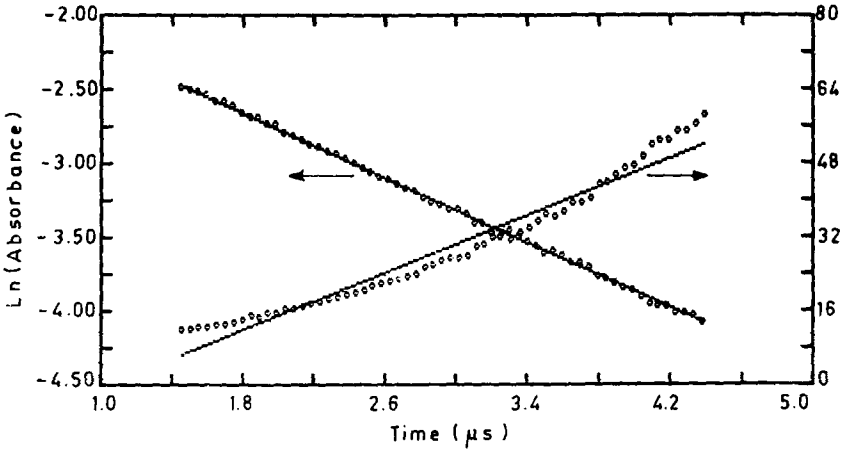


Figure 2B. Kinetic analysis of the decay of the hydrated electron absorbance at 700 nm in 0·1 mol dm^{-3} *t*-butanol and 10^{-4} mol dm^{-3} toluidine blue at pH 12·2.

pH 12·5, the above value was confirmed by measuring the rate constant with e_{aq}^- at pH 11·5 (neutral and protonated forms are, respectively, 65 and 30%) and at pH 11 (when equimolar concentrations of the two are present). From the observed pseudo first-order rate constants at these pH's and knowing the rate constant for the protonated form ($1{\cdot}7 \times 10^{10}$ dm^3 mol^{-1} s^{-1}), that for the neutral form was calculated to be $(2{\cdot}22 \pm 0{\cdot}2) \times 10^9$ dm^3 mol^{-1} s^{-1}. When we correct for the effect of the ionic strength on the basis of the Bronstead–Bjerrum equation (see e.g. Chiorboli *et al* 1988) the rate constant of the protonated form becomes $2{\cdot}5 \times 10^{10}$ dm^3 mol^{-1} s^{-1} at zero ionic strength. This value is close to the diffusion controlled bimolecular rate constant ($\mu = 0$) for oppositely charged species in aqueous solutions (radii assumed: 0·25 and 0·2 nm). However for the neutral form the above experimental value is about a factor of 3·3 lower than the diffusion controlled value ($7{\cdot}4 \times 10^9$ dm^3 mol^{-1} s^{-1}). In this connection it may be noted that in many cases rate constants for electron transfer reactions are much lower than the diffusion-controlled values that have been experimentally observed (see for e.g. Chiorboli *et al* 1988, for a recent reference). This is due to the fact that although formation of the activated complex is diffusion-controlled, its subsequent dissociation to give the products (in the present case electron transfer) has to compete with the dissociation to give the reactants. The observed rate constant for electron transfer will be equal to the diffusion-controlled value only when the electron transfer is much faster than dissociation to the reactants, whereas when the two are of comparable magnitude the observed value will be lower than the diffusion-controlled value (Chiorboli *et al* 1988). Our experimental results indicate that in the case of reaction between oppositely charged species TBH^+ and e_{aq}^- the electron transfer is much faster than the dissociation, which could be attributed to the strong electrostatic attraction between these oppositely charged species. When there is no such driving force as in the case of the reaction of e_{aq}^- with neutral toluidine blue, the electron transfer rate is comparable to the rate of dissociation of the activated complex leading to an observed bimolecular rate constant appreciably lower than the diffusion-controlled value.

The absorbances of the species resulting from the reaction of e_{aq}^- with toluidine blue were monitored over the entire spectral region from 300–850 nm. Two absorption bands were observed with λ max at $\sim$ 400 nm and $\sim$ 830 nm (figure 3). The maximum absorbances at these wavelengths occurred 4 μs after the pulse. The formation of the transient absorbing at these wavelengths was found to be pseudo first-order with respect to toluidine blue concentration. From build up traces the bimolecular rate constant was computed and found to be close to the value derived from e_{aq}^- decay. The product was found to decay by second-order kinetics with rate constants of $0{\cdot}9 \times 10^9$ dm^3 mol^{-1} s^{-1} and $1{\cdot}03 \times 10^9$ dm^3 mol^{-1} s^{-1} at 400 nm and 800 nm respectively.

The spectrum obtained by reaction of isopropyl radicals with toluidine blue at pH 6·8 was found to be identical to the one observed by e_{aq}^- reaction (figure 3). The build up of the absorbances at these wavelengths reached a maximum $\sim$ 10 μs after the electron pulse. Kinetic analysis of the transient absorption signals at 400 nm and 830 nm indicated that the decay followed the second-order rate law with the same rate constant as in the case of species formed by direct e_{aq}^- reaction in the *t*-butanol matrix. The bleaching recovery at $\lambda = 580$ nm was to an extent of $\sim$ 50% indicating that the decay of the species is by dismutation. The closeness of the spectra and the decay kinetics suggest that the species formed by the hydrated electron reaction and by reaction of α-hydroxy

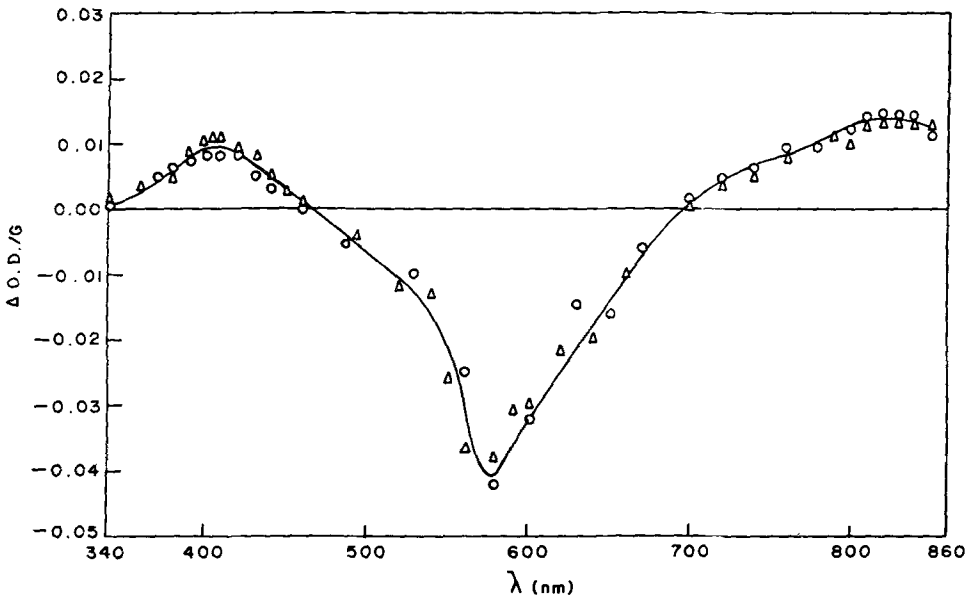


Figure 3. Absorption spectra of transient species obtained on pulse radiolysis of 10^{-4} mol dm^{-3} toluidine blue solutions (pH 6·8) using (○) isopropanol (1 mol dm^{-3})–N_2O matrix, and (Δ) *t*-butanol (0·1 mol dm^{-3}) matrix.

isopropyl radicals are the same. In the N_2O saturated isopropanol matrix the absorbances were found to be twice those observed in the N_2 saturated *t*-butanol matrix since in the former case e^-_{aq}, H and OH are converted to $(CH_3)_2$ COH radicals unlike in the latter where OH radicals are scavenged by *t*-butanol and only e^-_{aq} and H-atoms are available for reaction with toluidine blue.

The spectrum of the product formed by reaction of e^-_{aq} or isopropanol radicals is thus very similar to that of semithionine (Guha *et al* 1987) and semimethylene blue (Solar *et al* 1982) generated by similar reactions with thionine and methylene blue respectively.

4. pK of semitoluidine blue

The extinction coefficient and λ_{max} values of semireduced toluidine blue species showed a marked change with pH suggesting the existence of the species in different conjugate acid–base forms. The effect of pH on the absorbance was more marked at 830 nm. The transient absorbance changes at 830 nm were monitored as a function of pH (figure 4) using a deoxygenated isopropanol–acetone matrix containing 10^{-4} mol dm^{-3} toluidine blue. Two inflexion points at pH 1·9 and 8·5 were observed suggesting the existence of three conjugate acid–base forms which can be formally represented as,

$$TBH_3^{2+} \underset{1\cdot9}{\overset{-H^+}{\rightleftharpoons}} TBH_2^{+} \underset{8\cdot5}{\overset{-H^+}{\rightleftharpoons}} TBH, \tag{3}$$

or

$$TBH_2^{+} \underset{1\cdot9}{\overset{-H^+}{\rightleftharpoons}} TBH \underset{8\cdot5}{\overset{-H^+}{\rightleftharpoons}} TB^-, \tag{4}$$

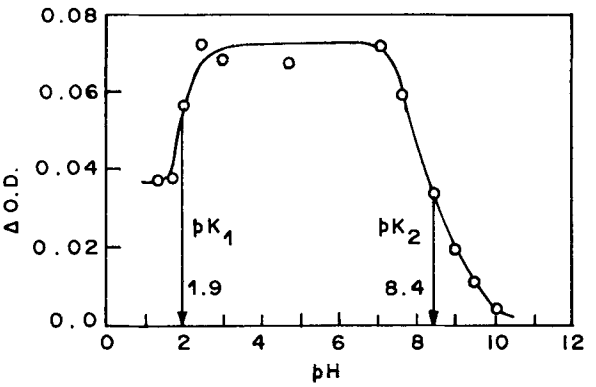


Figure 4. pH dependence of absorbance of semireduced toluidine blue at 830 nm in a deoxygenated isopropanol (1 mol dm^{-3})–acetone (0·1 mol dm^{-3}) matrix containing 10^{-4} mol dm^{-3} toluidine blue.

where TB represents a neutral toluidine blue molecule. A third possibility,

$$\underset{}{\mathrm{TBH}} \underset{1\cdot9}{\overset{-\mathrm{H}^+}{\rightleftharpoons}} \mathrm{TB}^- \underset{8\cdot5}{\overset{-\mathrm{H}^+}{\rightleftharpoons}} \mathrm{TB}^{--}, \tag{5}$$

is ruled out because TBH^+ has only one ionisable proton (see TBH^+ in scheme 1).

The above two equilibria (3) and (4) were distinguished from each other by studying the effect of ionic strength on the second-order decay rate constant. The results are shown in table 1 and indicate the presence of a singly-charged species at neutral pH and a neutral species at alkaline pH. The higher rate constant obtained at higher ionic strength at pH 6·8 was indicative of a reaction between two like-charged species. The rate constants obtained at pH 10 in the presence and absence of added salt were close to each other indicative of a neutral species at this pH. The absence of a salt effect at the highly acidic pH may perhaps be attributed to the possibility that the species TBH_2^+ exists as an iron-pair with the counterion and hence effectively behaves as a neutral species. Also, the TBH form is ruled out at this pH as discussed above. Hence it can be inferred that the equilibria represented by (3) explain the protonation of semitoluidine blue species.

Table 1. Absorption maxima, extinction coefficients and decay kinetics of semireduced toluidine blue.

Species	pH	λ_m	Extinction coefficient (dm^3 mol^{-1} cm^{-1})	$2k/\varepsilon$(s^{-1})	
				In absence of salt	In presence of salt
TBH_3^{2+}	1.4	390	6250		
		830	6370		
TBH_2^+	6·8	400	5700	0·1902 × 10^6	0·314 × 10^6
		830	10760	(0·028)*	(0·128)
TBH	10	410	7170	0·138 × 10^6	0·156 × 10^6
				(0·03)	(0·13)

* Numbers in parentheses refer to ionic strengths in mol dm^{-3}

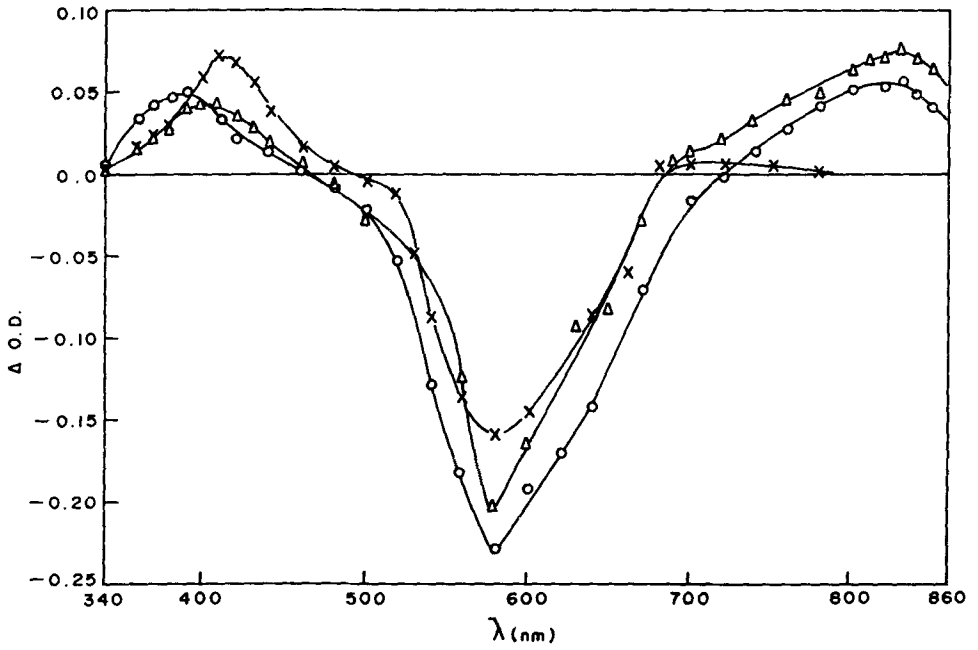


Figure 5. Absorption spectra of transient semitoluidine blue species in *e*-pulsed irradiated isopropanol (1 mol dm^{-3}) matrices containing 10^{-4} mol dm^{-3} toluidine blue (× pH 9·8; ○ pH 1·4; Δ pH 6·8).

The spectra of all the three forms were monitored 10–12 μs after the pulse (figure 5) in N_2O saturated matrices of 10^{-4} mol dm^{-3} toluidine blue and 1 mol dm^{-3} isopropanol. A characteristic feature of all the three forms is a band in the 400 nm region. The other band in the longer wavelength region (800 nm) is observed only at pH's 1·4 and 6·8, but at pH 9·6 this band appears to be blue-shifted and merges with the absorption band of toluidine blue. The transient spectrum recorded at a still higher pH was identical to the one obtained at pH 9·8 except that the longer wavelength band was better defined and indicated a clear maximum at 680 nm. This was possible because at this pH the spectral band of the ground state dye molecule is appreciably blue-shifted and hence does not interfere with the product transient species. A similar observation has been made in the case of thionine (Guha *et al* 1987). The characteristic features of different acid-base forms are summarized in table 1. The extinction coefficients have been calculated from the observed absorbance of the species using an isopropanol–acetone matrix (G total reducing species = 6) and lower doses to minimise radical–radical reactions. The extinction coefficient values determined are absolute ones obtained knowing the concentration of the transients formed from the G values of the reducing radicals and the dose per pulse. Further since it is known that only 85% of the radicals produced are reducing in nature this factor has been taken into account while calculating the extinction coefficient. Also at 390 nm where toluidine blue has appreciable absorption, appropriate corrections have been made to account for ground state bleaching.

5. Reaction with H-atoms

The absorption spectra of the short-lived species produced by reaction of H-atoms with toluidine blue in N_2-saturated acidic solutions were monitored from 300 to 850 nm. The product transient showed two absorption bands in the 400 nm and 800 nm region (figure 6) which were similar to the ones observed in the case of semitoluidine blue species generated by electron transfer from $(CH_3)_2$ COH radicals. The rate constant for the formation of the transient species by the reaction of H-atoms was determined to be $1{\cdot}0 \times 10^{10}$ dm^3 mol^{-1} s^{-1}. The transient species decayed by second-order kinetics with a rate constant of $1{\cdot}0 \times 10^9$ dm^3 mol^{-1} s^{-1}. By quantitative comparison of the transient absorbances in the 800 nm band in the N_2-saturated *t*-BuOH matrix and in the isopropanol–N_2O matrix, it was inferred that only 85% of the H-atoms react with toluidine blue by one-electron transfer.

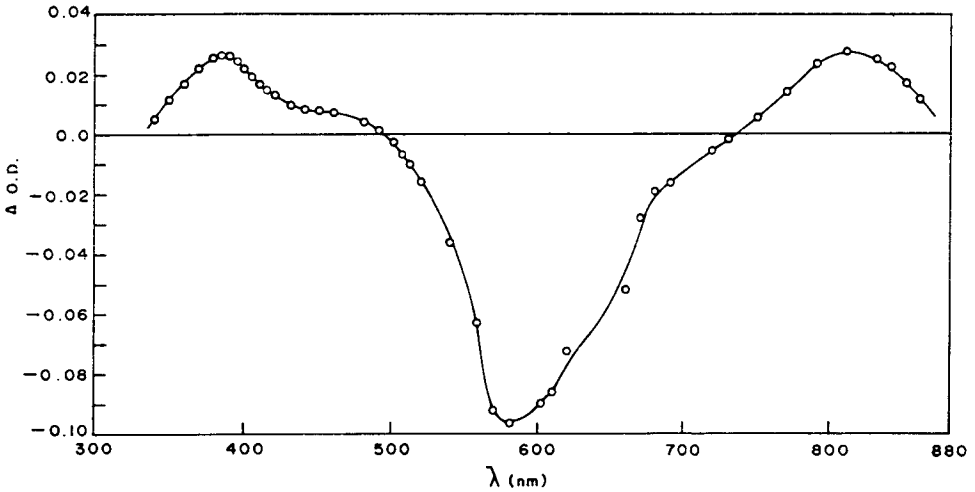


Figure 6. Absorption spectrum of semireduced toluidine blue species produced by reaction with H-atoms in N_2-saturated *t*-butanol (1 mol dm^{-3}) matrix containing 2×10^{-4} mol dm^{-3} toluidine blue (pH 1·6).

6. Reaction of toluidine blue with other organic reducing radicals

The 830 nm band of semitoluidine blue was observed in electron-pulsed N_2O-saturated neutral toluidine blue solutions containing 0·1 mol dm^{-3} of various organic compounds indicating that the radicals obtained from these compounds are also capable of bringing about one-electron reduction of toluidine blue as observed in the case of α-hydroxy isopropyl radicals. The absorbance changes and the kinetics of the semireduced species were monitored at this wavelength where the absorbance due to the ground state molecule is negligibly small. The rate constants for the one-electron reduction were evaluated from build up traces. The results are shown in table 2. The reducing ability of these radicals are related to their reduction potential values and the extent of semitoluidine blue formed by these organic radicals followed the trend in their one-electron reduction potentials. A similar trend was observed for the rate

Table 2. Rate constants for one-electron reduction of toluidine blue by organic radicals (pH 6·8).

Reducing radical obtained from	k ($dm^3 mol^{-1} s^{-1}$)	E^0 (vs. NHE) (Volts)
Isopropanol	$3·7 \times 10^9$	−1·05
EtOH	$4·0 \times 10^9$	−0·95
MeOH	$3·7 \times 10^9$	−0·73
THF	$3·66 \times 10^9$	−0·55
Dioxan	$2·60 \times 10^9$	+0·05
Glucose	$2·20 \times 10^9$	+0·06
t-Butanol	0·0	+0·15
Cytosine	$3·0 \times 10^9$	−0·12

constant for the reaction of these radicals with toluidine blue. From the inflexion point of semitoluidine blue absorbance vs. redox potential and the reaction rate constant vs. redox potential (figure 7), the one-electron reduction potential of toluidine blue was calculated to be +0·05 ±0·01 V (vs. NHE). The literature value for this potential as determined by the EMF method (Nikolskii and Palchevskii 1958) is +0·034 V vs. NHE. In view of the uncertainties associated with experimental procedures, the two values are considered to be in agreement.

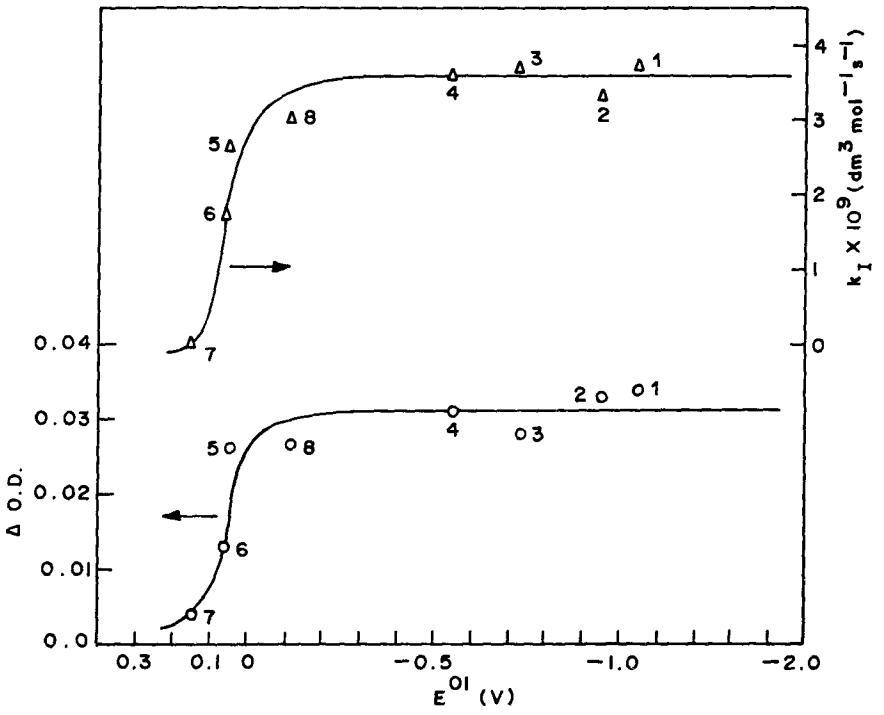


Figure 7. Redox titration curve for toluidine blue (numbers on the curve correspond to those in table 2). (○ OD values; △ rate constants.)

7. Conclusion

Toluidine blue, methylene blue and thionine contain the parent phenothiazine ring and hence show similar trends in their behaviour towards various reducing radicals. The reactivity of e_{aq}^- towards toluidine blue ($1{\cdot}7 \times 10^{10}\,dm^3\,mol^{-1}\,s^{-1}$), thionine ($3 \times 10^{10}\,dm^3\,mol^{-1}\,s^{-1}$) (Guha *et al* 1987) and methylene blue ($2{\cdot}2 \times 10^{10}\,dm^3\,mol^{-1}\,s^{-1}$) (Solar *et al* 1982) is very high and similar in nature. Further, the spectra of the semireduced species formed in all three cases closely resemble each other since all the three dyes are derivatives of phenothiazine and differ in their structure only with respect to the methyl substituents on the amino groups. Their *pK* values and redox potential values are also very similar. However in the case of the H-atom reaction a different reactivity has been observed. Unlike in the case of thionine where only two transients have been observed, viz. the semiquinone and H-atom adduct, in the case of methylene blue a third species resulting from the attack of the H-atom on the S-atom and having λ_{max} at 280,400 and 800 nm has been reported and attributed to the presence of methyl substituted amino groups in the molecule (Solar *et al* 1982). Since toluidine blue like methylene blue also contains a methyl-substituted amino group a similar observation is expected. However because of instrumental limitations we have not been able to look into the wavelength region below 300 nm to positively identify this species.

Acknowledgements

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