Pulse radiolysis study of one-electron oxidation of thionine in aqueous solutions

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Abstract. One-electron oxidation of thionine has been studied using specific oxidizing radicals such as Cl_2^-, $Tl(II)$ and N_3 generated by pulse radiolysis of aqueous solutions. The semioxidized thionine exhibited three pK's indicating four conjugate acid-base forms. N_3 radicals were found to be less efficient in oxidizing thionine as compared to Cl_2^-, Tl^{2+} and $Tl(OH)^+$. The rate constants for electron abstraction from thionine by Cl_2^-, Tl^{2+}, $Tl(OH)^+$, $Tl(OH)_2$ and N_3 were evaluated. The spectra of different protonated forms of semioxidized thionine and the extinction coefficients at λ_{max} are presented. Reaction of OH radicals with thionine gave transient products whose spectra and acid-base properties were different from those of semioxidized thionine. The rate constant for formation of the product transient agrees well with competition kinetic value for reaction of OH with thionine reported earlier.

Keywords. Pulse radiolysis; one-electron oxidation; thionine oxidation.

1. Introduction

The one-electron reduction of thionine has been the subject of many investigations in the past, employing flash photolysis and pulse radiolysis techniques. Recently it has been shown (Guha *et al* 1987) that many organic radicals generated in pulse radiolysis experiments are able to bring about one-electron reduction of this molecule, and from a correlation of the efficiency of reduction of thionine and the standard potential for oxidation of the radical, the standard potential for the one-electron reduction of this compound was inferred. This value is in fairly good agreement with the indirect estimate made in the past (Rabinowitch 1940). There are no reports of one-electron oxidation of thionine in the literature. Kamat and Lichtin (1982), had however, attributed a transient absorption spectrum with a maximum at 480 nm observed in laser flash photolysis of thionine in aqueous solution (at pH ~ 2) to the semioxidized form resulting from a net one electron transfer between the ground and excited triplet states of the molecule. There is, however, no direct proof to show that the species so observed is in fact the semioxidized form. We have therefore carried out pulse radiolysis experiments to find out whether one-electron oxidation of thionine can be directly observed via the use of well-known one-electron oxidants such as OH, Cl_2^-, Tl^{2+}, I_2^-, Br_2^- and $\dot{N}_3$ that can be generated in aqueous solutions. The results of these investigations are reported

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here. Reaction of SO_4 with thionine could not be studied as there was precipitation on addition of $K_2S_2O_8$ to aqueous thionine solution.

2. Experimental

Thionine used in this study was from Fluka and was used after purification as described earlier (Guha *et al* 1982). Other chemicals were either BDH 'Analar' or E Merck 'GR' grade. 'IOLAR' grade N_2, O_2 and N_2O from Indian oxygen were used for saturating the solutions. The pH of the solution was adjusted using H_2SO_4, NaH_2PO_4 and $Na_2B_4O_7$. 10 H_2O and NaOH in the appropriate ranges. The pulse radiolysis experimental set up used in this study has been fully described earlier (Guha *et al* 1987). In all the experiments 25 nS electron pulses were employed and dosimetry was carried out using air saturated 0·05 mol dm^{-3} KCNS for which $G\epsilon = 21,522$ dm^3 mol^{-1} cm^{-1} per 100 eV at 500 nm (Fielden 1982).

3. Results

Irradiation of water by ionizing radiations generates both oxidizing (OH) and reducing (e_{aq}^-, H) species. In oxygen saturated acid media (pH < 4) the reducing species are completely converted to HO_2 radicals within about 3 μs following a 25 nS pulsed electron irradiation. On pulsing solutions, which also contained 0·1 mol dm^{-3} *t*-butanol as scavenger for the OH radical and 5×10^{-5} mol dm^{-3} of thionine, no transient light absorbing species were observed, thus revealing the inertness of HO_2 towards thionine. However, when *t*-butanol was absent, a transient spectrum was observed (figure 1, curve a) which had maxima at 385, 475 and 760 nm, and a shoulder at 450 nm. These features are very similar to the ones
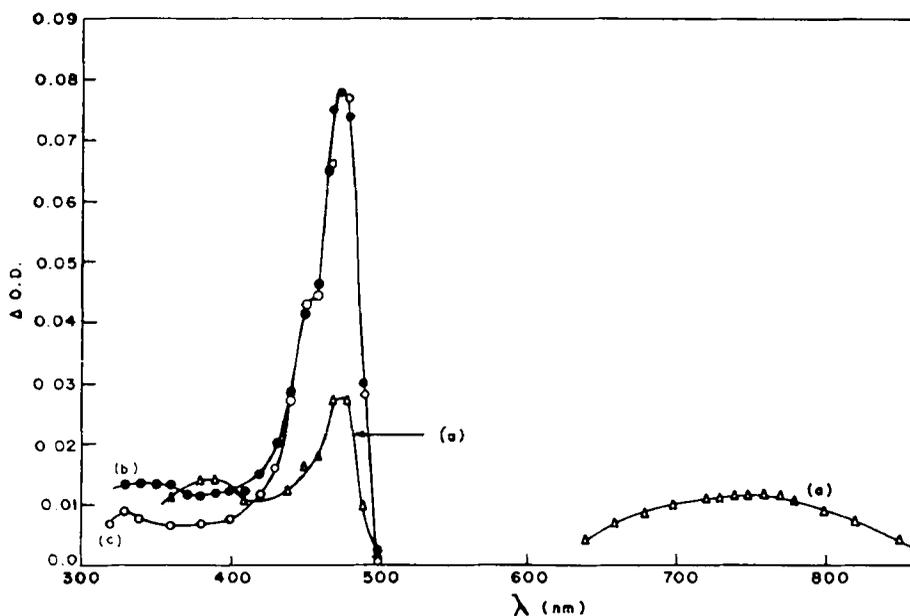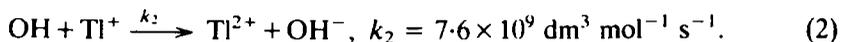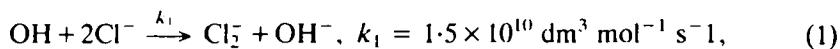

Figure 1. Absorption spectra of transient products formed by the reaction of OH (a), Cl_2 (b) and Tl^{2+} (c) in *e*-pulse irradiated aqueous thionine solutions at pH 1·7.

reported by Kamat and Lichtin (1982) and attributed by them to an equimolar mixture of semioxidized and semireduced forms of thionine formed by inter-molecular electron transfer between ground and triplet excited thionine. In our system (O_2-saturated acid medium), as noted above, the HO_2 radicals are inert, and reaction of thionine with the oxidising species (OH) can give rise to only the semioxidized thionine species. Although OH is a very strong oxidant ($E° = 2·8$ V; Buxton 1982), it can also bring about reactions other than oxidation such as addition to the heteroaromatic ring of thionine, abstraction of hydrogen atom etc. Hence we were led to try more specific and known one-electron oxidants (Bonifacic and Asmus 1976) viz. Cl_2^-, Tl^{2+}, Br_2^-, N_3 etc.

In oxygen saturated acidic solutions OH radicals can be quantitatively converted to Cl_2^- and Tl^{2+} via reactions:

$$OH + 2Cl^- \xrightarrow{k_1} Cl_2^- + OH^-, \quad k_1 = 1·5 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}, \tag{1}$$

$$OH + Tl^+ \xrightarrow{k_2} Tl^{2+} + OH^-, \quad k_2 = 7·6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}. \tag{2}$$

The transient spectra observed on pulsing acidic solutions containing 5×10^{-5} mol dm^{-3} thionine and $0·01$ mol dm^{-3} Cl$^-$ or $0·002$ mol dm^{-3} Tl$^+$ are shown in figure 1 (curves b and c). These spectra show a maximum at ~ 480 nm and a shoulder at ~ 450 nm, but no broad maxima in the 760 nm region, and are remarkably similar to the semioxidized thionine species reported by Kamat and Lichtin (1982). Further evidence to suggest the generation of the species by reaction with Cl_2^- can be seen in figure 2 which depicts the time resolved transient
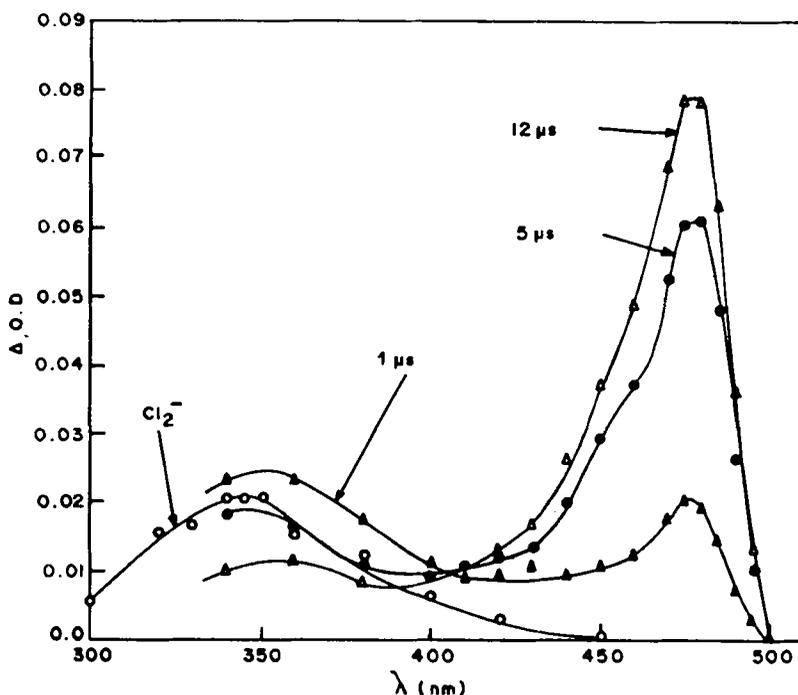

Figure 2. Time resolved absorption spectra of semioxidized thionine species formed by the reaction of Cl_2 ($\blacktriangle$-1 μs; $\bullet$-5 μs; $\triangle$-12 μs after the e-pulse) and the absorption spectrum of Cl_2 radical (O-1 μs after the e-pulse).

spectra of electron beam pulsed O_2 saturated acidic solutions containing $0 \cdot 01$ mol dm^{-3} Cl^- and 5×10^{-5} mol dm^{-3} thionine. It is seen that with passage of time the Cl_2^- absorption peak at 345 nm progressively diminishes in intensity accompanied by a simultaneous increase in the 480 nm peak from the product. The transient signals at 345 nm due to the Cl_2^- species in the absence and presence of thionine in oxygen-saturated acidic solution containing $0 \cdot 01$ mol dm^{-3} Cl^- are given in figure 3. From this it is seen that Cl_2^- decay becomes appreciably faster in the presence of thionine, indicating the reaction of Cl_2^- with that compound. From the kinetics of build-up of product transient absorbance at 480 nm which was found to be pseudo first-order with respect to thionine concentration, a value of $3 \cdot 3 \times 10^9$ dm^3 mol^{-1} s^{-1} was calculated for the rate constant of this reaction.

The Tl^{2+} species has an absorption spectrum which overlaps with that of thionine; hence the effect of the latter on its decay could not be studied. The product transient build-up at 480 nm was again found to be pseudo first-order with respect to thionine concentration and a value of $\cdot 3 \times 10^9$ dm^3 mol^{-1} s^{-1} was obtained for the bimolecular rate constant.

Reaction of Cl_2^- or Tl^{2+} cannot be studied in the neutral pH region, because the reaction of Cl^- with OH is extremely slow at pH > 3 (Anbar and Thomas 1964) whereas Tl^{2+} hydrolyses to $Tl(OH)^+$ and $Tl(OH)_2$ in less acidic media; $pK_1 = 4 \cdot 6$, $pK_2 = 7 \cdot 7$ (Bonifacic and Asmus 1976b). One-electron oxidants useful in the higher pH region are N_3, Br_2^-, I_2^- which can be generated by electron beam pulsing of N_2O saturated aqueous solutions of NaN_3, KBr and KI, respectively, via reactions:
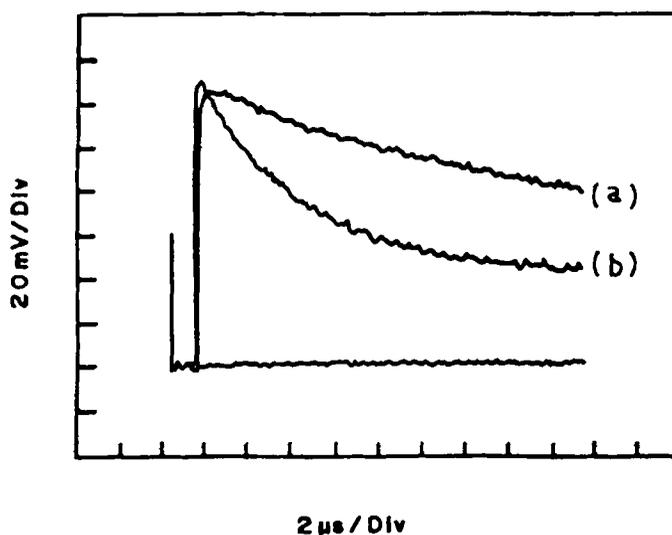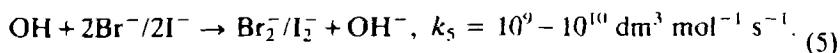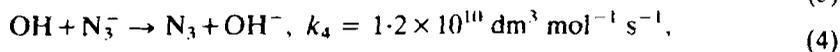
$$e_{aq}^- + N_2O + H_2O \rightarrow N_2 + OH + OH^-, \quad k_3 = 8 \cdot 9 \times 10^9 \ dm^3 \ mol^{-1} \ s^{-1},$$

(3)

$$OH + N_3^- \rightarrow N_3 + OH^-, \quad k_4 = 1 \cdot 2 \times 10^{10} \ dm^3 \ mol^{-1} \ s^{-1},$$

(4)

$$OH + 2Br^-/2I^- \rightarrow Br_2^-/I_2^- + OH^-, \quad k_5 = 10^9 - 10^{10} \ dm^3 \ mol^{-1} \ s^{-1}.$$

(5)

2 µs / Div

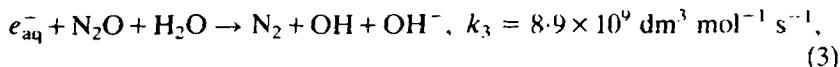**Figure 3.** Decay of Cl_2^- radical absorption ($\lambda = 345$ nm) in the absence (a) and presence (b) of thionine (5×10^{-5} mol dm^{-3}), pH $1 \cdot 7$.

N_2O saturated aqueous solution (pH 6–10) containing 0.05 mol dm^{-3} KBr or KI and 10^{-4} mol dm^{-3} thionine on e-beam pulsing did not reveal the formation of any transient light absorbing species indicating that Br_2^- and I_2^- are not able to oxidize thionine. However, N_2O saturated solutions containing 0.005 mol dm^{-3} NaN_3 and 10^{-4} mol dm^{-3} thionine did produce transient light absorbing species on e-beam pulsing. These had features in the 400–500 nm region characteristic of the semioxidized thionine species. Hence it is inferred that N_3 radicals are able to oxidize thionine. Typical absorption spectra of product species are given in figure 4. The transient spectra obtained by the Tl(II) reaction with thionine at pHs 5.8 and 9.6 (figure 4) were similar to those obtained by N_3 reaction at pHs 4.8 and 9.6, supporting the above observation.

In order to evaluate the pK of the semioxidized thionine species, transient absorbance at 480 nm of e-beam pulsed solutions were measured as a function of pH. Because of the fact that (1) is very slow above pH 3 and N_3^- has a pK = 4.7, the HN_3 species present at pH < 4 being rather unreactive towards OH, a single matrix could not be employed for the entire pH region. Below pH 3, O_2-saturated 0.01 mol dm^{-3} NaCl was used, whereas above pH 4, the matrix employed was N_2O saturated 0.05 mol dm^{-3} NaN_3. The net G-values for the oxidizing species in these two matrices are 2.8 and 5.5, respectively; hence the measured absorbances were normalized to unit G-value of the oxidizing species and plotted (figure 5). The plot clearly reveals two inflexion points, corresponding to pK = 6.9 and 8.3. It is difficult to infer from this figure whether there is one more pK at more acidic pH. This region was investigated by using Tl(II) as the oxidizing species. The results plotted in figure 5 clearly reveal the presence of another pK at 4.3 for the
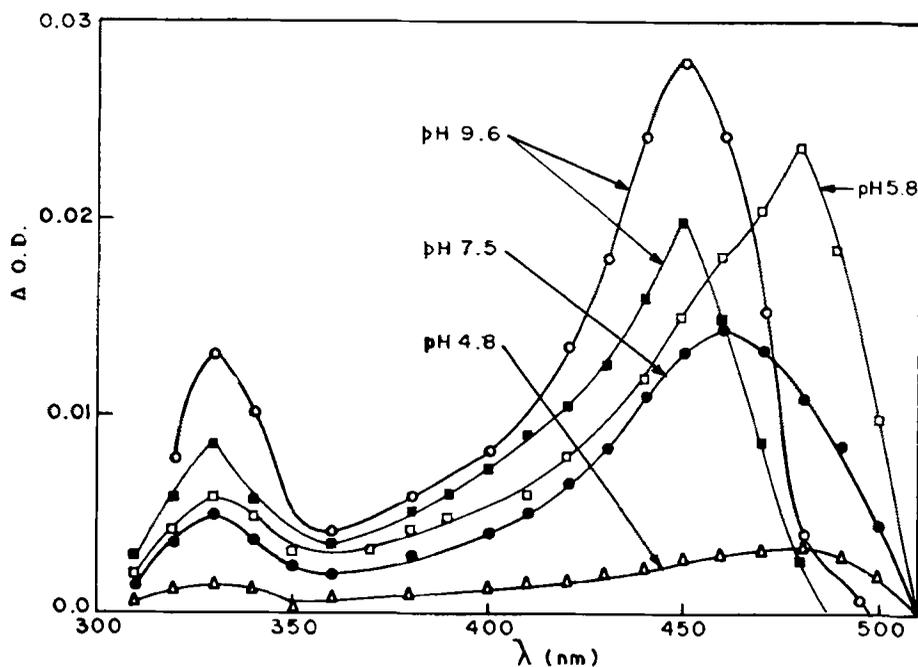

Figure 4. Absorption spectra of different conjugate acid-base forms of semioxidized thionine species produced by the reaction of N_3 ($\triangle$, $\bullet$, $\bigcirc$) and Tl(II) ($\square$, $\blacksquare$).
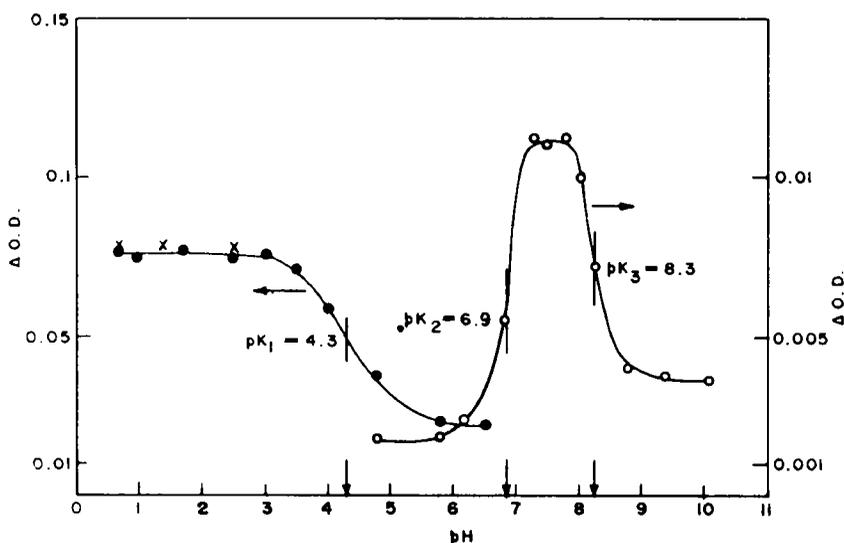
Figure 5. Absorbance changes with pH at 480 nm for the semioxidized thionine species formed by Cl_2 ($\times$), Tl(II) ($\bullet$) and N_3 ($\bigcirc$).

semioxidized thionine species. That this is a genuine pK for this species and not an artefact due to the different hydrolytic forms of Tl(II) with pK at 4·6 is supported by the following observations. First, both at pH 2·5 and 5·8, the two forms of Tl(II) were found to oxidize thionine with the same rate constant of 3×10^9 dm^3 mol^{-1} s^{-1}. Hence the extent of oxidation by $Tl(OH)^+$ form cannot be expected to be very much less than that by the Tl^{2+} form. Bonifacic and Asmus (1976b) have reported that the oxidizing efficiency of $Tl(OH)^+$ is $\sim 85\%$ of that of Tl^{2+} in the case of Me_2S_2 whereas $Tl(OH)_2$ form has only 20% efficiency. In another system (Moorthy *et al* 1987) semioxidized riboflavin, which has no pK in this region, exhibited no change in the transient absorption as a function of pH on pulsing O_2-saturated solutions containing Tl^+, thus indirectly showing that the inflexion observed in the case of thionine is not an artefact. The λ_{max} and ε_{max} values of the different conjugate acid-base forms are given in table 1.

Although the spectrum of the product species formed from thionine by reaction with OH radicals has some resemblance to that of the semioxidized species, further experiments revealed that the two species are quite different. Thus the absorbance of the OH reaction product at 770 and 480 nm changes with pH (figure 6) in a manner quite different from the one observed in the case of the semioxidized species (figure 5). Also the spectra at pH 9·5 and 3 observed for the OH reaction product (figure 7) are quite different from those of the semioxidized species (figures 2–3). Although the 770 nm band is indicative of the semireduced thionine species, this band present in the spectrum of the OH reaction product cannot be ascribed to the semireduced species for the following reason. In the pulse radiolytic reduction studies on thionine reported earlier (Guha *et al* 1987), the 770 nm band was totally absent in the spectra in alkaline pHs, whereas this band is observed in the case of OH reaction product at all pHs. The absorption spectrum at pH 9·5 appears to be that due to a mixture of species.

Table 1.

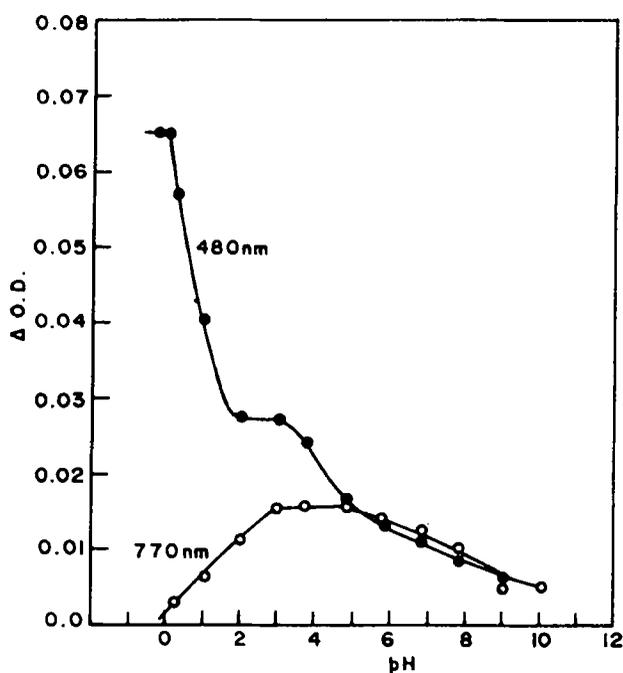| Protonated form of semioxidized thionine	pH	λ_{max} (nm)	Corrected ε (10^4 dm^3 mol^{-1} cm^{-1})
TH_2^{3+}	1·7	480	3·5
TH^{2+}	5·8	480	1·35
T^+	7·5	460	2·8
$T(-H^+)$	9·6	450	5·3

Figure 6. Absorbance changes with pH for the OH-reaction product of thionine at 770 and 480 nm.

4. Discussion

The standard potentials for reduction of the various one-electron oxidants as reported in the literature are summarized in table 2. Generally the more positive this potential, the stronger the species is expected to be as an oxidizing agent. The rates and efficiencies of oxidation are expected to follow this trend. Although there are divergent values for the potential of the N_3/N_3^- couple, the lower value is supported by recent equilibrium pulse radiolysis experiments and confirmed by cyclic voltammetry (Alfassi *et al* 1987). Our observation that thionine is oxidizable by Cl_2^-, Tl^{2+} and N_3 but not by I_2^- would place the potential of the semioxidized thionine/thionine couple between $+2·3$ and $+1·0$ volt. From the normalized
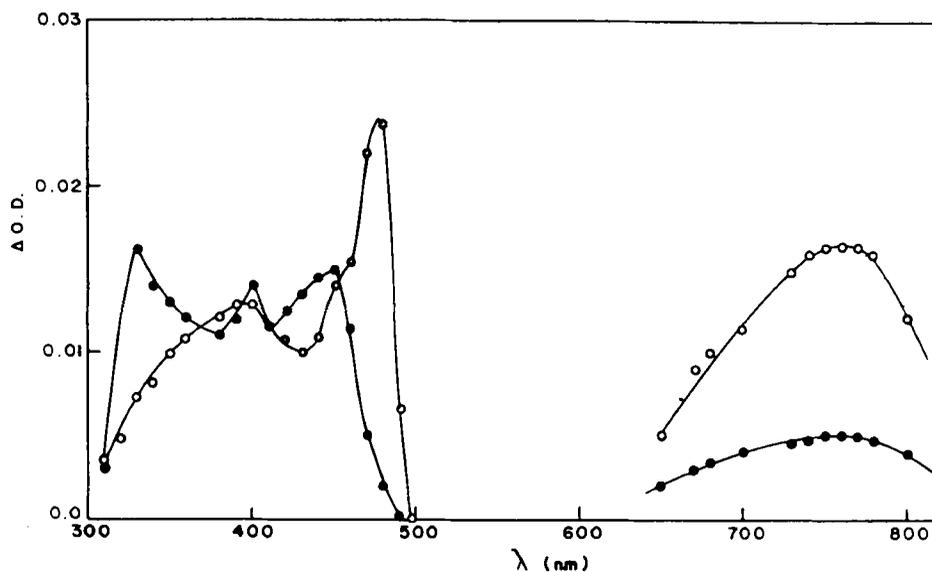
Figure 7. Absorption spectra of OH-reaction product at pH 3 (○) and pH 9·6 (●).

**Table 2.** One-electron oxidation potential of various radicals.

Radical	$E^{o\prime}$ (V vs. NHE)	Reference
Tl^{2+}	2·22	Schwarz et al (1974)
Cl_2^-	2·3	Henglein (1980)
Br_2^-	1·7	Henglein (1980)
I_2^-	1·0	Henglein (1980)
N_3	1·32	Alfassi et al (1987)

absorbance vs. pH curves (figure 5) for the semioxidized species generated by using different oxidant species, it would appear that the efficiency of N_3 for oxidizing thionine is considerably less than that of Cl_2^-, Tl^{2+} or $Tl(OH)^+$, but is comparable to that of $Tl(OH)_2$. (However, the rate constants of these oxidation reactions do not reflect this trend, being all $2-4 \times 10^9$ dm^3 mol^{-1} s^{-1}). Hence the potential of the semioxidized thionine/thionine couple is likely to be close to but more positive than that of the N_3/N_3^- couple (table 1), i.e. 1·3 V, as the efficiency of this couple in oxidizing thionine is considerably less than 50%. Considering this, the inability of Br_2^- to oxidize thionine would at first sight suggest that the potential of the $Br_2^-/2Br^-$ couple must be much lower than 1·7 volts. However the equilibrium pulse radiolysis experiments by Alfassi et al (1987) between the N_3/N_3^- and $Br_2^-/2Br^-$ couples lead to the value of 1·3 V for the former on the basis of a value of 1·63 V for the latter couple. Hence the inability of Br_2^- to oxidize thionine appears to be inexplicable on the basis of redox potentials alone.

The extinction coefficient of semioxidized thionine at the λ_{max} of 480 nm at pH 1·7 (evaluated by assuming the extent of oxidation of thionine by Tl^{2+} or Cl_2^- at this pH

to be 100%) is 35,000 dm^3 mol^{-1} cm^{-1}. This value is considerably higher than the one reported by Kamat and Lichtin (1982). It may be noted that their estimate is based on the assumption that under photochemical excitation intermolecular electron transfer is the only route for the formation of semioxidized and semireduced thionine. If, as reported by Somer and Green (1973), photoexcited thionine, particularly at high concentrations, is reducible by water also, contributing to an additional yield of semithionine, the extinction coefficient based on the above assumption is expected to be lower. From our results the extent of oxidation of thionine by N_3 was found to be $\sim 18\%$; this value is made use of to evaluate the extinction coefficient of semioxidized thionine at the λ_{max} values of its spectra at pH 9·6 and 7·5, respectively. The value for the other two forms were calculated on the basis of 100% efficiency of oxidation by $Tl(OH)^+$ and Cl_2^-.

The present experiments do not throw any light on the site of oxidation of thionine; it may be the heteroaromatic thiazine ring. In neutral solutions the molecule is present as the monocation (TH^+) and has pK values of 0·3 and 11:

$$TH_2^{2+} \underset{}{\overset{0.3}{\rightleftharpoons}} TH^+ + H^+ \underset{}{\overset{11}{\rightleftharpoons}} T + 2H^+.$$
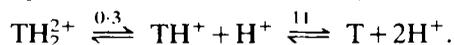
As the semioxidized species is electron deficient, it can be expected to lose protons more readily than the parent thionine. Hence the pK values of the former are assigned as follows:

$$TH_2^{3+} \underset{}{\overset{4.3}{\rightleftharpoons}} TH^{2+} + H^+ \underset{}{\overset{6.9}{\rightleftharpoons}} T^+ + 2H^+ \underset{}{\overset{8.3}{\rightleftharpoons}} T(-H^+) + 3H^+.$$

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