Bull. Chem. Soc. Jpn., 60, 1113—1117 (1987)

A Fast Kinetic Study of Formation and Decay of 2,2′-Azinobis(3-ethylbenzothiazole-6-sulfonate) Radical Cation in Aqueous Solution

Pichai Maruthamuthu,* Lakshmanan Venkatasubramanian, and Periasamy Dharmalingam
Department of Energy, University of Madras, A. C. College Campus, Madras 600 025, India
(Received November 28, 1985)

The kinetics of oxidation of 2,2′-azinobis(3-ethylbenzothiazole-6-sulfonate) (ABTS) to the radical cation, $ABTS^{\cdot+}$ by $S_2O_8^{2-}$, HSO_5^-, MnO_4^-, $Cr_2O_7^{2-}$, Ce(IV), and molecular Cl_2, Br_2, and I_2 and further oxidation to the dication, $ABTS^{2+}$ by the above oxidants except HSO_5^-, Br_2, and I_2 have been carried out by the stopped-flow spectrophotometric technique. The first-stage oxidation was followed by monitoring the absorption of $ABTS^{\cdot+}$ at 417 nm and the second-stage oxidation, by following the disappearance of $ABTS^{\cdot+}$ or the appearance of $ABTS^{2+}$ at 513 nm. Both the reactions, formation and decay of $ABTS^{\cdot+}$ were found to obey a total second-order kinetics, first-order each with respect to [ABTS] or [$ABTS^{\cdot+}$] and [oxidant]. With I_2, ABTS underwent reversible reaction, the stoichiometry of which being,

$$I_2 + 2ABTS \rightleftharpoons 2ABTS^{\cdot+} + 2I^- \text{ with } K = 142.8,$$

whereas Br_2 generated only the radical cation and Cl_2 gave a decomposed product via radical cation and dication intermediates. $ABTS^{2+}$ formed in presence of excess MnO_4^-, $Cr_2O_7^{2-}$, and Ce(IV) decayed as in the case of Cl_2 reaction but in presence of $S_2O_8^{2-}$ the dication was stable for more than three days. In the absence of excess $S_2O_8^{2-}$, $ABTS^{2+}$ went back to $ABTS^{\cdot+}$, possibly via oxidation of water. The radical cation was converted back to the parent compound ABTS by reducing agents such as dithionite and sulfite ions.

Recently much attention has been focussed on the reactions of radical cations in an aqueous solution to explore the possibility of using them in solar energy conversion processes.[1,2] Würster's salt (TMPD) which forms $TMPD^{\cdot+}$ on one-electron oxidation and Weitz's salt (Methylviologen) which forms a radical cation on one-electron reduction are assessed to be electron-relays in chemical routes to solar energy conversion. We have already reported on the fast kinetics of the formation and decay of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine raical cation ($TMPD^{\cdot+}$) in aqueous solution by several oxidizing agents.[3] Previous studies on the polarographic oxidation[4,5] of 2,2′-azinobis(3-ethylbenzothiazole) have revealed the formation of a radical cation and a dication. The utility of ABTS as an analytical reagent for colorimetric determination of periodic acid has also been reported.[6] However no kinetic measurements were carried out. Here we present the results of fast kinetics of formation and decay of radical cation, $ABTS^{\cdot+}$ by one-electron as well as multielectron oxidizing agents.

Experimental

All kinetic measurements were made with Applied Photophysics Model-1705 stopped-flow-spectrophotometer. Absorption output was digitised with a Datalab Model-902 Transient recorder equipped with variable input sensitivity and variable sampling interval, and was monitored by Trio Model-CS-1562A Oscilloscope. About 500—700 points were collected for each kinetic measurement and at least six kinetic runs were made for each rate constant measurement. The data were stored and analyzed by CBM-3032 Personal Computer. The kinetic plots were plotted on a Hewlett-Packard graphics plotter. All the measurements were carried out at room temperature (25 °C), with the reagent solutions prepared using doubly distilled water. Cyclic voltammogram studies were carried out with a PAR Model 173/175 electrochemical setup using a platinum wire as the working electrode and KCl as the supporting electrolyte. The chemicals used were of the best research grade commercially available. ABTS was from Boehringer, Mannheim (West Germany) in the form of diammonium salt and was used as such. Peroxomonosulfate (PMS) in the form of a triple salt ($2KHSO_5$-$KHSO_4$-K_2SO_4) with the trade name "OXONE" was obtained from Dupont de Nemours & Co (USA). Other chemicals such as peroxodisulfate (PDS), $KMnO_4$, $K_2Cr_2O_7$, $Ce(NH_4)_2(SO_4)_3$, and molecular I_2, Br_2, and Cl_2 were obtained from Sarabhai M. Chemicals. The reagent solutions were thoroughly degassed by purified nitrogen or argon. The extent of formation and decay of radical cation ($ABTS^{\cdot+}$) were monitored at 417 nm which

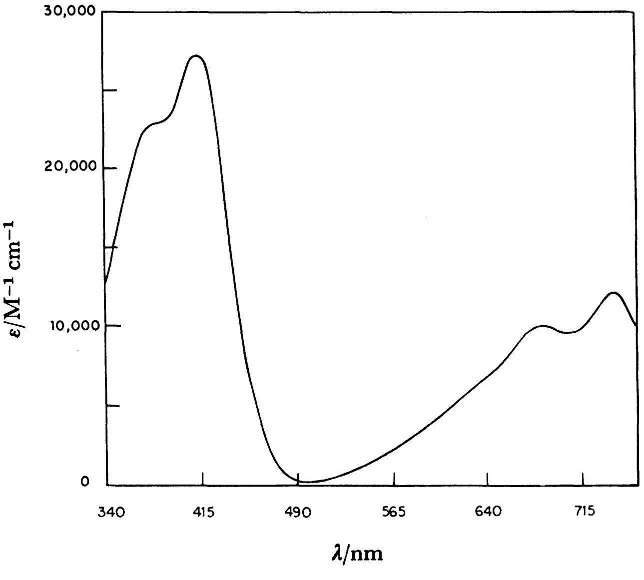


Fig. 1. Molar absorption spectrum of $ABTS^{\cdot+}$.

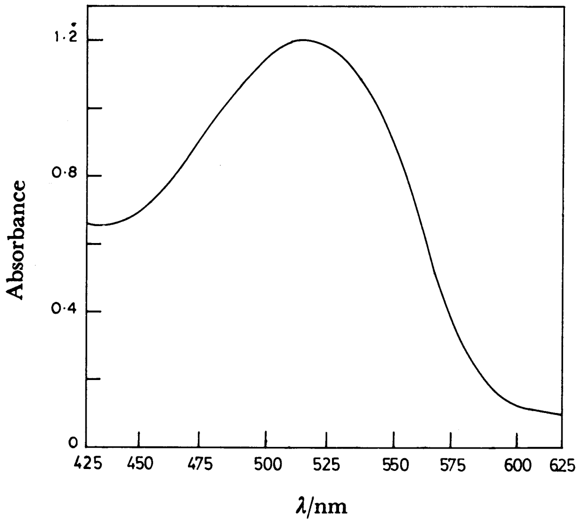


Fig. 2. Transient spectrum of $ABTS^{2+}$.

corresponds to its maximum absorbance (ε_{417}=2.70×10^4 M^{-1} cm^{-1} -Fig. 1) (1 M=1 mol dm^{-3}). Formation of dication $ABTS^{2+}$ was monitored by its absorbance maximum at 513 nm (Fig. 2). The radical cation ($ABTS^{\cdot +}$) used was prepared by the reaction of ABTS with PMS (HSO_5^-) in the ratio of 2:1 respectively in deaerated water. The radical cation formed was found to be stable for more than a week.

Results and Discussion

All the kinetic measurements were carried out under pseudo-first order conditions, with the concentration of the oxidant in at least ten times excess over that of ABTS. The changes in the concentration of the transient with time were followed at different time scales depending on the reactivity of the oxidant. Plots of log (absorbance) against time were found to be linear up to ≈70% of the reaction with the correlation coefficient ≥0.99, showing that the reaction is first order with respect to ABTS. Plots of the pseudo first order rate constants k'/s^{-1} against concentrations of oxidant at fixed [ABTS] were found to be linear passing through the origin (Fig. 3), showing the first-order dependence of the rate on the oxidant. From the slopes of the above plots, the second-order rate constants $k_f/M^{-1}\,s^{-1}$ were calculated to be presented in Table 1. The proposed rate law for the formation of radical cation is

$$\frac{d[ABTS^{\cdot +}]}{dt} = k_f[ABTS][Oxidant]. \qquad (1)$$

For the determination of the rate constants of the decay of the radical cation either to the dication ($ABTS^{2+}$) or back to ABTS, experiments were carried out with the $ABTS^{\cdot +}$ preprepared as mentioned above. The kinetics were spectrophotometrically followed either at 417 or 513 nm, by varying [$ABTS^{\cdot +}$] as well as [Oxidant] under pseudo-first-order conditions. Rate law analysis similar to the above (Fig. 4) gave the kinetic rate law for the decay process to be

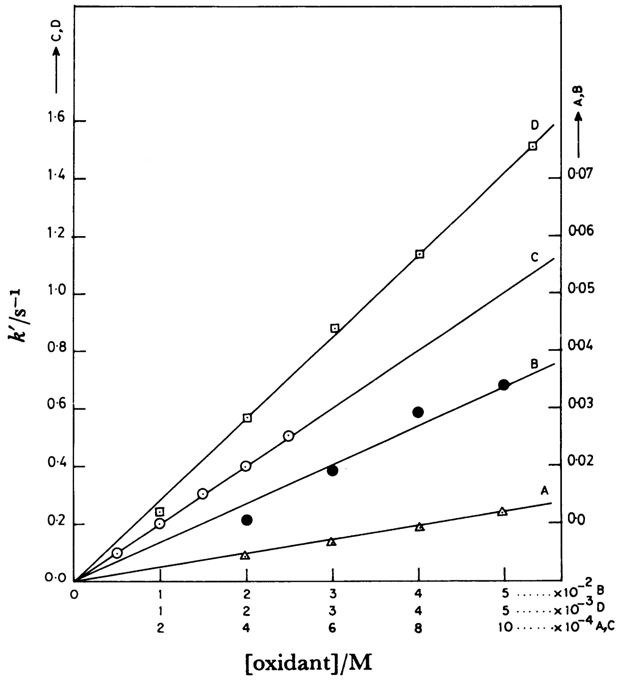


Fig. 3. Evaluation of k_f.

k' vs. [Oxidant]; T=25 °C; A: PMS; B: PDS; C: $Cr_2O_7^{2-}$; D: Ce(IV).

Table 1. Kinetic Data for the Formation and Decay of $ABTS^{\cdot +}$ at 25 °C

Oxidant	$k_f/M^{-1}\,s^{-1}$	$k_d/M^{-1}\,s^{-1}$
PMS	11.16	—
PDS	0.63	0.20
MnO_4^-	2.50×10^4	1.50×10^3
$Cr_2O_7^{2-}$/0.2 M H^+	1.01×10^3	4.00×10^2
Ce(IV)/0.2 M H^+	3.01×10^2	3.00×10^1
I_2	6.28×10^2	—
Br_2	1.00×10^5	—
Cl_2	—	2.40

$$\text{Rate} = -\frac{d[ABTS^{\cdot +}]}{dt} = \frac{d[ABTS^{2+}]}{dt}$$
$$= k_d[ABTS^{\cdot +}][Oxidant]. \qquad (2)$$

The calculated rate constants k_d are shown in Table 1.

Reaction with Peroxodisulfate (PDS) and Peroxomonosulfate (PMS). The kinetics of reactions of these two peroxosalts of sulfur with ABTS were found to be first-order each with respect to the oxidant and ABTS. Although these two oxidants are two-electron transferring agents, they behave like a one-electron oxidant in forming the radical cation, $ABTS^{\cdot +}$. On short time scales, (5—20 ms), PDS (1—5×10^{-2} M) and ABTS (1—5×10^{-5} M) generated only the radical cation ($ABTS^{\cdot +}$). However at longer time scales (20—50 ms)

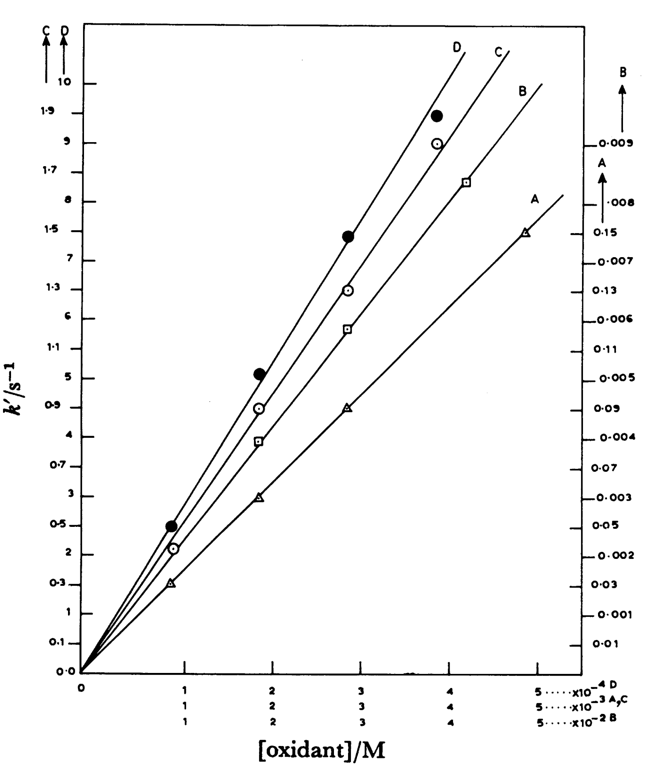
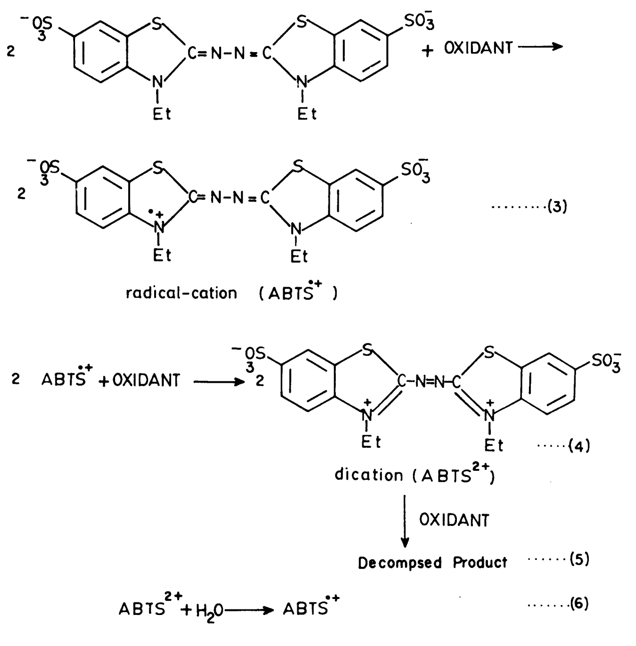


Fig. 4. Evaluation of k_d.
k' vs. [Oxidant]; T=25 °C; A: Ce(IV); B: PDS; C: $Cr_2O_7^{2-}$; D: MnO_4^-.

the radical cation formed decayed to the dication, $ABTS^{2+}$. The rate constant determined for the reaction (3), formation of $ABTS^{\cdot+}$, was 0.63 $M^{-1}\,s^{-1}$ and that for the second-stage oxidation (4) was 0.20 $M^{-1}\,s^{-1}$.



From the magnitudes of the rate constants, it is seen that the oxidation of $ABTS^{\cdot+}$ to $ABTS^{2+}$ is approximately three times slower than that of ABTS to $ABTS^{\cdot+}$. The dication which is pink-red brittle precipitate is stable for more than three days in the presence of excess $S_2O_8^{2-}$. The precipitate, separated and free from $S_2O_8^{2-}$, when dissolved in water turned back into $ABTS^{\cdot+}$ (6). On the other hand, the reaction of HSO_5^- with ABTS, whatever be the concentrations of both of them, stopped with the formation of $ABTS^{\cdot+}$. This unusual stability of the radical cation is explained by strong delocalization of the odd electron into the π-cloud, thus causing a high stability and long wavelength absorption. The rate constant determined for the oxidation of ABTS by PMS is 11.16 $M^{-1}\,s^{-1}$. Even though the oxidation potential for PMS (1.81 V) is less than that of PDS (2.01 V), the rate constant for PMS with ABTS is approximately 18 times higher than that of PDS. This observation is similar to the one encountered in many thermal reactions[7] wherein the rate constants for the oxidation by PMS were higher than those of PDS. In some cases, such as oxidation of methoxybenzenes[8] the reaction of PMS took place at measurable rates at 25 °C, whereas PDS reacted only in presence of Ag^+ catalyst. Hence, even though the thermodynamic factor (oxidation potential) is favorable for PDS to react with many substrates, the reactions are kinetically inhibited. This might be true in the present investigation also. However, PMS does not react further with the radical cation unlike $S_2O_8^{2-}$.

Reactions of Halogens. Molecular iodine oxidised ABTS to $ABTS^{\cdot+}$ instantaneously, but I_2 in excess KI (5%) did not bring the reaction instantaneously, suggesting the equilibrium reaction (7),

$$2\text{ABTS} + I_2 \underset{k_r}{\overset{k_f}{\rightleftharpoons}} 2\text{ABTS}^{\cdot+} + 2I^- . \tag{7}$$

The forward reaction was measured by following the formation of radical cation for various concentrations of ABTS with different amounts of I_2 in water at natural pH (pH=6.9) in 2—10 ms time scale. The second-order rate constant, k_f was calculated to be 628.1 $M^{-1}\,s^{-1}$. The reverse process was monitored by following the disappearance of $ABTS^{\cdot+}$ for various concentrations of $ABTS^{\cdot+}$ (1—5$\times10^{-5}$ M) and I^- (2—8$\times10^{-2}$ M). The rate constant for the reverse reaction was, k_r=4.40 $M^{-1}\,s^{-1}$, then the equilibrium constant being $K=k_f/k_r$=142.8. The rate constants measured at pH 3—6 did not show any difference from those measured at the near neutral and natural pH. From the equilibrium constant, the change in the Gibbs free energy, $\Delta G°$ at 25 °C for the reaction (7) is calculated to be, $\Delta G°=-RT\ln K$

$$\Delta G° = -8.314 \times 298 \times 2.303 \times 2.155$$

$$\Delta G° = -1.23 \times 10^4\,\text{J mol}^{-1}.$$

The difference in the standard redox potentials between the two one-electron redox couples is $\Delta E° = \Delta G°/F = -0.127$ V. The standard redox potential

$E°$, for $ABTS^{\cdot +}/ABTS$ measured in aqueous medium by cyclic voltammetric technique was +0.43 V. Since the $E°$ for I_2/I^- is +0.54 V, theoretical value of the standard electrode potential for the reaction (7) should be −0.11 V which is in good agreement with the one estimated from the equilibrium constant. In the case of the reactions of Br_2 and Cl_2 with ABTS, such equilibrium was not observed. Molecular Br_2 in water or aqueous Br_2/KBr oxidized ABTS to $ABTS^{\cdot +}$ in 0.5—2.0 ms time scale and the rate constant for the reaction (8),

$$\mathrm{ABTS} + \mathrm{Br_2} \longrightarrow \mathrm{ABTS}^{\cdot +} + \mathrm{Br_2}^{\cdot -} \tag{8}$$

was $1.00 \times 10^5 \ M^{-1} s^{-1}$. There was no detectable reaction of $ABTS^{\cdot +}$ either with Br_2 or Br^-. No attempt was made to investigate the fate of $Br_2^{\cdot -}$ in the present work since it was postulated to decay by the bimolecular reaction at diffusion controlled rate,[9]

$$2\mathrm{Br_2}^{\cdot -} \longrightarrow \mathrm{Br}^- + \mathrm{Br_3}^- . \tag{9}$$

In the case of Cl_2, the first stage oxidation (3) was very fast and took place within the mixing time of the instrument (dead time 2 ms). The second-stage oxidation resulted in a decomposed product via the dication as the transient even for the lowest concentrations of the reactants $ABTS^{\cdot +}$ and Cl_2. The kinetics of the decay process was also found to be total second-order, first-order each with respect to $[ABTS^{\cdot +}]$ and $[Cl_2]$ and the rate constant was calculated to be $2.40 \ M^{-1} s^{-1}$.

$$\mathrm{ABTS}^{\cdot +} + \mathrm{Cl_2} \longrightarrow \mathrm{ABTS}^{2+} \longrightarrow \text{decomposed product} \tag{10}$$

The decomposed product formed in the reaction (10) could not be reverted back to any one of the species, $ABTS^{2+}$, $ABTS^{\cdot +}$, or ABTS by the addition of strong reducing agents such as sulfite or dithionite. Since our interest in this study is centered around the formation and reactivity of radical cations from ABTS, not much attention was paid on the complete identification of the decomposed product. However, IR and UV spectral investigation showed the formation of substituted thiophenol and disulfide depending upon the concentration of Cl_2 used. From the magnitudes of the rate constants (Table 1), the reactivity trend of the halogens with ABTS, $Cl_2 > Br_2 > I_2$ is similar to the trend of their oxidation potentials and the latter two halogens give only the one-electron oxidation product, $ABTS^{\cdot +}$. In the case of oxidation of $ABTS^{\cdot +}$ to $ABTS^{2+}$, judging from the magnitudes of the oxidation potentials of Cl_2 (1.35 V) and $ABTS^{\cdot +}/ABTS^{2+}$ (0.83 V) and the magnitude of the rate constant for the reaction (10), it is not surprising that Br_2 (oxidation potential 1.06 V) hardly react with $ABTS^{\cdot +}$.

Reaction of MnO_4^-, $Cr_2O_7^{2-}$, and Ce(IV). At low concentrations of the oxidants, ([ABTS]=1—4×10^{-5} M; [oxidant]=1—4×10^{-4} M), both MnO_4^- and $Cr_2O_7^{2-}$ (in 0.2 M H^+) were found to generate only the radical cation. However at higher concentrations of the oxidants and on longer time scales, the radical cation was found to decay at a measurable rate to pink-red dication and then to a decomposed product. In the case of Ce(IV) in 0.2 M H^+, at any concentrations of the reactants, ABTS was oxidized to the dication via the radical cation as a transient. The observed rate constant for both the processes are presented in Table 1. From the magnitudes of the rate constants the reactivity of these oxidants with ABTS as well as $ABTS^{\cdot +}$ is found to be $MnO_4^- > Cr_2O_7^{2-} > Ce(IV)$, the second-stage oxidation being one order of magnitude less than that for the radical cation formation in the case of MnO_4^- and Ce(IV). The rate constants estimated are in line with the oxidation potentials[10] of MnO_4^- (1.695 V) and $Cr_2O_7^{2-}$ (1.44 V). In the case of Ce(IV) (1.44 V) on thermodynamic grounds, the reactivity should be comparable to that of $Cr_2O_7^{2-}$. However, it is less reactive than $Cr_2O_7^{2-}$ probably due to its high ability of complexing[11] with groups containing electron donors.

Reduction of Radical Cation. The possibility of the reduction of the radical cation, $ABTS^{\cdot +}$ was tested by using dithionite and sulfite ions as reducing agents. The reaction (11) was brought about, producing the parent compound ABTS,

$$\mathrm{ABTS}^{\cdot +} + \text{reductant} \longrightarrow \mathrm{ABTS}\ \cdot \tag{11}$$

The kinetics of the reaction gave the second-order rate constants shown below:

$$\mathrm{ABTS}^{\cdot +} + \mathrm{SO_3}^{2-} \longrightarrow \mathrm{ABTS},\ k_2 = 1.06 \times 10^3 \ M^{-1} s^{-1},$$
$$\mathrm{ABTS}^{\cdot +} + \mathrm{S_2O_4}^{2-} \longrightarrow \mathrm{ABTS},\ k_2 = 6.30 \times 10^2 \ M^{-1} s^{-1}.$$

Other preliminary tests show that the radical cation $ABTS^{\cdot +}$ is capable of oxidizing many organic and inorganic substrates.[12]

The authors are thankful to the Department of Science and Technology, Government of India, New Delhi for financial support.

References

1) M. Grätzel, *Acc. Chem. Res.*, **14**, 376 (1981).

2) G. Levey, A. L. Rieges, and J. O. Edwards, *J. Org. Chem.*, **46**, 1255 (1981).

3) P. Maruthamuthu, L. Venkatasubramanian, and P. Dharmalingam, *J. Chem. Soc., Faraday Trans, 1*, **82**, 359 (1986).

4) S. Hüenig, H. Balli, H. Cornard, and A. Schott, *Justus Liebigs Ann. Chem.*, **676**, 36 (1964).

5) S. Hüenig, *Justus Liebigs Ann. Chem.*, **676**, 32 (1964).

6) G. Mahuzier, B. S. Kirkacharian, and C. Harfouche-obeika, *Anal. Chim. Acta*, **76**, 79 (1975).

7) a) R. Ranganathan and P. Maruthamuthu, *Int. J. Chem. Kinet.*, **18**, 49 (1986); b) R. Ranganathan and P.

Maruthamuthu, *J. Chem. Soc., Perkin Trans. 2,* **1986**, 285.

8) P. Dharmalingam and P. Maruthamuthu, *J. Chem. Soc., Perkin Trans. 2,* submitted for publication.

9) "Rate Constants for Reactions of Inorganic Radicals in Aqueous Solution," ed by A. B. Ross and P. Neta, U. S. Department of Commerce-National Bureau of Standards (1979), p. 38.

10) "Oxidation Potentials," ed by W. M. Latimer, Prentice-Hall Inc., Englewood Cliffs, NJ (1966).

11) J. Handwick and E. Robertson, *Can. J. Chem.*, **29**, 818 (1951).

12) P. Maruthamuthu and L. Venkatasubramanian, unpublished results.