ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 92 (1995) 181-188

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A:CHEMISTRY

Kinetics of one-electron reduction of acridine orange and characterization of its semireduced species in aqueous solutions by pulse radiolysis

S.N. Guha, J.P. Mittal *

Chemistry Group, Bhabha Atomic Research Centre, Bombay 400 085, India

Received 15 March 1995; accepted 14 June 1995

Abstract

Semireduced acridine orange was generated pulse-radiolytically by reactions of various one-electron reducing radicals (e_{aq}^-, $(CH_3)_2COH$, CO_2^-) with the cationic dye acridine orange. Rate constants for the reactions of all these radicals with the dye were determined and the yields of the transient, semireduced acridine orange in these reactions were estimated. Spectral and acid–base properties of the semireduced species formed by these reactions have been evaluated and the nature of its decay kinetics has been resolved. Rate constants for the interaction of the transient with the parent molecule have been determined. Using $(CH_3)_2CO$, $H^+/(CH_3)_2COH$ as the standard couple, the one-electron reduction potential of the dye was determined.

Keywords: Kinetics; One-electron reduction; Acridine orange; Pulse radiolysis

1. Introduction

Acridine orange (AO; 3,6-bis-dimethylaminoacridine), a cationic dye, finds application as a photosensitizer in redox devices for utilization of solar energy [1,2]. This dye is also important in biological studies in regard to its photodynamic effect [3]. Although there are some studies reported in the literature on its redox reactions leading to the generation of semireduced and semioxidized species using the flash photolysis technique [4–6], the spectroscopic data obtained are likely to be of insufficient accuracy owing to interference from the triplet states involved and the radicals derived from the redox reagents employed for the photoredox reactions. The pulse radiolysis technique, on the other hand, provides a clean method of generating such transient species exclusively and thus characterization becomes unambiguous. Surprisingly little pulse radiolysis work on this compound is reported in the literature. In an early study Balazs et al. [7] examined the interaction of metachromatic dyes, including acridine orange, with hyaluronic acid using pulse radiolysis and determined rate constants for hydrated electron reactions in this system. Other pulse radiolysis work by Solar et al. [8] deals with H atom reactions of the dye mainly in the acidic pH range. The authors have shown that H atoms react with AO by three different modes simultaneously, giving rise to H adduct, semireduced AO and radical (R) species. They resolved the spectral information on the various radical species from the superimposed spectra by a computer simulation method.

In this work we have generated exclusively the semireduced species by the reaction of pulse-radiolytically formed hydrated electrons whose concomitant oxidation product is water and hence is not expected to complicate characterization of the transient species. Reactions of isopropyl ketyl ($(CH_3)_2COH$) and carbon dioxide anion (CO_2^-) radicals were also studied over the pH range 1–10 and the results compared with those of e_{aq}^- reactions at corresponding pH values. The spectral parameters, pK_a and formation and decay kinetics of semireduced AO were evaluated. These data have been compared with those obtained in previous photochemical studies of this dye and discussed.

2. Experimental details

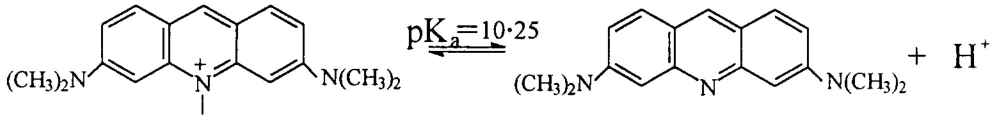
Acridine orange (Aldrich) was recrystallized from water. The product was checked for purity via its absorption spectrum and molar absorptivity [9] and found to be satisfactory. The dye has a pK_a of 10.25 [6] and exists predominantly in cationic form as AOH^+ below this pH (Scheme 1).

All other chemicals and reagents were AR or GR grade and were used without further purification. Gases (N_2, O_2

---

* Corresponding author.

Elsevier Science S.A.
SSDI 1010-6030(95)04133-8



(AO)

Scheme 1.

and N_2O) employed for purging the solutions were of IOLAR grade obtained from Indian Oxygen Ltd. Appropriate pretraps were used whenever the solutions contained tertiary butanol, isopropanol or acetone to compensate for losses due to evaporation. The pH of the solution was adjusted using phosphate or borate buffers in the pH region 4–10, whereas below pH 4 sulphuric acid was used [10]. Reducing radicals for reaction with AO were generated by short electron pulse irradiation of a suitable matrix as described at appropriate places in the text. Solutions were prepared in nanopure water with a conductivity of 0.1 μS cm^{-1} and placed in rectangular quartz cuvettes of 1 cm optical path length for exposure to 50 ns pulses of 7 MeV electrons from a linear accelerator obtained from Ray Technology, UK. Details of the pulse radiolysis set-up have been given elsewhere [10]. The absorbed dose per pulse was evaluated by standard thiocyanate dosimetry assuming $G\epsilon$ for the $(SCN)_2^-$ radical to be 21 522 dm^3 mol^{-1} cm^{-1} per 100 eV at 500 nm [11], where G is the radiation chemical yield expressed as the number of molecules formed or destroyed per 100 eV of energy absorbed and ϵ (mol^{-1} dm^3 cm^{-1}) is the molar absorptivity. The dose employed in the present study, unless otherwise stated, was typically 9 Gy per pulse. However, lower doses of about 7 Gy were used for the determination of formation rate constant and molar absorptivity values. The molar absorptivity of a radical (ϵ_R) was computed from its absorbance (A_R) measured at a given wavelength (λ) using the relationship

$$\epsilon_R = \epsilon_P + \frac{A_R \times 21\,522}{A_{(SCN)_2^-} G_R} \qquad (1)$$

where $A_{(SCN)_2^-}$ is the absorbance of the thiocyanate radical at 500 nm, ϵ_P is the molar absorptivity of the parent molecules and G_R is the radiation chemical yield of the radical. A value of 21 522 dm^3 mol^{-1} cm^{-1} per 100 eV is taken for $G\epsilon$ for $(SCN)_2^-$ at 500 nm as mentioned before.

3. Results and discussion

3.1. Hydrated electron reaction

Pulse irradiation of water leads to the formation of OH, H and e_{aq}^- as the major primary–reactive species:

$$H_2O \longrightarrow e_{aq}^-,\ H,\ \dot{O}H \qquad (2)$$

The hydrated electron (e_{aq}^-) reaction with AO was studied in an electron-pulsed O_2-free *tert*-butanol matrix at pH 7. In this matrix the OH radicals generated are readily scavenged by *tert*-butanol, which is converted to the unreactive $(CH_3)_2CH_2COH$ radical via

$$(CH_3)_3COH + OH \longrightarrow (CH_3)_2\dot{C}H_2COH + H_2O \qquad (3)$$

and the H atoms are mostly converted to e_{aq}^- at pH > 4. In the absence of oxygen the above matrix containing AO is therefore suitable for studying the reaction of hydrated electrons with the dye. However, a small fraction of H atoms with a G value of about 0.6 is expected to be present at higher pH. Since e_{aq}^- in this pH range is present in large excess with $G = 2.7$, interference by H atoms will be small and has been ignored in this case. Moreover, it should be noted that among the three different modes of H atom reaction stated in Section 1, electron transfer leading to semireduced AO is the most prominent process [8] and hence characterization of the semireduced species will not be affected to any significant extent. In a separate experiment, electron pulse irradiation of the above matrix at pH 7 containing 7×10^{-5} mol dm^{-3} AO but saturated with O_2 was carried out. This did not give rise to any light-absorbing transient signal in the entire wavelength range 300–800 nm or any bleaching of the dye in the region where the parent dye molecule shows optical absorption. Under this condition the only radicals formed are $(CH_3)_2CH_2COH$ (see Eq. (3)) and weakly reducing O_2^- via

$$O_2 + e_{aq}^- \longrightarrow O_2^- \qquad (4)$$

It can be inferred that neither $(CH_3)_2CH_2COH$ nor O_2^- has any appreciable reactivity with AO and hence in the electron-pulsed N_2-saturated *tert*-butanol matrix at pH 7 the reactive species is predominantly e_{aq}^-. The difference transient absorption spectrum recorded immediately after the electron pulse given to this matrix containing 7×10^{-5} mol dm^{-3} AO is shown in Fig. 1. The observed spectrum exhibited a prominent band with λ_{max} at 400 nm and bleaching of the dye in the wavelength regions around 320–280 and 440–520 nm attributable to the semireduced AO species. The transient spectrum when corrected for the dye bleaching and plotted as molar absorptivity (using Eq. (1)) vs. wavelength (λ) revealed distinct absorption bands with λ_{max} at 270 and 490 nm in addition to the 400 nm band, which on correction showed λ_{max} at 410 nm (Fig. 2). The maximum absorbance at λ_{max} occurred approximately 2 μs after the pulse, which indicates a high reactivity of e_{aq}^- with the dye. The rate constant for the reaction of e_{aq}^- with the dye as determined from the build-up of absorbance at 400 nm and also by following the decay of hydrated electrons at 720 nm in the

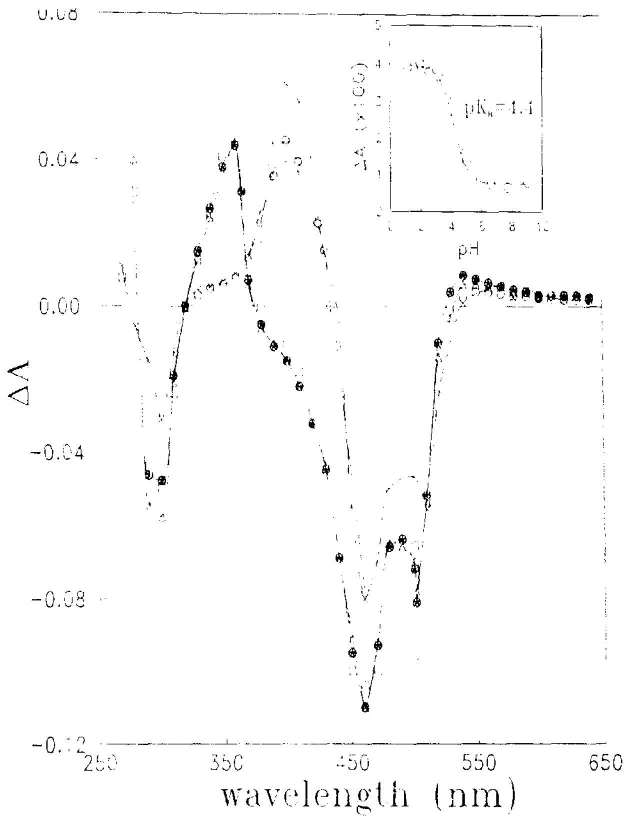


Fig. 1. Difference absorption spectra of semireduced AO obtained by electron pulse irradiation of acridine orange solutions under various conditions: e_{aq}^- reaction, (○) pH 7; $(CH_3)_2\dot{C}OH$ reaction, (×) pH 7, (△) pH 1, (⊛) pH 2.2, and (□) pH 3; CO_2^- reaction, (+) pH 7.

presence of AO was $(2.6 \pm 0.2) \times 10^{10}$ $dm^3\ mol^{-1}\ s^{-1}$. At pH 7 the dye exists as AOH^+ (see Scheme 1), hence its reduction with e_{aq}^- can be represented as

$$AOH^+ + e_{aq}^- \longrightarrow AOH \qquad (5)$$

3.2. Reaction of isopropyl ketyl ($(CH_3)_2\dot{C}OH$) radical

Electron pulse irradiation of an N_2O-saturated aqueous solution containing isopropanol gives rise to reducing ketyl radicals via

$$e_{aq}^- + N_2O + H_2O \longrightarrow \dot{O}H + OH^- + N_2 \qquad (6)$$

$$H/\dot{O}H + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2/H_2O \qquad (7)$$

At neutral pH the reaction of the ketyl radical with the dye was studied in a matrix containing 1 mol dm^{-3} isopropanol, 7×10^{-5} mol dm^{-3} AO and 5×10^{-3} mol dm^{-3} phosphate buffer. Pulse irradiation of this matrix gave rise to a transient spectrum (Fig. 1) showing a prominent absorption band with λ_{max} at 400 nm and bleaching in similar wavelength regions as observed for the *tert*-butanol matrix. The nature of the spectrum when corrected for the dye absorption also compared well with that obtained in the case of the e_{aq}^- reaction in the *tert*-butanol matrix (Fig. 2). However, the intensity of the 410 nm band observed in the isopropanol matrix was somewhat lower. It has been assumed in the past [12] that among the radicals formed by reaction (7), about 85% are strongly reducing α-hydroxy alkyl radicals and the remaining 15% are unreactive β-hydroxy alkyl radicals $(\dot{C}H_2(CH_3)CHOH)$. In computing the electron transfer efficiency of the ketyl radical for the yield of semireduced AO, the *G* value has been assumed to be 5.1, i.e. 15% lower than the total radical yield of 6.0. With the assumption of this *G* value and the corrected band intensity we computed a value of 76% for the yield of semireduced AO species considering the efficiency of the e_{aq}^- reaction with AO to be 100%. We therefore conclude that $(CH_3)_2\dot{C}OH$ radicals react with AO giving rise to the semireduced species as in the case of the e_{aq}^- reaction, though with somewhat lower efficiency:

$$(CH_3)_2\dot{C}OH + AOH^+ \longrightarrow AOH + (CH_3)_2CO + H^+ \qquad (8)$$

3.3. Reaction of CO_2^- with AO

The carbon dioxide anion (CO_2^-) is a strongly reducing radical and is generated pulse-radiolytically by the reaction of H and OH with formate ions in aqueous solution according to

$$HCOO^- + H/OH \longrightarrow CO_2^- + H_2/H_2O \qquad (9)$$

The reaction of this reducing radical with AO was studied in an N_2O-saturated matrix containing 0.1 mol dm^{-3} sodium formate and 7×10^{-5} mol dm^{-3} AO at pH 7. The difference absorption spectrum of the transient formed as a result of this reaction is shown in Fig. 1. The nature of the transient spectrum is comparable with that observed in the case of ketyl radicals or e_{aq}^- at neutral pH. However, the 410 nm band intensity was considerably lower and the yield of semireduced AO computed in this case was only about 58% (the

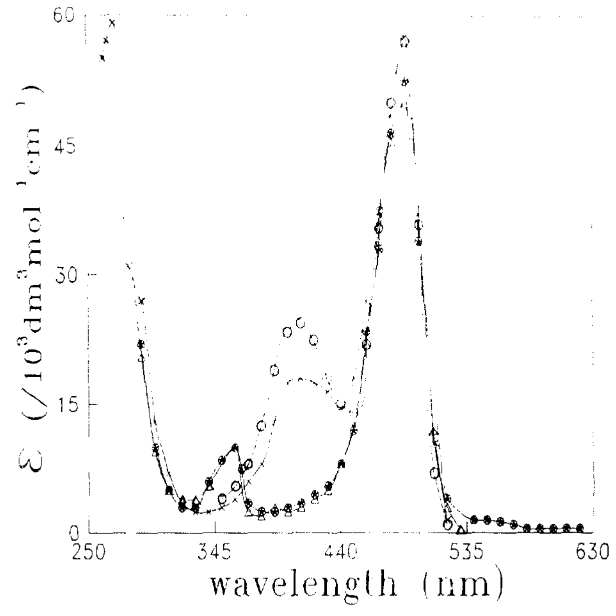


Fig. 2. Corrected absorption spectra of semireduced AO: e_{aq}^- reaction, (○) pH 7; $(CH_3)_2\dot{C}OH$ reaction, (×) pH 7, (△) pH 1, (⊛) pH 2.2.

Table 1
Spectral and kinetic parameters of semireduced species formed by reaction of various reducing radicals with acridine orange

Protolytic form of semireduced species	pH	λ_{max} (nm)	ϵ ($dm^3\ mol^{-1}\ cm^{-1}$) (corrected)	Reducing radical	k ($dm^3\ mol^{-1}\ s^{-1}$)
AOH	7	410	24000 ± 2000	e_{aq}^-	$(2.6 \pm 0.2) \times 10^{10}$
		270	51500 ± 12000		
		490	45000 ± 11000	$(CH_3)_2COH$	$(1.0 \pm 0.2) \times 10^9$
				CO_2^-	$(9.0 \pm 0.2) \times 10^8$
AOH_2^+	1–3	360	14000 ± 1500	$(CH_3)_2COH$	$(7.0 \pm 0.2) \times 10^8$
		270	–		
		490	–		

corrected spectrum is not included in Fig. 2), a value lower than even the one observed in the case of ketyl radicals. The reaction of CO_2^- by electron transfer leading to the semireduced species may be written as

$$CO_2^- + AOH^+ \longrightarrow AOH + CO_2 \qquad (10)$$

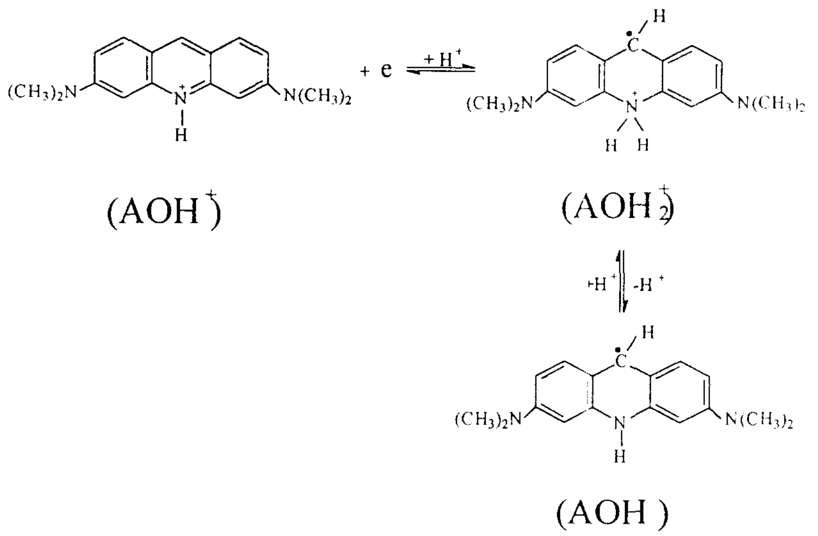
The AO molecule has two $-N(CH_3)_2$ groups attached to the side-rings and one N atom in the central ring. Electron addition to the N atom of the chromophore ring giving rise to the semireduced species (see structure in Scheme 2) appears to be more feasible. Moreover, comparison of our spectral data for ketyl and CO_2^- radicals with those for hydrated electrons indicates that the transient bands observed are due to the semireduced species only. This conclusion, as we shall see later, is supported by our findings that the decay of the absorption band of the transient formed by the reaction of ketyl and CO_2^- radicals follows exactly the same kinetics as that of the one formed by e_{aq}^-. Further, Solar et al. [8] observed, as stated before, three different transient species formed simultaneously by the reaction of H atoms with the AO molecule and by a computer simulation method they resolved the various transient spectra attributable to H adduct, semireduced AO and R species. Although the spectra of these species at neutral pH are not shown in their report, from the tabulated trend of λ_{max} shift with pH we can say that the bands observed by us in the reaction of ketyl radicals and CO_2^- tally with those of the semireduced species only.

In a photochemical study by Kellmann on AO [4] the author reported a superimposed absorption spectrum in AO aqueous solution at pH 12 and inferred that a band with λ_{max} at 405 nm was due to both triplet state and semireduced species of AO. The presence of the semireduced species was confirmed by the reaction of AO triplet with the well-known reducing agent TMPD (tetramethyl-*para*-phenylenediamine). This reaction gave rise to a composite spectrum pertaining to both semireduced AO and the oxidized $TMPD^+$ radical. The author assigned 405 nm to the semireduced species and all the remaining bands to the $TMPD^+$ radical. As we shall see later, the semireduced AO has a pK_a of 4.4 and hence the protolytic form present at pH 7 studied by us and at pH 12 studied by Kellmann should be the same. We infer that the semireduced AO generated by us in the reaction of e_{aq}^-, ketyl or CO_2^- radicals has two bands with λ_{max} at 270 and 490 nm in addition to the 410 nm band. The molar absorptivities of the semireduced species computed at 270 and 490 nm are expected to have very high error owing to the intense parent absorption. The values (with error limits) at the various λ_{max} are given in Table 1.

Regarding the lower efficiency of electron transfer in the case of ketyl and CO_2^- radicals, it should be noted that the one-electron reduction potential of AO (-1.16 V vs. NHE (normal hydrogen electrode), as we shall see later) is sufficiently less negative than the oxidation potential [13] of either CO_2^- (-1.9 V (NHE)) or the ketyl radical (-1.39 V (NHE)). The latter two species are expected to react with AO by electron transfer with almost 100% efficiency. There is a possibility of self-reaction of ketyl or CO_2^- radicals competing with the reactions of these radicals with the dye, which could explain the lower yield of semireduced species. This possibility, however, can be ruled out, since in an experiment which was carried out keeping the matrix the same and varying only the AO concentration (in the range $(3–7) \times 10^{-5}$ mol dm^{-3}) we observed no significant change in the transient yield as far as the CO_2^- reaction is concerned. In the case of the ketyl radical reaction the small variation in the yield is attributable to equilibrium (13), which is discussed fully in Section 3.6. Under the circumstances it is difficult to explain the lower yield on the basis of competition by self-reaction. Although isopropyl ketyl and CO_2^- radicals are strongly reducing species with high negative oxidation potentials and react with many compounds by one-electron transfer [10,14,15], their interactions with some molecules, e.g. pyridinol, have been reported to involve addition [16,17]. In some cases it has been reported that isopropyl ketyl radicals also undergo H atom abstraction [17]. Although we have not observed any additional transient bands in the reactions of these radicals with AO, it is anticipated that the ketyl and CO_2^- radicals also react with AO by modes other than electron transfer and presumably their reaction products do not absorb in the near-UV or visible region.

3.4. Effect of pH on semireduced AO

We consider only the ketyl radical reaction for the study of the pH effect on semireduced AO. The advantage in this



Scheme 2.

case, in addition to the higher yield of semireduced AO, is that a single matrix can be employed over the entire pH range 1–10 (pK_a of the ketyl radical is reported to be 12.3 [15] and the radical below this pH exists in one form) studied. Spectra of the transient formed by the reaction of $(CH_3)_2COH$ radicals with AO at acidic pH values of 1.0, 2.2 and 3.0 are shown in Figs. 1 and 2. The corrected spectra presented in Fig. 2 are derived using Eq. (1) and the observed optical densities. However, for computing the true extinction coefficient values at the respective λ_{max}, the yield of semireduced AO in the reaction of ketyl radicals has been considered and it has been assumed that the yield remains the same as that for neutral pH. In his flash photolysis study of this molecule Kellmann generated semireduced species at pH 3 by the reduction of AO triplet with TMPD and reported its absorption spectrum 0.25 ms after the flash. Among the transient bands with λ_{max} at 330, 360, 560 and 610 nm observed by the author, the 360 nm band was attributed to the semireduced AO species and the rest to TMPD$^+$. In the present pulse radiolysis study the transient derived from AO by the reaction of ketyl radicals at acidic pH 1–3 revealed intense bands with λ_{max} at 270, 360 and 490 nm (see corrected spectra in Fig. 2). The absorption at λ_{max} of these bands reached a maximum about 50 μs after the electron pulse. On this time scale the ketyl radicals are expected to decay almost completely in giving rise to the product transient species. Moreover, they do not show any absorption in the visible region to interfere with the transient spectrum. We therefore infer that the bands with λ_{max} at 270, 360 and 490 nm are exclusively due to semireduced AO species at acidic pH. On comparison of the transient spectra at the various pH values we find that their nature is quite different (see Figs. 1 and 2). Such an effect of pH can be attributed to the existence of an acid–base equilibrium of the transient involved.

In order to determine the protolytic equilibrium constant (pK_a) of semireduced AO, a single matrix consisting of N_2O-saturated 1×10^{-4} mol dm^{-3} AO and 1.0 mol dm^{-3} isopropanol was employed over the pH range 1–9. In this matrix e_{aq}^- is converted either to OH radicals at higher pH or to H atoms in the acidic region. These radicals along with pulse-radiolytically generated H and OH will be converted to reducing $(CH_3)_2COH$ radicals (Eq. (7)) with G(red) = 5.1. The optical density of the transient measured at 360 nm wavelength as a function of pH gave a curve with one inflection point from which pK_a of semireduced AO was inferred to be 4.4 (see inset of Fig. 1). Since acridine orange exists as AOH^+ in the ground state in the pH range 1–10 studied, the pK_a of 4.4 of the transient semireduced species can be assigned as

$$AOH_2^+ \xrightleftharpoons{pk_a=4.4} AOH + H^+ \qquad (11)$$

The electron addition to AO followed by deprotonation may be depicted as in Scheme 2.

The spectra recorded at acidic pH values and pH 7 (Figs. 1 and 2) may be assigned to the two different forms of semireduced species AOH_2^+ and AOH respectively.

3.5. Decay kinetics of semireduced AO

Semireduced species of various other photogalvanic dyes such as thionine [17], toluidine blue [14] and neutral red [18] are reported to decay by second-order kinetics attributable to disproportionation. In the present case, however, the decay of the transient semireduced species monitored in the electron-pulsed N_2-saturated *tert*-butanol matrix containing AO in the range $(3–7) \times 10^{-5}$ mol dm^{-3} followed first-order kinetics and the observed rate constant depended on the initial AO concentration. Fig. 3 shows the typical decay of the transient absorption at 400 nm becoming faster in the presence of increasing AO concentration at neutral pH. As is seen from the analysis of these traces, the decay follows a very good first-order kinetic fit. The first-order rate constant is found to be linear with the AO concentration employed.

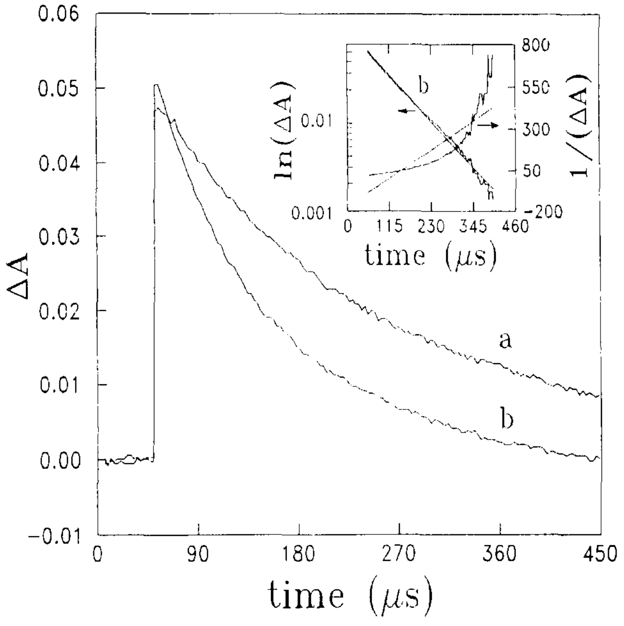


Fig. 3. Oscilloscope traces showing decay of transient absorption (400 nm) in presence of (a) 3.5×10^{-5} and (b) 5.3×10^{-5} mol dm^{-3} AO. Inset: kinetic analysis showing first- and second-order fits.

We can therefore infer that semireduced AO interacts with the parent dye molecule itself and decays by pseudo-first-order kinetics. Such an interaction could be shown by

$$AOH + AOH^+ \longrightarrow (AOH)_2{}^+ \qquad (12)$$

The bimolecular rate constant for the interaction of semireduced AO with the parent dye derived from the slope of this curve was found to be 1×10^8 dm^3 mol^{-1} s^{-1}. The decay of the semireduced species formed in the electron-pulsed N_2O-saturated 1.0 mol dm^{-3} isopropanol or 0.1 mol dm^{-3} formate matrix at pH 7 also followed the same kinetics with rate constant values close to that observed for the *tert*-butanol matrix within experimental error. In the case of other dyes, as stated before [10,14,18], a disproportionation mechanism for the decay of the semireduced species has been inferred on the basis of 50% recovery of the dye bleaching monitored at some suitable wavelength where the parent molecule absorbs. In the present case, however, the second-order decay of AOH was not observable owing to the prominent pseudo-first-order reaction (12). The decay kinetics of the transient formed by the reaction of ketyl radicals at acidic pH showed a more or less similar trend, except that the rate constant for the interaction of the transient semireduced species with AO was somewhat lower (6×10^7 mol^{-1} dm^3 s^{-1}) than that observed at neutral pH. In order to keep the ionic strength (μ) constant at the two pH values, adjustment of pH 7 was made by adding appropriate equimolar phosphate concentrations (5.5×10^{-3} mol dm^{-3}). This maintained μ of the solution at 0.03 mol dm^{-3}, the same as that of the acidic solution (1×10^{-2} mol dm^{-1} H_2SO_4). The lowering of the rate constant at acidic pH may therefore not be due to the effect of ionic strength. At pH below 4.4 (Eq. (11)) the predominant form of semireduced species is $AOH_2{}^+$ and therefore its interaction with the dye, which exists as AOH^+ (Scheme 1), is expected to be slower, in view of electrostatic repulsion between two approaching like-charged species, as compared with that involving the neutral AOH radical at pH above 4.4. The observed change in decay kinetics at various pH values thus appears to be due to an electrostatic effect.

3.6. One-electron reduction potential of AO

The one-electron reduction potentials of most acridine dyes have been determined by polarographic methods. These values along with those of other dyes have been systematically tabulated by Chan and Bolton [20]. It should be noted that these dyes undergo two-electron reductions electrochemically and in many cases it may be difficult to separate the first and second polarographic waves to infer an accurate value of the one-electron potential. We have attempted here to estimate the one-electron reduction potential of acridine orange by pulse radiolysis using the equilibrium method [21]. We studied the equilibrium between two redox couples, one being a standard with known potential and the other the AOH^+/AOH couple whose potential is to be determined. Equilibrium could not be established with some of the reference couples tried, such as $O_2/O_2{}^-$, MV^{2+}/MV^+ (MV, methyl viologen) and NB/NB^- (NB, nitrobenzene), since electron transfer was favoured only in one direction. In fact, Chan and Bolton in their flash photolysis study of AO [22] showed that semireduced AO could completely react with M^{2+} to give MV^+ and we have seen that the reverse reaction was not at all detectable. These results clearly indicate a high negative potential of acridine orange. Other standard couples with higher negative potentials employed were BP/BP^- (BP, benzophenone) and $(CH_3)_2CO,H^+/(CH_3)_2\dot{C}OH$.

3.6.1. $(CH_3)_2CO,H^+/(CH_3)_2\dot{C}OH$ as standard couple

The equilibrium established in this case can be represented as

$$AOH^+ + (CH_3)_2\dot{C}OH \rightleftharpoons AOH + (CH_3)_2CO(+H^+) \qquad (13)$$

We have discussed earlier the reaction of the ketyl radical with AO and mentioned its electron transfer efficiency and possible formation of an adduct radical. Here, if we assume that the adduct formed does not interfere with the measurement and also that the yield of the adduct remains constant, then any variation in absorbance due to AOH on changing the concentration of acetone can be attributed to the above equilibrium (13). It may be noted that in our study with the ketyl radical earlier, no acetone was employed externally and hence in the reaction of this radical with AO (Eq. (7)) the matrix will have only the stoichiometric concentration of acetone. This will favour the forward reaction with no excess acetone being present in the matrix. In order to create a favourable condition for the back reaction to take place, we

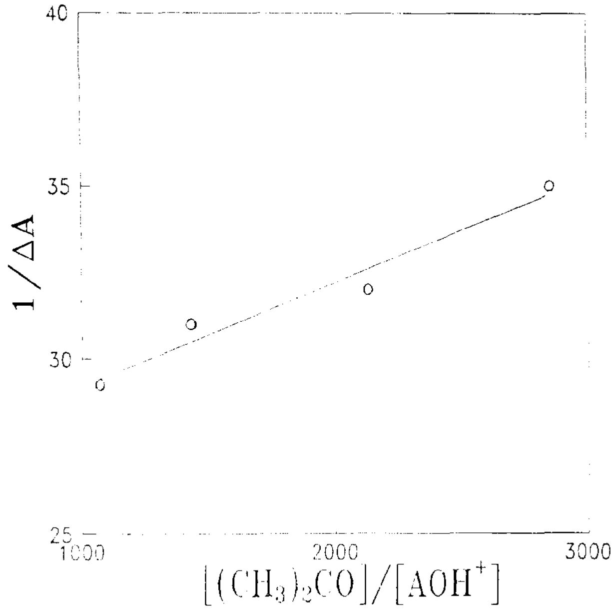


Fig. 4. Plot of $1/\Delta A$ (400 nm) vs. [acetone]/[AO] in N_2-saturated 1 mol dm^{-3} isopropanol matrix (pH 7) containing 7×10^{-5} mol dm^{-3} AO and varying concentrations of acetone.

employed a matrix containing fixed concentrations of 1 mol dm^{-3} isopropanol and 7×10^{-5} mol dm^{-3} AO and varying concentrations (in the range 0.075–0.20 mol dm^{-3}) of acetone. Electron pulse irradiation of the above matrix saturated with N_2 will lead to ketyl radicals via Eqs. (14) and (7):

$$(CH_3)_2CO + e_{aq}^- + H^+ \longrightarrow (CH_3)_2\dot{C}OH \qquad (14)$$

The ketyl radicals so formed will react with AOH^+ leading to the equilibrium (13). The equilibrium constant K is related to the concentrations of the species by

$$K = \frac{[AOH][(CH_3)_2CO]}{[AOH^+][(CH_3)_2COH]} \qquad (15)$$

When we monitor the absorbance (A) at 400 nm where semireduced AO (AOH) absorbs and assuming that the total radical concentration [R] (at a given dose and G value) is constant throughout (see Ref. [23] for details), it can be shown that

$$\frac{1}{A} = \frac{[(CH_3)_2COH]}{K\epsilon[R][AOH^+]} + \frac{1}{\epsilon[R]} \qquad (16)$$

where ϵ is the molar absorptivity of semireduced AO at 400 nm. A plot of $1/A$ vs. $[(CH_3)_2CO]/[AOH^+]$ is expected to be linear with intercept/slope = K. Although the experimental data fluctuate considerably owing to the back reaction (Eq. (13)) being less favoured, they fit a linear plot (Fig. 4). From this plot the equilibrium constant K was computed as 8.5×10^3. The magnitude of K is related to the difference ΔE^0 in the reduction potentials of the two couples AOH^+/AOH and $(CH_3)_2CO,H^+/(CH_3)_2\dot{C}OH$ by

$$\Delta E^0 = E^0_{AOH^+/AOH} - E^0_{(CH_3)_2CO,H^+/(CH_3)_2COH} = 0.059 \log K \qquad (17)$$

Knowing the values of K and $E^0_{(CH_3)_2CO,H^+/(CH_3)_2COH}$ (1.39 V (NHE) [13]), $E^0_{AOH^+/AOH}$ was computed as -1.16 ± 0.05 V (NHE) at pH 7. Determination of the equilibrium constant by the kinetic method [23,24] was tried, but as the error in the measurement of the rate constant was very high, we have not included the result here. The reported polarographic value of -0.92 V [20] for $E^0_{AOH^+/AOH}$ appears to be somewhat less negative. This will also be evident from the results of our next experiment carried out with BP/BP^- as the reference couple.

3.6.2. *BP/BP⁻ as standard couple*

The one-electron reduction potential of benzophenone is reported [25] to be -1.0 V (NHE). If we assume the value of $E^0_{AOH^+/AOH}$ to be -0.92 V as reported by Chan and Bolton [20], then the equilibrium between these couples should be established with ease. On the contrary, the BP^- generated was unable to reduce AO to give the semireduced species. Thus the decay of the BP^- anion radical absorption monitored at 330 nm was found to be unaffected by the presence of AO at a concentration as high as 1×10^{-4} mol dm^{-3}, whereas in the presence of a trace quantity of O_2 ($E^0_{O_2/O_2^-}$ being -0.155 V (NHE) it became considerably faster. A higher concentration of AO was not employed since its dimer formation becomes more and more favoured as the dye concentration is increased. We feel that the one-electron reduction potential of AO should be somewhat higher than -1.0 V (NHE).

4. Conclusions

The rate constant for the reaction of hydrated electrons with acridine orange is very high and close to the diffusion-controlled limit. Isopropyl ketyl and carbon dioxide anion radicals react by one-electron transfer to give semireduced species in yields of 76% and 58% respectively.The semireduced species generated in these reactions revealed three prominent absorption bands with λ_{max} at 270, 410 and 490 nm at neutral pH. The 410 nm band of the semireduced species is blue shifted by 50 nm at acidic pH owing to the involvement of a protolytic equilibrium with a pK_a of 4.4. The semireduced acridine orange decays by pseudo-first-order kinetics with respect to the parent dye concentration. The one-electron reduction potential of acridine orange is -1.16 V (NHE). The high negative potential and strong visible absorption band of the dye could be of advantage for its utilization as a sensitizer in reactions designed for the photolysis of water by solar radiation.

References

[1] A.I. Krasna, *Photochem. Photobiol.*, *31* (1980) 75.
[2] L.I. Grossweiner, *Photochem. Photobiol.*, *10* (1969) 183.

[3] (a) H. Schmidt, A. Al-Ibrahim, U. Dietzel and L. Bieker, *Photochem. Photobiol., 33* (1981) 127; (b) J.D. Spikes and R. Straight, *Ann. Rev. Phys. Chem.*, (1967) 409.
[4] A. Kellmann, *Photochem. Photobiol., 14* (1971) 85.
[5] A. Kellmann, *Photochem. Photobiol., 20* (1974) 103.
[6] A. Kellmann and Y. Lion, *Photochem.. Photobiol., 29* (1979) 217.
[7] E.A. Balazs, J.V. Davies, G.O. Philips and D.S. Scheufele, *J. Chem. Soc. C*, (1968) 1429.
[8] S. Solar, W. Solar and N. Getoff, *Z. Natur. A, 37* (1982) 78.
[9] V.Z. Zanker, *Phys. Chem. (Lpz.), 199* (1952) 225.
[10] S.N. Guha, P.N. Moorthy, K. Kishore, D.B. Naik and K.N. Rao, *Proc. Indian Acad. Sci. (Chem. Sci.), 99* (1987) 99, 261.
[11] E.M. Fielden, in J.H. Baxendale and F. Busi (eds.), *Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*, Reidel, Dordrecht, 1982, pp. 49–62.
[12] K.D. Asmus, H. Mockel and A.J. Henglein, *Phys. Chem., 73* (1969) 2573.
[13] H.A. Schwarz and R.W. Dodson, *J. Phys. Chem., 93* (1989) 409.
[14] J. Mahadevan, S.N. Guha, K. Kishore and P.N. Moorthy, *Proc. Indian Acad. Sci. (Chem. Sci.), 101* (1989) 43.
[15] G.V. Buxton, in J.H. Baxendale and F. Busi (eds.), *Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*, Reidel, Dordrecht, 1982, pp. 321–349.
[16] D.B. Naik and P.N. Moorthy, *J. Chem. Soc., Perkin Trans. 2*, (1990) 705.
[17] A.J. Swallow, in J.H. Baxendale and F. Busi (eds.), *Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*, Reidel, Dordrecht, 1982, pp. 317–345.
[18] S.N. Guha, P.N. Moorthy and J.P. Mittal, *J. Chem. Soc., Perkin Trans., 2* (1993) 409.
[19] A. Kira and M. Koizumi, *Bull. Chem. Soc. Jpn., 42* (1969) 625.
[20] M.S. Chan and J.R. Bolton, *Solar Energy, 24* (1980) 561.
[21] P. Wardman, *J. Phys. Chem. Ref. Data, 18* (1968) 1637.
[22] M.S. Chan and J.R. Bolton, *Photochem. Photobiol., 34* (1981) 34, 537.
[23] S.N. Guha, P.N. Moorthy and J.P. Mittal, *Radiat. Phys. Chem., 39* (1992) 183.
[24] K. Kishore, S.N. Guha, J. Mahadevan, P.N. Moorthy and J.P. Mittal, *Radiat. Phys. Chem., 34* (1989) 721.
[25] P.S. Rao and E.J. Hayon, *J. Am. Chem. Soc., 96* (1974) 1287.