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Reactions of sulphacetamide with pulse radiolytically generated reducing radicals

S SABHARWAL[1]*, K KISHORE[2] and P N MOORTHY[2]

[1]Isotope Division, and [2]Applied Chemistry Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085

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Abstract. Pulse radiolysis technique has been used to characterise the transients formed by the reaction of sulphacetamide with e^-_{aq} and subsequently study the electron transfer reactions from the transient to various electron acceptors such as thionine, safranine-T and methyl viologen. The results indicate that the semi-reduced sulphacetamide species are highly reducing in nature as they transfer electrons to various dyes with near diffusion controlled rates ($k > 10^9 dm^3 mol^{-1} s^{-1}$) in alkaline and acidic solutions. The influence of oxygen on the decay behaviour of semi-reduced species has been investigated and the results show that O_2 reaction with SA^- is very fast ($k = 1{\cdot}5 \times 10^9 dm^3 mol^{-1} s^{-1}$) and leads to the formation of a permanent-coloured product. Reactions of H atoms resulted in the formation of two transient species whose spectral, kinetic and acid–base characteristics have also been investigated.

Keywords. Sulphacetamide; pulse radiolysis; electron-transfer reaction.

1. Introduction

The photochemical studies of sulphonamide drugs are of importance as these drugs undergo photodegradation and also cause phototoxic and photoallergic reactions (Weinstein 1975). In recent years, the fast techniques of laser flash photolysis and pulse radiolysis have been applied to study and understand the molecular basis for phototoxicity and photoallergy of many drugs including sulphacetamide (Land *et al* 1982; Hamouldi *et al* 1984; Navartnam *et al* 1985; Jones *et al* 1988). From these studies, the major short-lived species viz. the drug cation radical, the hydrated electron, the superoxide anion radical and singlet oxygen were identified as the probable agents which can cause damage to biological material. These studies have mainly dealt with the identification of transient species produced from the one-electron redox reactions of the drug and the factors which control their formation. The subsequent electron transfer reactions from these transients to suitable acceptor/donor molecules have not been reported for these drugs principally because of the overlapping absorption of the species involved and interference from the products of these reactions. However, such studies can provide useful information about the possible reactions these species can undergo in biological systems.

*For correspondence

In our earlier paper, we have reported the electron transfer reactions from semi-oxidised sulphacetamide radicals to biological antioxidant ascorbic acid (Sabharwal *et al* 1994). In the present paper we give a detailed report on some properties of radicals formed in the reaction of sulphacetamide with reducing radicals viz. e_{aq}^- and H atoms. The technique of pulse radiolysis has been used to generate and characterize the transients formed in the one-electron reduction of sulphacetamide and subsequently study the one-electron transfer reactions from semi-reduced sulphacetamide to acceptor compounds such as thionine, safranine-T and methyl viologen at different pHs. Recent studies have shown that the anion radicals of certain drugs are auto-oxidised rapidly in the presence of molecular oxygen to regenerate the parent compound and super-oxide anion ($O_2^{-\cdot}$). The net result of this reaction is the catalytic reduction of molecular oxygen to $O_2^{-\cdot}$, which may cause oxidative stress (Suntres and Shek 1992). Therefore, reactions of O_2 with the semi-reduced sulphacetamide radicals have also been investigated.

2. Experimental

Sulphacetamide was obtained from Sigma Chemicals and its purity checked by TLC. All other reagents were of AnalaR grade. Nanopure water (conductivity 0·6μ siemens cm^{-1}) obtained by passing distilled water through a Barnstead Nanopure Cartridge System was used to prepare all solutions. The solutions were purged with Iolar grade (Indian Oxygen) gases. The *p*H values of the solutions were adjusted using H_2SO_4, KH_2PO_4, Na_2HPO_4 $2H_2O$, $Na_2B_4O_7$ $10H_2O$ and NaOH in appropriate concentrations. Details of the pulse radiolysis experimental set-up used have been described in detail elsewhere (Guha *et al* 1987). 50 ns single pulses of 7-MeV electrons were used for irradiating the solutions in 1 cm square suprasil cuvettes. The typical dose as measured by thiocynate dosimeter was 16 Gy taking GE for the $(CNS)_2^-$ radical generated as 21522 $dm^3\,mol^{-1}\,cm^{-1}$ per 100 eV at 500 nm (Fielden 1984). For studying the reactions of e_{aq}^- and H atoms, *t*-butyl alcohol was used as OH radical scavenger. In neutral solutions H atoms were generated by the reaction of e_{aq}^- with 1 mol dm^{-3} KH_2PO_4 via

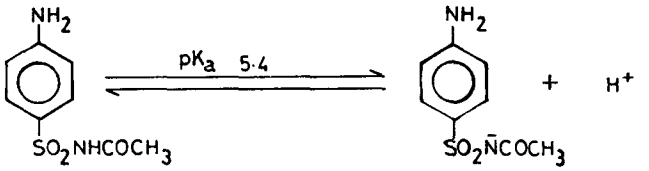
$$H_2PO_4^- + e_{aq}^- \longrightarrow H + HPO_4^{-2}. \qquad (1)$$

For gamma irradiation studies a Co-60 source (dose rate: 14 Gy min^{-1}) was employed.

3. Results and discussion

Sulphacetamide has a pk_a value of 5·4. It has an acidic N–H linkage adjacent to the sulphonyl group (scheme 1) and above *p*H 7 is practically completely ionized (Bells and Robin 1942).

Its reactions therefore were studied at *p*H 9·2 and 3·8 where it is present in distinct conjugate acid or base form.



Scheme 1.

3.1 *Reactions of e^-_{aq} with sulphacetamide*

The reaction of hydrated electrons with the two acid–base forms of sulphacetamide was studied by pulse irradiating its nitrogen saturated solutions containing 0·1 mol dm^{-3} *t*-butanol at *p*H 3·8 or 9·2. The rates of reaction were measured by following the decay of absorbance due to e^-_{aq} at 720 nm in the presence of 1×10^{-4} to 4×10^{-4} mol dm^{-3} sulphacetamide. At *p*H 3·8, the rate of reaction of neutral form of sulphacetamide (SA) with hydrated electron was $2{\cdot}5 \times 10^{10}$ dm^3 mol^{-1} s^{-1}. The absorption spectrum of the transient observed 2 μs after the pulse (figure 1a) shows the presence of one optical absorption band with $\lambda_{max} = 370$ nm and extinction coefficient (ε) $= 4 \times 10^3$ dm^3 mol^{-1} cm^{-1}. The transient species at 370 nm decayed by second-order kinetics with $2k/\varepsilon l = 1{\cdot}1 \times 10^6$ s^{-1} giving a value of $4{\cdot}4 \times 10^9$ dm^3 mol^{-1} s^{-1} for the bimolecular decay rate constant of this species. The reaction with the deprotonated form of sulphacetamide (SA^-) present at *p*H 9·2 was an order of magnitude slower ($k = 1{\cdot}95 \times 10^9$ dm^3 mol^{-1} s^{-1}). This is understandable because the reaction at *p*H 9·2 is between two negatively charged species and the resulting electrostatic repulsion may slow down the reaction considerably. The absorption spectrum of the transient species at *p*H 9·2, shown in figure 1b is similar to the one at *p*H 3·8 except that the λ_{max} is shifted to 390 nm and the extinction coefficient is much lower ($\varepsilon = 1{\cdot}35 \times 10^3$ mol dm^{-3}). The species decayed by second order kinetics with $2k/\varepsilon l = 1 \times 10^6$ dm^3 mol^{-1} s^{-1}. The spectral and kinetic parameters of the transient (given in table 1) can be assigned to the semi-reduced sulphacetamide radicals and their formation can be represented by the reaction:

$$SA + e^-_{aq} \longrightarrow SA^{-\cdot}, \text{ (pH 3·8)}, \tag{3}$$

$$SA^- + e^-_{aq} \longrightarrow SA^{-\cdot\,-}, \text{ (pH 9·2)}, \tag{4}$$

The difference in the extinction coefficient of the transient at 370 nm in weakly acidic and alkaline solutions were made use of in determining its pk_a. From the plot of absorption vs *p*H (inset in figure 1), the pk_a of the semi-reduced species was 6·2 which is about 0·8 units away from that of the parent molecule pk_a of 5·4. This indicates that electron addition to sulphacetamide is in the vicinity of the acidic –NH– linkage. It is known that sulphonamides which possess groups capable of donating electrons to the –NH–linkage have higher pk_a values (Bells and Robin 1942). The reaction of e^-_{aq} with sulphonamides is known to cleave the S–N bond resulting in the formation of sulphanilic acid as a final degradation product (Phillips *et al* 1973). However, initially the electron adduct of sulphacetamide appears to be favoured as an intermediate.

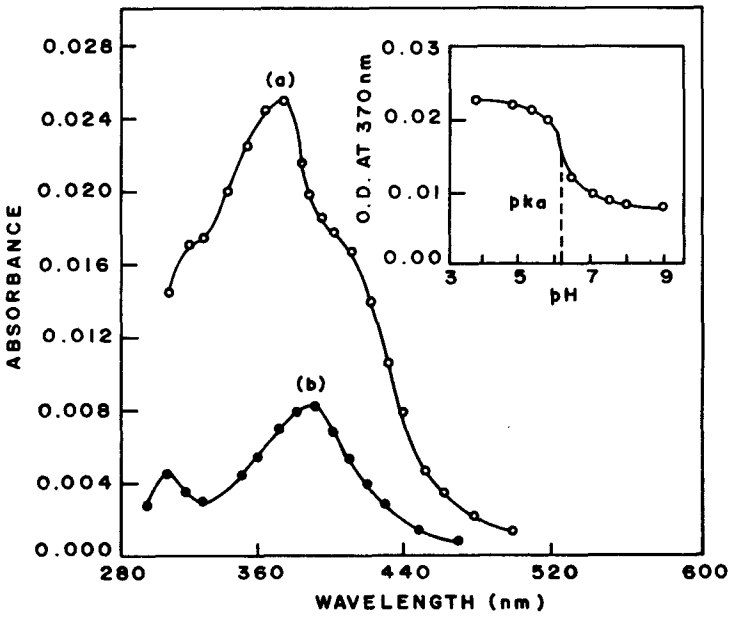


Figure 1. Absorption spectra of sulphacetamide–e^-_{aq} adducts (a) at *p*H 3·8 and (b) at *p*H 9·2 monitored with 1×10^{-4} mol dm^{-3} sulphacetamide aqueous solution 2 μs after the 50 ns pulse. *Inset*: Absorption changes as a function of *p*H at 370 nm for the sulphacetamide–e^-_{aq} adduct.

Table 1. Kinetic and spectroscopic data of the transients formed by reaction of e^-_{aq} and H atoms with sulphacetamide.

Reductant	*p*H	λ_{max} (nm)	ε (dm^3 mol^{-1} cm^{-1})	Reaction rate (dm^3 mol^{-1} cm^{-1})	$2k/\varepsilon l$ (s^{-1})
e^-_{aq}	3·8	390	3985	$2{\cdot}5 \times 10^{10}$	1×10^6
	9·2	370	1340	1×10^9	$1{\cdot}1 \times 10^6$
H	2·0	380	4200	8×10^8	$8{\cdot}9 \times 10^5$
	2·0	330	7900	$1{\cdot}3 \times 10^9$	$3{\cdot}5 \times 10^5$
	5·8	380	3850	$3{\cdot}6 \times 10^9$	6×10^5

3.2 *Reducing properties of semi-reduced sulphacetamide*

Specific one-electron reductants such as $(CH_3)_2C{\cdot}OH$ and $CO_2^{-\cdot}$ were unable to bring about reduction of sulphacetamide in the *p*H range 2–13. Therefore, the one-electron reduction potential of sulphacetamide was inferred to be $> -2{\cdot}0$ V versus NHE indicating that the semi-reduced species, formed by reaction of hydrated electron, must be a strong reductant. In order to confirm this, its reaction with various electron-acceptors, such as thionine ($E^{0\,1} = -0{\cdot}04$ V) (Guha *et al* 1987), safranine-T ($E^{0\,1} = -0{\cdot}185$ V) (Naik and Moorthy 1990) and methyl viologen ($E^{0\,1} = -0{\cdot}450$ V) (Nahar and Rabani 1985) were studied at *p*H 3·8 and 9·2.

$$SA^{-\cdot}/SA^{-\,-\cdot} + \text{dye} \longrightarrow SA/SA^- + \text{dye}^{-\cdot} \qquad (5)$$

Table 2. Rate constant for electron transfer from e^-_{aq} reaction product of sulphacetamide to various electron acceptors.

Electron acceptor	E_0	pH	Rate constant ($dm^3\,mol^{-1}\,s^{-1}$)
Thionine	−0·045	3·8	$4·6 \times 10^9$
		9·2	$> 5 \times 10^{10}$
Safranine-T	−0·185	3·8	$5·4 \times 10^9$
		9·2	$7·2 \times 10^9$
Methyl viologen	−0·535	3·8	$1·4 \times 10^{10}$
		9·2	$1·1 \times 10^{10}$
Oxygen	—	3·8	$1·6 \times 10^9$
		9·2	$1·5 \times 10^9$

Confirmation of electron transfer was done by comparing the transient absorption spectra obtained by this reaction with that obtained directly by reaction of the above acceptors with e^-_{aq} under identical conditions. The rate constants were determined by following the build-up of the product absorption and are shown in table 2. These results show that the semi-reduced species transfers electrons to all the acceptors with near diffusion-controlled rates, irrespective of their redox potentials, at both the *p*H values and hence, the reduction potential of both the forms $SA^{-\cdot}/SA^{-\,-\cdot}$ is much more negative than −0·450 V versus NHE.

3.3 *Reactions of oxygen with the semi-reduced species*

The reaction of many drugs or their metabolites with oxygen bring about incomplete reduction of oxygen resulting in the formation of species such as O_2^- which have been implicated in the oxidative stress-induced toxicity (Holtzman 1982). A study of the reactions of the semi-reduced sulphacetamide with O_2 is therefore of importance. For this purpose, the effect of oxygen on the decay behaviour of the pulse-radiolytically generated semi-reduced sulphacetamide species, was investigated at appropriate λ_{max} under the experimental conditions in a manner such that (i) OH radicals are scavenged by *t*-butanol (ii) majority (>95%) of the electrons react with sulphacetamide to produce the semi-reduced species which subsequently decay by reacting with oxygen. It is evident from the decay profiles of the transients in presence and absence of oxygen at *p*H values of 3·8 and 9·2 (figures 2 and 3) that at both the *p*H levels, the semi-reduced species react with oxygen. The rate constants for the reactions were $1·6 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$ and $1·5 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$ at *p*H 3·8 and at *p*H 9·2 respectively. In the absence of oxygen, the species decayed by a second order kinetics at both the *p*H levels. The interference from the small amount of O_2^- formed can be neglected as its reaction with sulphacetamide has been reported to be only $7 \times 10^7\,dm^3\,mol^{-1}\,s^{-1}$ and hence it will not react in this time scale (Land *et al* 1982).

Further, the formation of an unidentified permanent yellow coloured product was observed in oxygenated sulphacetamide solutions pulsed at *p*H 3·8. This was also indicated by the transient decay profile ($\lambda = 370$ nm) at this *p*H as shown in figure 2, where the absorbance increases at latter times. It has been suggested that singlet oxygen as well as superoxide anion may oxidize sulphacetamide and produce the

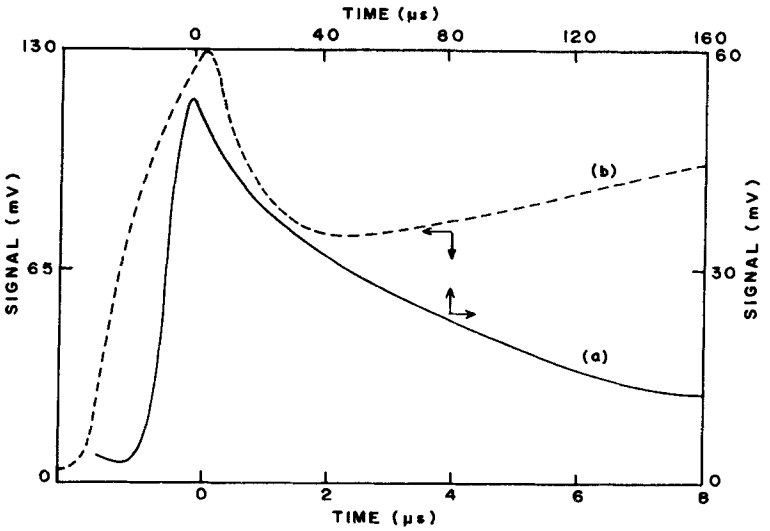


Figure 2. Kinetic traces of decay of sulphacetamide–e^-_{aq} adduct monitored at 370 nm with 1×10^{-2} mol dm^{-3} sulphacetamide containing 1 mol dm^{-3} *t*-butanol under (a) nitrogen. (b) oxygen at *p*H 9·2.

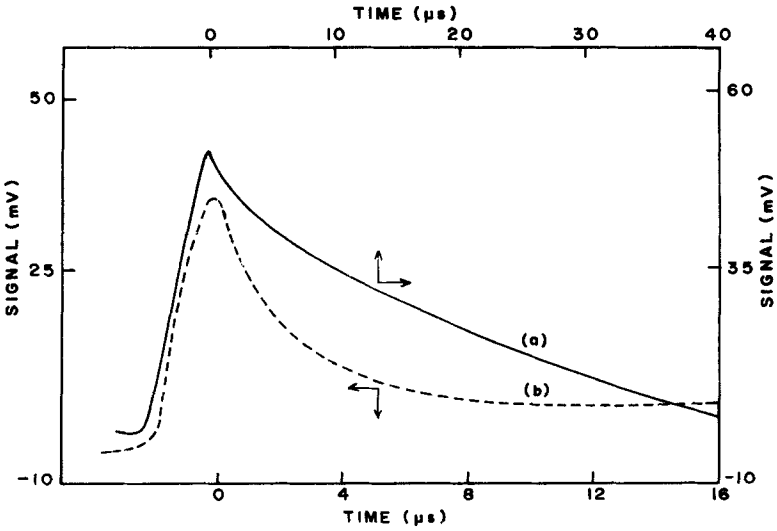


Figure 3. Kinetic traces of decay of sulphacetamide–e^-_{aq} adduct monitored at *p*H 3·8 under (a) nitrogen, (b) oxygen. Other conditions as in figure 2.

observed colouring (Land *et al* 1982). In order to elucidate the role of $O_2^{-\cdot}$ in this reaction, the gamma-radiolysis of sulphacetamide (1×10^{-4} mol dm^{-3}) in presence of 0·1 mol dm^{-3} formate in oxygenated solutions was investigated at *p*H 9·2 and *p*H 3·8. The non-formation of permanent yellow colour product even after irradiating to a dose of 660 Gy suggests that $O_2^{-\cdot}$ reaction with sulphacetamide does not lead to formation of this product(s).

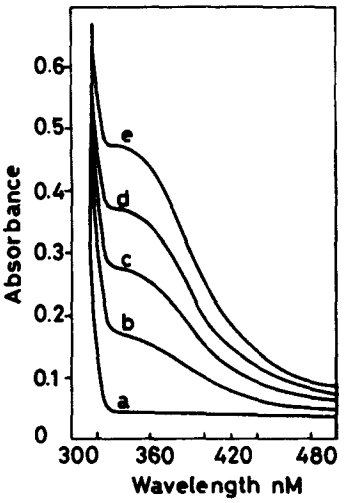


Figure 4. Absorption spectra of 1×10^{-2} mol dm^{-3} sulphacetamide solution containing 1·0 mol dm^{-3} *t*–butanol at *p*H 3·8 irradiated to various gamma radiation doses (a) unirradiated, (b) 105, (c) 210, (d) 315 and (e) 420 Gy.

Figure 4 shows the optical absorption spectrum of an aerated solution of sulphacetamide (1×10^{-2} mol dm^{-3}) at *p*H 3·8 in the presence of 1·0 mol dm^{-3} *t*-butanol irradiated to various gamma doses. On irradiation, the absorption in the 330–400 nm region is observed to increase as in the case of pulse radiolysis results. This increase in absorption is due to the formation of stable products on gamma-irradiation since the blank solution did not show any such change at the same time. Under these irradiation conditions, semi-reduced sulphacetamide is the predominant species that is initially formed which subsequently reacts with oxygen to form the coloured product(s). The reactions occurring under the conditions of figures 3 and 4 can be represented as:

$$OH + (CH_3)_3COH \longrightarrow CH_2(CH_3)_2COH + H_2O, \quad (6)$$

$$e^-_{aq} + SA \longrightarrow SA^{-\cdot} \quad (7)$$

$$SA^{-\cdot} + O_2 \longrightarrow \text{coloured product(s)}. \quad (8)$$

This result indicates that the sulphacetamide anion radical reacts with O_2 irreversibly resulting in the formation of permanent-coloured product.

3.4 *Reaction of sulphacetamide with H atoms*

One electron reduction of sulphacetamide by H atoms was attempted as an alternative way of producing the semi-reduced species. Figure 5(a) shows the spectrum obtained after the pulse radiolysis of nitrogen saturated 1×10^{-3} mol dm^{-3} sulphacetamide solution containing 0·1 mol dm^{-3} of *t*–butanol at *p*H 2. The absorption spectrum exhibited two bands with λ_{max} at 390 nm and 330 nm. H atoms were found to react with sulphacetamide with rate constants of 8×10^8 dm^3 mol^{-1} s^{-1} and $1{\cdot}3 \times 10^9$ dm^3 mol^{-1} s^{-1} respectively at 390 nm and 330 nm. Both the transients decayed by second-

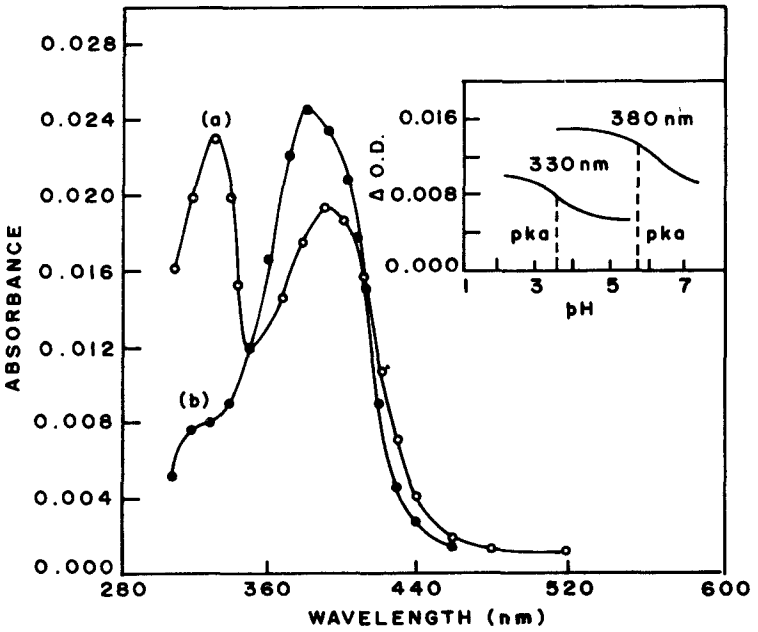


Figure 5. Absorption spectra of the sulphacetamide–H adducts at *p*H values (a) 2 and (b) 5·8 monitored with 1×10^{-4} mol dm^{-3} sulphacetamide solution containing 0·1 mol dm^{-3} *t*–butanol, nitrogen saturated, 10 μs after 50 ns pulse.

order kinetics with $2k/\varepsilon l = 8{\cdot}9 \times 10^5\ dm^3\ mol^{-1}\ s^{-1}$ (390 nm) and $3{\cdot}5 \times 10^5\ dm^3\ mol^{-1}\ s^{-1}$ (330 nm). These results indicate the formation of two intermediates at *p*H 2. The reactions of H atoms at neutral *p*H can also be studied by pulse radiolysis by making use of the reaction of e^-_{aq} with $H_2PO_4^-$ to produce H atoms, (1). Thus H atom reaction with sulphacetamide was studied in N_2-saturated solutions containing 1 mol dm^{-3} KH_2PO_4 and 0·25 mol dm^{-3} Na_2HPO_4 (*p*H 5·8) and 0·1 mol dm^{-3} *t*–butanol. The spectrum of the transient, figure 5b, shows the formation of only one species with λ_{max} at 380 nm. The rate constant for the reaction was found to be $4 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$ and the species was found to decay by second order kinetics with $2k/\varepsilon l = 6 \times 10^5\ s^{-1}$. The pk_a values of the transient species, determined by monitoring the absorbance of the transients at their respective λ_{max} were found to be 6·2 (380 nm) and 3·7 (330 nm). From the results of these studies, summarized in table 1, it is inferred that in neutral solutions, the H atom also reacts mainly by electron transfer reaction and the transient formed has characteristics similar to that produced by e^-_{aq}. This inference was further confirmed by monitoring the electron transfer reaction from the transient at *p*H 5·8 to methyl viologen. As in the case of semi-reduced sulphacetamide, this species also reduced methyl viologen at a fast rate. At *p*H 2, however, the H atom probably reacts by both addition/abstraction as well as electron transfer process, with the former being the more predominant reaction.

4. Conclusion

The reaction of sulphacetamide with e^-_{aq} results in the formation of semi-reduced species which are highly reducing in nature and react with oxygen at a fast rate. At

*p*H 3·8, the reaction of $SA^{-\cdot}$ with O_2 produces a permanent-coloured product. H atoms in neutral solutions also predominantly react by electron transfer reaction whereas in acidic solutions the predominant reaction is abstraction/addition.

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