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Redox chemistry of *o*- and *m*-hydroxycinnamic acids: A pulse radiolysis study

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Abstract. Radiation chemical reactions of $\bullet\text{OH}$, $\text{O}\bullet^-$, $\text{N}_3\bullet$ and e_{aq}^- with *o*- and *m*-hydroxycinnamic acids were studied. The second-order rate constants for the reaction of $\bullet\text{OH}$ with ortho and meta isomers in buffer solution at pH 7 are $3.9 \pm 0.2 \times 10^9$ and $4.4 \pm 0.3 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ respectively. At pH 3 the rate with the ortho isomer was halved ($1.6 \pm 0.4 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) but it was unaffected in the case of meta isomer ($k = 4.2 \pm 0.6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The rate constant in the reaction of $\text{N}_3\bullet$ with the ortho isomer is lower by an order of magnitude ($k = 4.9 \pm 0.4 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The rates of the reaction of e_{aq}^- with ortho and meta isomers were found to be diffusion controlled. The transient absorption spectrum measured in the $\bullet\text{OH}$ with *o*-hydroxycinnamic acid exhibited an absorption maximum at 360 nm and in meta isomer the spectrum was blue-shifted (330 nm) with a shoulder at 390 nm. A peak at 420 nm was observed in the reaction of $\text{O}\bullet^-$ with the *o*-isomer whereas the meta isomer has a maximum at 390 and a broad shoulder at 450 nm. In the reaction of e_{aq}^- , the absorption peaks were centred at 370–380 nm in both the isomers. The underlying reaction mechanism is discussed.

Keywords. Pulse radiolysis; transient absorption spectra; *o*- and *m*-hydroxycinnamic acids, $\bullet\text{OH}$ and $\text{N}_3\bullet$ radicals.

1. Introduction

Pulse radiolysis is an invaluable tool for investigating the effect of radiation on matter, particularly on the identities and molecular structure of short lived intermediates and in unravelling the underlying reaction mechanisms.^{1–3} Pulse radiolysis technique coupled with optical detection, and product analysis by chromatographic methods like GC-MS, HPLC and HPIC, form an important tool for characterisation and quantification of reactive intermediates. Of particular interest are the reactions of the reactive water radicals ($\bullet\text{OH}$ or e_{aq}^-) and secondary radicals (e.g. $\text{N}_3\bullet$, $\text{SO}_4\bullet^-$) derived from them with a variety of organic and biologically important molecules.

There is a growing interest in naturally occurring phenolic compounds that display biological antioxidant properties such as *p*-hydroxycinnamic acid,^{4–7} ferulic acid,^{5–8,9} caffeic acid^{5–7,10,11} and curcumin^{12–16} which are ubiquitous in plant food. Cinnamic acid

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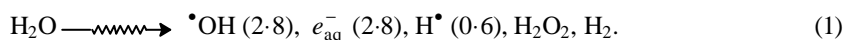
derivatives inhibit oxidative modification of human low density lipoprotein.⁶ It has been demonstrated^{11,17,18} that the interaction of the oxidising OH adduct of DNA, poly A and poly G with hydroxycinnamic acid derivatives proceed via electron transfer. Cinnamic acid derivatives have been shown to be able to scavenge superoxide, peroxy and hydroxyl radical.^{11,19,20}

The hydroxyl radical is known to react with substituted benzenes predominantly by addition to the ring, yielding hydroxycyclohexadienyl radicals and not by interaction with the substituent. In this study, *o*- and *m*-isomers of hydroxycinnamic acid were chosen as model compounds because two possible reaction pathways can occur i.e addition to the aromatic ring and/or addition to the double bond. Earlier work²¹ on the reaction of the $\bullet\text{OH}$ radical with cinnamic acid in aqueous solution has shown that addition to both ring and olefinic group occurs, the relative extent of the two pathways being 3 : 7 respectively. Similarly it has been shown⁴ that $\bullet\text{OH}$ reacts with *p*-hydroxycinnamic acid predominantly by the addition to the unsaturated substituent, forming benzyl type radicals. To understand the effect of substitution on the reaction mechanism, we have undertaken a comprehensive study on the reaction of oxidising and reducing radicals with derivatives of cinnamic acid. In this preliminary study, the results from the reaction of oxidising and reducing radicals with the *o*- and *m*-hydroxycinnamic acids are reported.

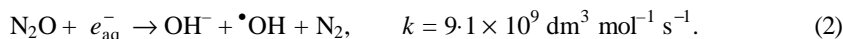
2. Experimental

o- and *m*-Hydroxycinnamic acids were obtained from Aldrich and the other chemicals used were commercially available analytical grade reagents of high purity. All solutions were freshly prepared using deionised water purified by the Millipore – Milli-Q system having a resistivity of $18 \text{ M}\Omega \text{ cm}^{-1}$. The pH of the solution was adjusted by using phosphate buffer (pH = 7), perchloric acid (pH = 3) or sodium hydroxide (pH = 13). Pulse radiolysis experiments were carried out using high energy 7 MeV electron pulses from the linear accelerator facility at the Radiation Chemistry and Chemical Dynamics Division, BARC, Mumbai. Thiocyanate dosimetry was used to determine the dose rate using $10^{-2} \text{ mol dm}^{-3}$ KSCN. The absorbance of $(\text{SCN})_2^{\bullet-}$ was monitored at 500 nm with $G_{500} = 21522 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}/100 \text{ eV}$. In all experiments, 50 ns pulse width was used and the dose per pulse was kept in the range 13–15 Gy.

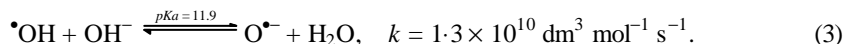
The oxidising and reducing radicals were generated by pulse radiolysis using appropriate solution conditions. Pulse radiolysis of aqueous solution produces reactive radicals primary $\bullet\text{OH}$, e_{aq}^- , $\bullet\text{H}$ and molecular products like H_2O_2 , H_2 , H_3O^+ etc. The values in parentheses are the *G* values per 100 eV.



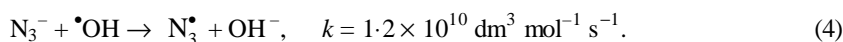
Irradiation of N_2O saturated aqueous solution converts e_{aq}^- into $\bullet\text{OH}$ radicals doubling its yield.



The reaction of $\text{O}^{\bullet-}$ was studied in N_2O saturated basic solutions (pH ~13). At this pH, almost all $\bullet\text{OH}$ radicals are converted into $\text{O}^{\bullet-}$.



N_3^\bullet radicals were generated by irradiation of N_2O saturated $2 \times 10^{-2} \text{ mol dm}^{-3}$ of NaN_3 where all the $\bullet\text{OH}$ radicals were converted into N_3^\bullet with *G* ~5.6 and was used for oxidation in the pH range 4–10.



3. Results and discussion

3.1 Kinetics

3.1a *Reactions of $\cdot\text{OH}$, $\text{O}^{\bullet-}$ and N_3^\bullet radicals:* The rates of the reaction of the $\cdot\text{OH}$ radical with *o*- and *m*-isomers of hydroxycinnamic acids were monitored at the absorption maxima of the intermediates formed and a typical trace depicting the rate of formation in ($1 \times 10^{-3} \text{ mol dm}^{-3}$) *o*-hydroxycinnamic acid in buffer solution (*pH* 7) is given in figure 1a. The rates of formation of the intermediate species was found to be linear with the solute concentration in the range $(2-8) \times 10^{-4} \text{ mol dm}^{-3}$. The second order rate constant was found to be $(3.9 \pm 0.2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1})$, and a similar value ($k = 4.4 \pm 0.3 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) was observed with the *m*-isomer. These values are lower than reported by Bobrowski⁴ in the *para* isomer ($k = 8.2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). In acidic medium (*pH* 3), the rate of the $\cdot\text{OH}$ reaction with the *ortho* isomer was found halved ($k = 1.6 \pm 0.4 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The rate constant values measured in neutral solution along with those reported in for cinnamic^{21,22} benzoic,²³ and *p*-hydroxycinnamic⁴ acid and are given in table 1. A comparison of these values shows that rates are similar in these systems.

The rates of the $\text{O}^{\bullet-}$ reaction with hydroxycinnamic acids were also determined in basic medium (*pH* = 13) where >90% of the reacting species exists as $\text{O}^{\bullet-}$. The rates

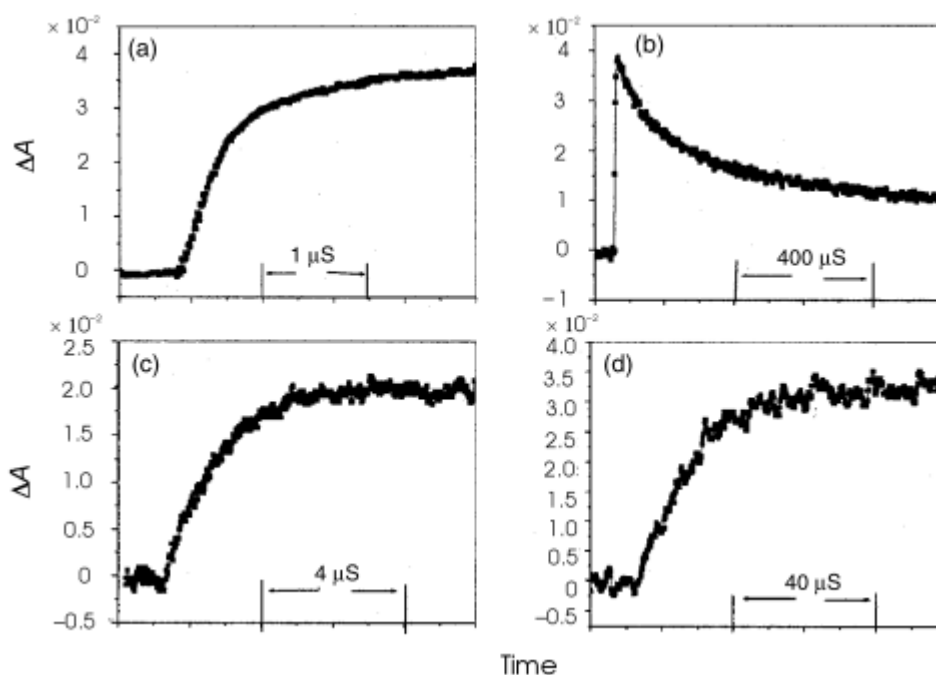


Figure 1. (a) Absorption build up and (b) decay at 360 nm; (c) build up of $\text{MV}^{\bullet+}$ (605 nm) and (d) $\text{TMPD}^{\bullet+}$ (610 nm) in *o*-hydroxycinnamic acid at *pH* 7. Dose/pulse = 15 Gy.

Table 1. Second-order rate constants ($k/\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$) and absorption maxima (I_{max}/nm). Values in parentheses are those recorded at pH 3. Values for benzoic, cinnamic and *p*-hydroxycinnamic acid are taken from published work.

Compounds	•OH		O•-		N ₃ •		e_{aq}^-	
	$k/10^6$	I_{max}	$k/10^8$	I_{max}	$k/10^8$	I_{max}	$k/10^9$	I_{max}
Benzoic acid ²³	(4.3)							
Cinnamic acid ^{1,22}	8.1	310, 365	nd		nd		10.1 ± 1.0 (22.0)	370
<i>o</i> -Hydroxycinnamic acid	3.9 ± 0.2 (1.6 ± 0.4)	360 (360, 400)	8.0 ± 0.7	420	4.9 ± 0.4	360	7.2 ± 0.6	370
<i>m</i> -Hydroxycinnamic acid	4.4 ± 0.3 (4.2 ± 0.6)	330, 390 (330, 430)	7.2 ± 1.3	390, 450	nd	nd	8.9 ± 0.7	380
<i>p</i> -Hydroxycinnamic acid ⁴	8.2	330, 410	3.1	545, 595	nd	nd	nd	nd

nd – not determined

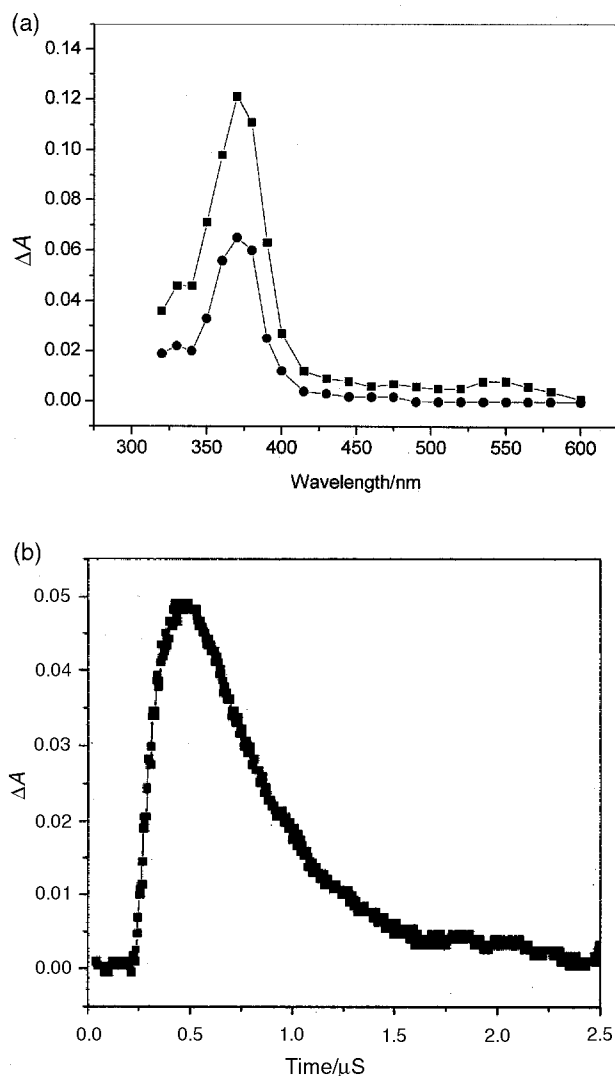


Figure 2. (a) Transient absorption spectrum obtained in the reaction of e_{aq}^- with *o*-hydroxycinnamic acid at pH 7, (■) 2.5 and (●) 40 μs . (b) Decay of e_{aq}^- absorption with solute.

with *o*- and *m*-isomers were comparable with $k = 8.0 \pm 0.7 \times 10^8$ and $7.2 \pm 1.3 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ respectively. The decrease in rates as compared with $\bullet\text{OH}$ radical is due to electrostatic repulsion between the dianion of the hydroxycinnamic acid and $\text{O}^{\bullet-}$ such a lowering was observed by us in other substituted benzenes^{24,25} and purines.²⁶

The reaction of the N_3^\bullet radical with *o*-hydroxycinnamic acid was studied at pH 7 and the second order rate constant measured from the plot of k_{obs} as a function of [*o*-hydroxycinnamic acid] and the fit was found to be good. ($k = 4.9 \pm 0.4 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$). The difference in the reaction rates between $\bullet\text{OH}$ and N_3^\bullet radicals indicates that the latter

reacts more selectively and such a selectivity has been reported²⁷ by us recently in azo dyes where $\rho^{\ddagger} = -2.0$ was obtained from the Hammett plot.

3.1b *Reactions of e_{aq}^-* : The rates of the reaction of e_{aq}^- with *o*- and *m*-hydroxycinnamic acids were monitored at 700 nm in the concentration range $(2-8) \times 10^{-4} \text{ mol dm}^{-3}$. The trace showing the decay of e_{aq}^- in its reaction with *o*-hydroxycinnamic acid ($1 \times 10^{-3} \text{ mol dm}^{-3}$) is given in figure 2b. The rate constants obtained from the linear plot of k_{obs} versus [solute] were found to be diffusion controlled. With $k = 7.2 \pm 0.6 \times 10^9$ and $8.9 \pm 0.7 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ for *o*- and *m*-isomers respectively.

3.2 Transient absorption spectra

The spectrum obtained in the reaction of $\bullet\text{OH}$ with *o*-hydroxycinnamic acid ($1 \times 10^{-3} \text{ mol dm}^{-3}$) in phosphate buffer solution (*pH* 7) exhibited an intense absorption

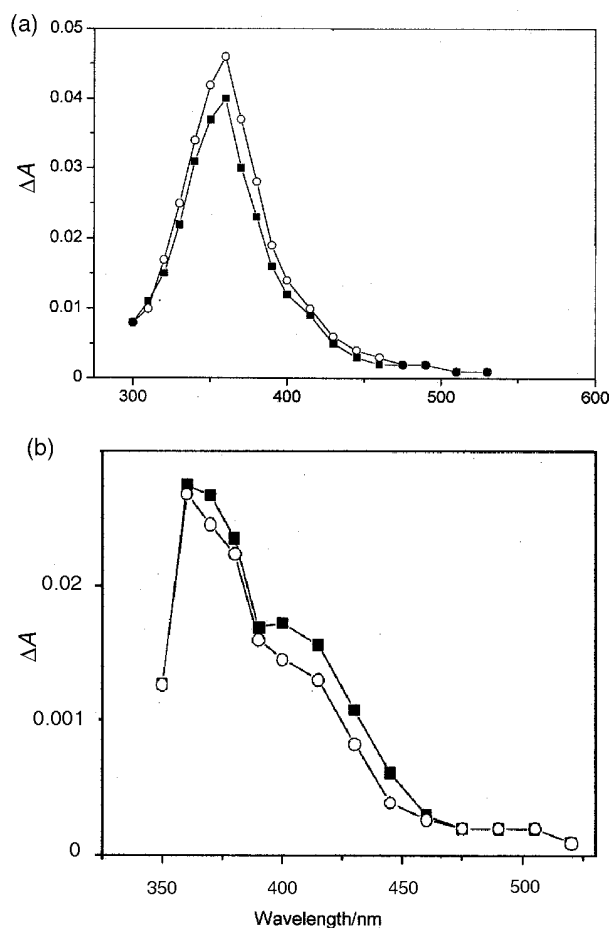


Figure 3. (a) Time-resolved absorption spectra obtained in the reaction of $\bullet\text{OH}$ with *o*-hydroxycinnamic acid ($1 \times 10^{-3} \text{ mol dm}^{-3}$) at *pH* 7: (O) 7.5 and (■) 40 μs and (b) *pH* 3: (■) 3 and (O) 40 μs after the pulse. Dose/pulse = 15 Gy.

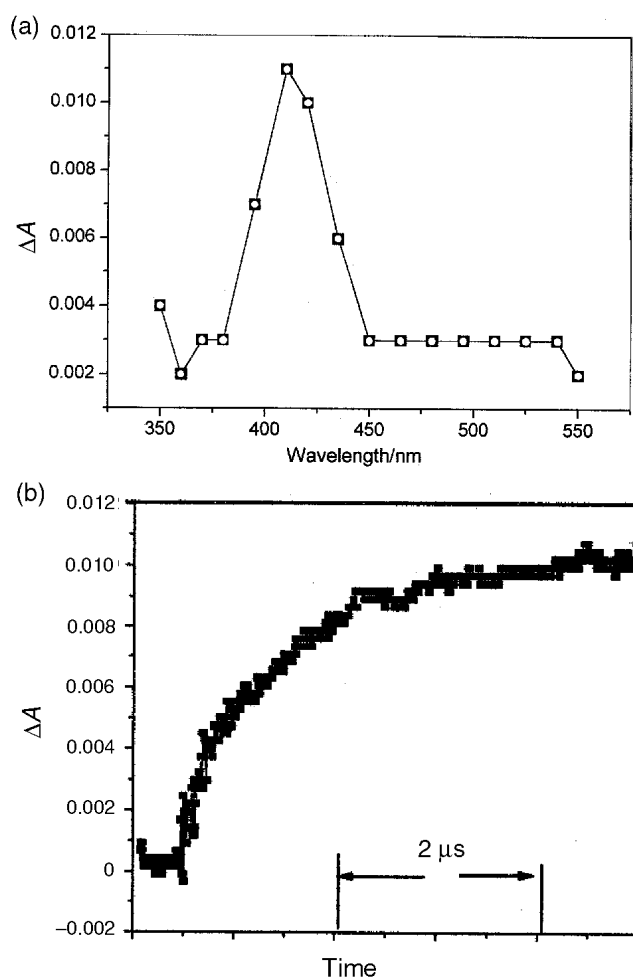


Figure 4. (a) Transient absorption spectrum obtained in the reaction of $O^{\bullet -}$ with *o*-hydroxycinnamic acid ($1 \times 10^{-3} \text{ mol dm}^{-3}$), at (■) 3 and (○) 40 ns after the pulse. (b) Absorption buildup at 430 nm.

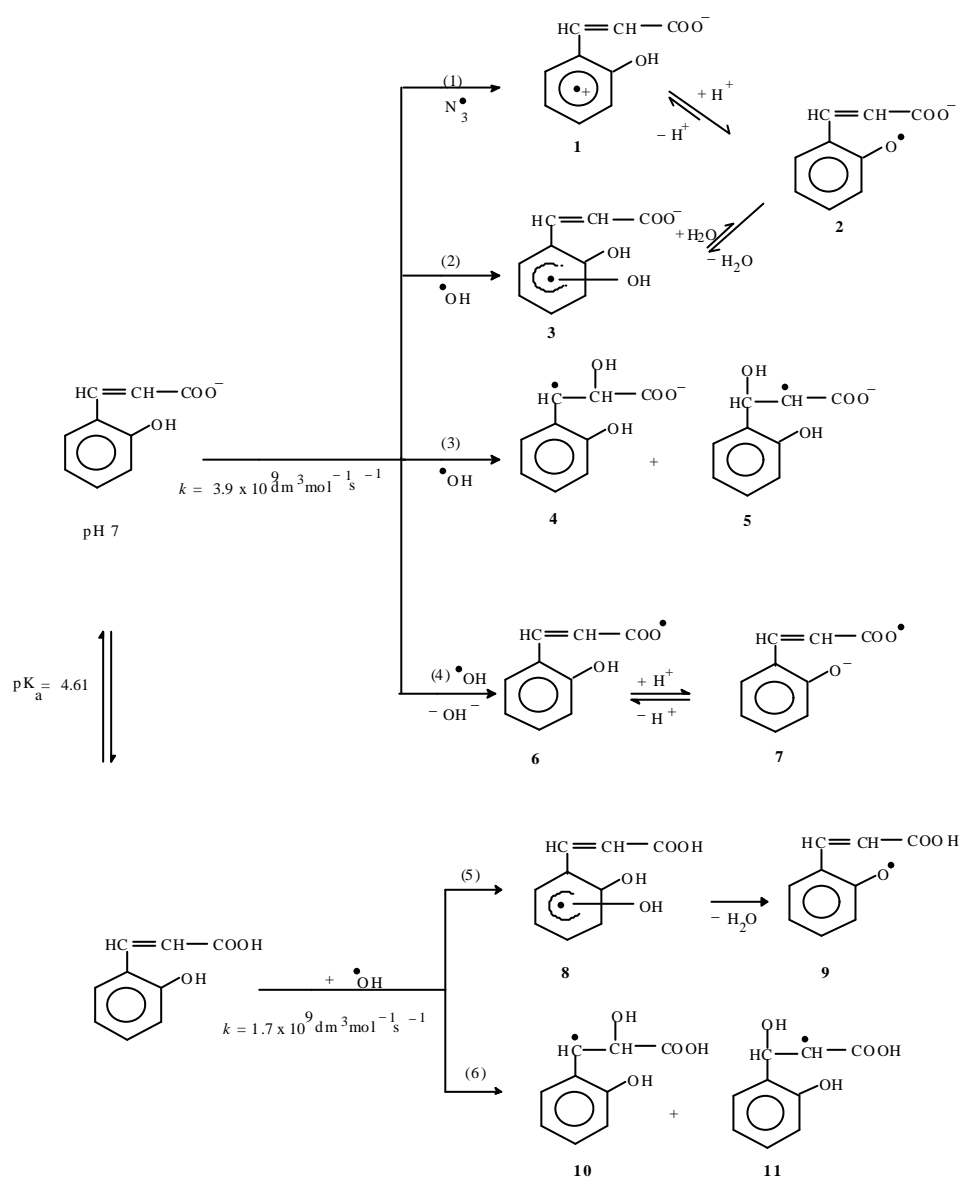
maximum at 360 nm (figure 3a). The time resolved spectrum has not shown any changes (figure 3a) and only bimolecular decay was noticed (figure 1b). The spectrum recorded in acid solution at pH 3 is different with a less intense peak at 360 nm and a broad shoulder centred around 400 nm (figure 3b). In contrast with behaviour observed in ortho isomer at pH 7, the spectrum was blue shifted ($\lambda_{\text{max}} = 330 \text{ nm}$) with a broad shoulder at 400 nm in the meta isomer. However, spectra are identical in the two isomers at pH 3.

The transient absorption spectrum obtained in the reaction of $O^{\bullet -}$ with the ortho isomer exhibited a sharp peak at 420 nm (figure 4a), whereas the meta isomer exhibited two peaks at 390 and 450 nm. When the one electron oxidant, N_3^{\bullet} radical was employed, the spectrum recorded in buffer solution at pH 7 is similar to that observed in the reaction of the $\bullet OH$ with *o*-hydroxycinnamic acid at the lower pH (figure 5). The transient absorption

spectrum measured in the reaction of e_{aq}^- with *o*- and *m*-hydroxycinnamic acids exhibited peaks at 370–380 nm (figure 2a).

3.3 Reaction mechanism

The azidyl radical, N_3^\bullet , reacts by one electron transfer process and its reaction with *o*-hydroxycinnamic acid proceeds via one electron transfer predominantly from the ring [reaction (1), scheme 1] giving rise to the corresponding radical cation **1**. It immediately



Scheme 1. Pathways for the reaction of the $\bullet\text{OH}$ radical with the *o*-hydroxycinnamic acid.

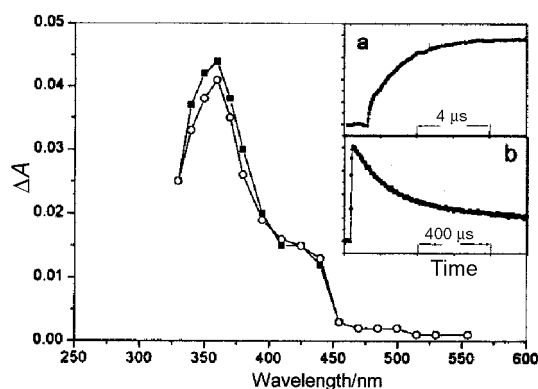


Figure 5. Transient absorption spectrum obtained in the reaction of N_3^\bullet with *o*-hydroxycinnamic acid ($1 \times 10^{-3} \text{ mol dm}^{-3}$) at pH 7, (■) 8 and (○) 40 ns after the pulse. Dose/pulse = 15 Gy. Inset: (a) Absorption build up and (b) decay at 360 nm.

deprotonates to give the corresponding phenoxyl radical **2** because the radical cations of substituted benzenes are not stable except in systems containing electron donating substituents²⁸ (e.g. $-\text{OCH}_3$). Thus, the spectrum observed in this reaction (figure 5) is attributed to the phenoxyl radical.

The reaction of the $\bullet\text{OH}$ radical with *o*-hydroxycinnamic acid at pH 7 can proceed by three ways i.e. addition to the aromatic ring [reaction (2), radical **3**], addition to the aliphatic double bond [reaction (3)] and by electron transfer [reaction (4)]. Based on the second order rate constant, which is nearly diffusion controlled, and the nature of the transient absorption spectra, all the three reactions seem to be operative. The addition to the olefinic double bond [reaction (3)] can give rise to two probable carbon centered radicals **4** and **5**. The carbon centered radical **4** formed by the addition of $\bullet\text{OH}$ at the ∞ carbon is expected to be more stable than that formed by the addition to the **b**carbon radical **5** because the benzylic radical **4** is stabilised by resonance.

In order to quantify these reactions, experiments were carried out using the weak oxidant methyl viologen and the reductant N,N,N',N', tetramethyl -1,4 phenylenediamine (TMPD). The rates for the reduction of MV^{2+} at 605 nm ($\epsilon = 12800 \text{ M}^{-1} \text{ cm}^{-1}$), and the oxidation of TMPD at 610 nm ($\epsilon = 12000 \text{ M}^{-1} \text{ cm}^{-1}$) were monitored. The traces depicting the build up of $\text{MV}^{\bullet+}$ and $\text{TMPD}^{\bullet+}$ when $(1-2) \times 10^{-3} \text{ mol dm}^{-3}$ of *o*-hydroxycinnamic acid and $1 \times 10^{-4} \text{ mol dm}^{-3}$ MV^{2+} or TMPD at pH 7 were pulse radiolysed are shown in figure 1(c) and (d) respectively. The rates of the formation of $\text{MV}^{\bullet+}$ and $\text{TMPD}^{\bullet+}$ were found to be 6.6×10^9 and $6.2 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ respectively and the yields corresponded to 17% and 33% $\bullet\text{OH}$.

The oxidation of TMPD to $\text{TMPD}^{\bullet+}$ can occur from its reaction with a hetroatom centred radical, the two possible radicals being **2** and **7**. However, the formation of phenoxyl radical at pH 7 is less likely as can be seen from the spectrum obtained in the $\bullet\text{OH}$ reaction with *o*-hydroxycinnamic acid which is different from that obtained by azidyl reaction at pH 7. Thus, the $\bullet\text{OH}$ radical reacts via one electron transfer [reaction (4)] giving rise to the hetroatom centered radical **6** which deprotonates to give the more stable species **7**. The reaction with methyl viologen (17% $\bullet\text{OH}$) is attributed to the formation of the radical **4**. The remaining 50% $\bullet\text{OH}$ is expected to react by reaction (2) giving rise to

isomeric OH adducts. In order to verify the extent of attack on the ring reaction was carried out with the stronger oxidant IrCl_6^{2-} , but it has not been successful due to its facile thermal reaction with *o*-hydroxycinnamic acid. At pH 3, where *o*-hydroxycinnamic acid is in its undissociated form,²⁹ ($pK_a = 4.61$), the reaction seems to proceed by two pathways. Addition of the $\cdot\text{OH}$ radical to the ring [reaction (5)] gives the OH adduct **8** which undergoes dehydration to give **9**. The formation of the radical **9** is evident from the similarity in spectrum obtained in the reaction of $\cdot\text{OH}$ and $\text{N}_3\cdot$ (pH 7) (Figures 3 and 5). In addition to this, the $\cdot\text{OH}$ radical adds to the olefinic double bond which is evident from the observed yield (17% $\cdot\text{OH}$) with the oxidant methyl viologen.

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