Reaction of oxide radical ion (O•−)
with substituted pyrimidines

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Abstract—Pulse radiolysis technique has been used to investigate the reaction of oxide radical
ion (O•−) with 4,6-dihydroxy-2-methyl pyrimidine (DHMP), 2,4-dimethyl-6-hydroxy pyrimidine
(DMHP), 5,6-dimethyl uracil (DMU) and 6-methyl uracil (MU) in strongly alkaline medium.
The second-order rate constants for the reaction of O•− with these compounds are in the range
$2–5 \times 10^8$ dm^3 mol^{-1} s^{-1}. The transient absorption spectra obtained with DHMP have two maxima
at 290 and 370 nm and with DMHP have maxima at 310 and 470 nm. The transient spectrum from
DMU is characterized by its absorption maxima at 310 and 520 nm and that of MU by its single
maximum at 425 nm. The intermediate species were found to react with N,N,N′,N′-tetramethyl-*p*-
phenylenediamine (TMPD) with high G(TMPD•+) values ranged between 3.9×10^{-7} mol J^{-1} and
4.8×10^{-7} mol J^{-1}. These radicals undergo decay by second-order kinetics ($2k/\varepsilon = 1.0–1.7 \times
10^6$ s^{-1}). The reaction of O•− with the selected pyrimidines is proposed to proceed through a hydrogen
abstraction from the methyl group forming allyl type radicals. These are mainly oxidizing radicals
and hence readily undergo electron transfer reactions with TMPD.

INTRODUCTION

Reports on the reaction of oxide radical ion (O•−) with DNA model systems such
as purine and pyrimidine bases and their nucleosides are relatively fewer in number
compared to hydroxyl radicals as such reactions take place only in highly alkaline
medium and therefore these are believed to be biologically less important. However,
the radical chemistry initiated by O•− is important from the chemical point of view

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as this will be quite different from that initiated by $^\bullet$OH. $^\bullet$OH is rapidly converted to its conjugate base, $O^{\bullet-}$, at high pH, $(pK_a(^\bullet OH) = 11.9)$ [1]. The reactivities of these two species are different. For example, $O^{\bullet-}$ has a low tendency to add to double bonds or to aromatic rings of organic molecules, but its hydrogen abstracting ability does not significantly differ from that of $^\bullet$OH [2–4]. $O^{\bullet-}$ can act as an oxidant, $(E^0(O^{\bullet-}, H^+/OH^-) = 1.77$ V [5].

The reactions of $O^{\bullet-}$ with pyrimidines and purines have been reported [4, 6–12]. However, the rate of its reaction is less (by almost 10 times) than the rate of reaction of $^\bullet$OH with purines and pyrimidines owing to a different reaction mechanism [6, 7]. An enhanced yield of base release from uracil nucleosides at higher pH has also been reported which was explained in terms of the participation of $O^{\bullet-}$ in their preferential attack at the sugar moieties [4, 7]. One-electron oxidation of both purines and pyrimidines and H-abstraction, if a methyl group is present, are reported as the major reaction pathways [4, 6, 7].
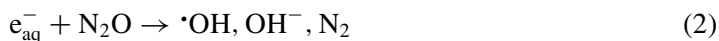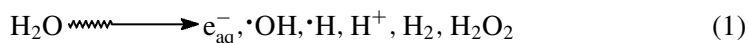
It is reported in pulse radiolysis and ESR studies that $O^{\bullet-}$ reacts with uracil and cytosine by adding to the pyrimidine ring and with thymine and 5-methyl cytosine by abstracting a hydrogen from the methyl group to form an allyl type radical [8–12]. However, later it was reported that the major reaction of $O^{\bullet-}$ with uracil at pH > 13 is an electron transfer leading to an oxy radical [6]. The OH-adduct of uracil in basic solutions undergoes a first order decay to produce a new radical whose spectrum is similar to uracil radical anion obtained by one-electron oxidation of uracil with the powerful oxidant, $SO_4^{\bullet-}$. From pulse radiolysis and EPR studies it was suggested that a base-catalysed dehydration reaction takes place from the $^\bullet$OH adduct of uracil [13, 14]. Similar results were obtained for cytosine by pulse radiolysis [15]. It is possible, therefore, that the OH^- induced conversion of the radicals produced by $^\bullet$OH attack of pyrimidines could be similar to that obtained by $O^{\bullet-}$ attack. On the other hand, the reaction could be very different if a methyl group is present. In the present paper, we have undertaken a study of $O^{\bullet-}$ with dihydroxy and dimethyl substituted pyrimidines in order to understand more about the substituent effect on the electron transfer or H abstraction reactions. The selected compounds include 4,6-dihydroxy-2-methyl pyrimidine (DHMP), 2,4-dimethyl-6-hydroxy pyrimidine (DMHP), 5,6-dimethyl uracil (DMU) and 6-methyl uracil (MU). The reductant, N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) was used to demonstrate the oxidizing property of the intermediate radicals.
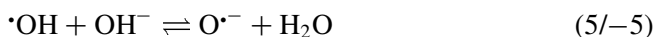
EXPERIMENTAL

DHMP, DMHP, DMU, MU and TMPD (Aldrich) were commercially available and have been used without further purification. Solutions were prepared in Millipore-Milli-Q filtered water. The concentration of the substrates was maintained at 1×10^{-3} mol dm^{-3}. Pulse radiolysis experiments were carried out using a linear accelerator delivering electron pulses of 7 MeV energy of 50 ns duration. An aerated

aqueous solution of KSCN (1×10^{-3} mol dm^{-3}) was used to monitor the dose per pulse [16–18] with $G \times \varepsilon_{500} = 2.15 \times 10^{-4}$ m^2 J^{-1}, which was kept at 14 Gy. Low dose experiments (5 Gy/pulse) were performed to study the electron transfer reaction between the intermediate radicals and TMPD, and the resulting TMPD$^{\bullet+}$ build-up was monitored at 565 nm [13]. The transient species formed on pulse radiolysis was monitored by using a 450 W pulsed xenon arc lamp, monochromator (Kratus GM-252) and a Hamamatsu R-955 photomultiplier as the detector. The photomultiplier output was digitised with a 100 MHz storage oscilloscope interfaced to a computer for kinetic analysis. The rate values are the averages of at least three experiments and the variation was within 15%. The rate constant values were taken from those kinetic analyses for which very good correlation was observed between the experimental and calculated results. The details of the pulse radiolysis set-up have been described elsewhere [16–18].

Radiolysis of water produces three highly reactive species (e_{aq}^-, $\bullet$OH and $\bullet$H) in addition to the formation of inert or less reactive molecular products (H$_2$ and H$_2$O$_2$). By saturating with N$_2$O, e_{aq}^- is converted into $\bullet$OH.

$$H_2O \longrightarrow e_{aq}^-, \bullet OH, \bullet H, H^+, H_2, H_2O_2 \tag{1}$$

$$e_{aq}^- + N_2O \rightarrow \bullet OH, OH^-, N_2 \tag{2}$$

In basic solutions, $\bullet$H is converted into e_{aq}^- with a rate constant of 2.2×10^7 dm^3 mol^{-1} s^{-1} (equation (3)) whereas in the presence of N$_2$O, solvated electrons transform into O$^{\bullet-}$ (equation (4), $k_4 = 9.1 \times 10^9$ dm^3 mol^{-1} s^{-1}) since $\bullet$OH is in equilibrium with its basic form, the O$^{\bullet-}$ (reaction 5, p$K_a = 11.9$). The rate constants of the forward and reverse reactions (equations (5) and (-5), respectively) are 1.2×10^{10} dm^3 mol^{-1} s^{-1} and 1×10^8 dm^3 mol^{-1} s^{-1}, respectively [19].

$$\bullet H + OH^- \rightarrow e_{aq}^- \tag{3}$$

$$e_{aq}^- + N_2O \rightarrow O^{\bullet-} + N_2 \tag{4}$$

$$\bullet OH + OH^- \rightleftharpoons O^{\bullet-} + H_2O \tag{5/-5}$$

In the present study, the transformation of $\bullet$OH into O$^{\bullet-}$ was accomplished at pH 13 and the aqueous solutions of these substrates were saturated with N$_2$O. Under these conditions, we assumed that $G(O^{\bullet-}) = G(\bullet OH) + G(\bullet H) + G(e_{aq}^-) = 6.1 \times 10^{-7}$ mol J^{-1}. In alkaline solutions the pyrimidine bases are also partially ionized [20]. The pK_a values of DMHP and DMU were not known. Therefore the pK_a values in basic pHs were experimentally determined by UV-VIS spectrophotometry as, pK_a (DMHP) $= 9.9$ and pK_a (DMU) $= 9.8$.

RESULTS AND DISCUSSION

Kinetics

The second-order rate constants for the reaction of $O^{\bullet-}$ with DHMP, DMHP, DMU and MU have been determined by monitoring the pseudo-first order build-up (k_{obs}) of the intermediates (at their absorption maxima) as a function of substrate concentrations. A typical k_{obs} *versus* [Py] plot obtained in the case of DMHP is shown in Fig. 1. The rate constants are in the range $2-5 \times 10^8$ dm^3 mol^{-1} s^{-1} (Table 1). These rate constants are almost by an order of magnitude lower than the rate constants obtained for $^{\bullet}$OH with pyrimidine (10^9 dm^3 mol^{-1} s^{-1}) [1] and are in agreement with
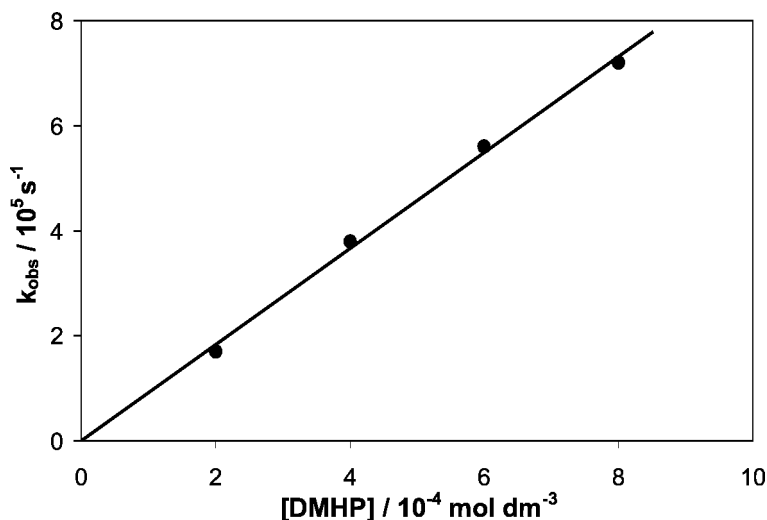

Figure 1. Plot of the rate of build-up (k_{obs}) *versus* concentration observed at 300 nm after delivering electron pulses to N$_2$O saturated solution of 2,4-dimethyl-6-hydroxypyrimidine (DMHP) at pH 13.

Table 1.
The bimolecular rate constants (k_2), absorption maxima (λ_{max}), and decay constants ($2k/\varepsilon$)[#] obtained for the reaction of $O^{\bullet-}$ with the selected pyrimidines

Substrate	$k_2/10^8$ (dm^3 mol^{-1} s^{-1})	λ_{max} (nm)	$2k/\varepsilon/$ 10^6 (s^{-1})
DHMP	2.4	290 370	1.7
DMHP	5.3	310 470	1.0
DMU	3.3	310 520	1.1
MU	3.6	425	1.6

[#] The decay constant is $2k/\varepsilon l$ where $l = 1$ cm and hence is written as $2k/\varepsilon$ with the unit s^{-1}.

the rate constants reported for $O^{\cdot-}$ with other pyrimidines [6]. Such reductions in rate constants are generally understood as due to two reasons [4, 7]. One is the change in the mode of reaction and the second is the electrostatic repulsion between the nucleophilic $O^{\cdot-}$ and the deprotonated pyrimidines (at pH $\geqslant$ 13).

Transient spectra

The transient absorption spectrum obtained with DHMP is characterized by its maxima at 290 and 370 nm (Fig. 2). The transient spectra from DMHP (6 μs after the pulse) has similar features to those of DHMP with two maxima at 310 and 470 nm (Fig. 2). In the case of DMU, a spectrum with absorption maxima at 310 nm and at 520 nm is obtained (Fig. 3). The spectrum recorded 10 μs after the pulse in the case of MU has a single absorption maximum at 425 nm with shoulders at around 310 nm and 570 nm (Fig. 3). All these spectra obtained from the selected pyrimidines undergo second-order decay without showing any noticeable changes up to about 500 μs. The bimolecular rate constants (k_2), absorption maxima (λ_{max}),
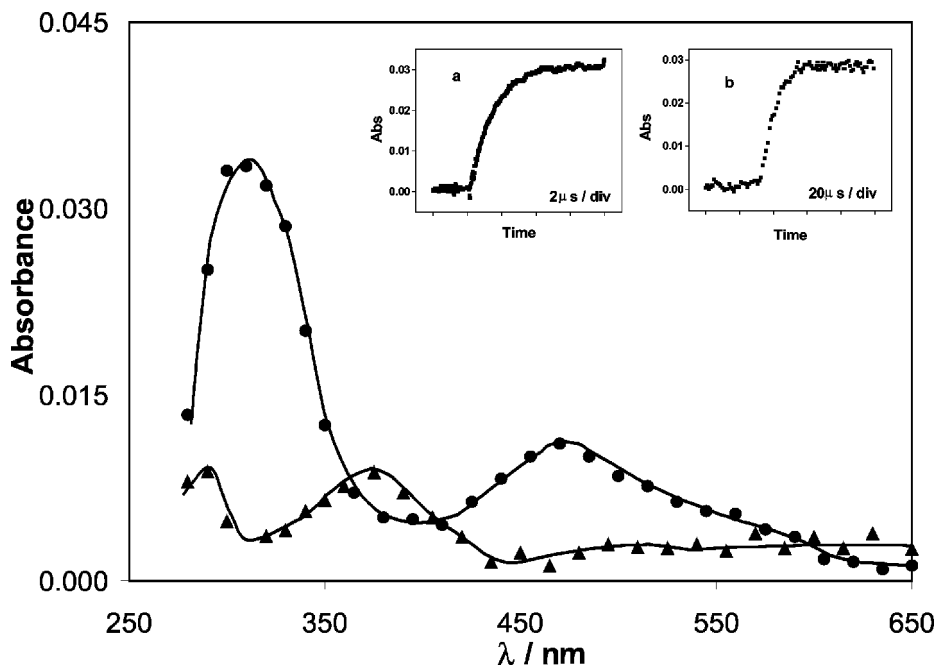

Figure 2. Transient absorption spectra obtained at 10 μs after delivering electron pulse to N_2O saturated solutions of 1×10^{-3} mol dm^{-3} 4,6-dihydroxy-2-methyl pyrimidine (DHMP) (▲) and to N_2O saturated solutions of 1×10^{-3} mol dm^{-3} 2,4-dimethyl-6-hydroxypyrimidine (DMHP) (6 μs after the pulse) (●) at pH 13. Inset: (a) the transient build-up at 300 nm from DMHP (b) the TMPD$^{\cdot+}$ build-up at 565 nm obtained with DMHP (2×10^{-3} mol dm^{-3}) in the presence of TMPD (5×10^{-5} mol dm^{-3}).
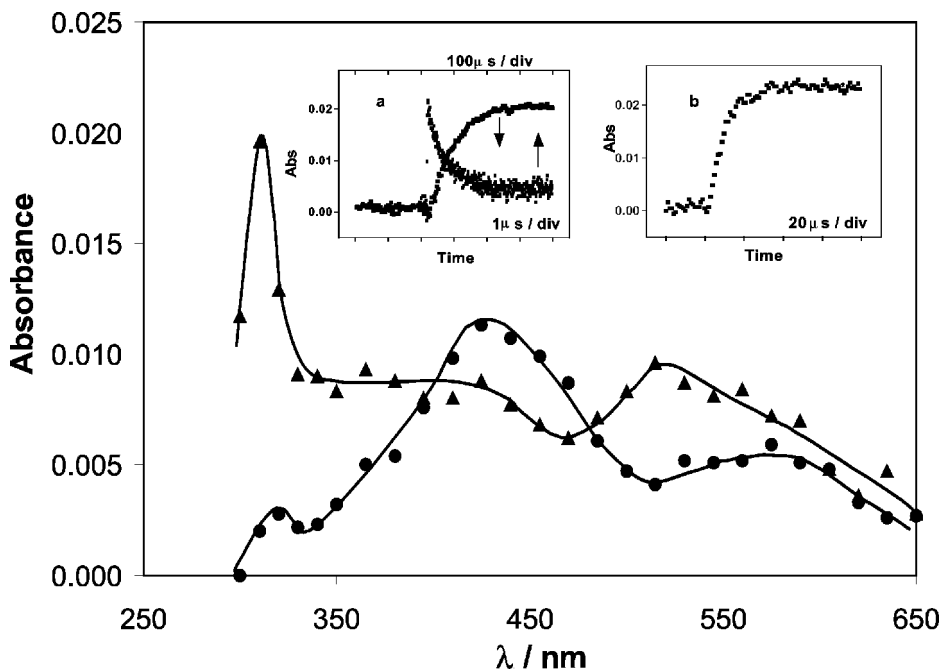
Figure 3. Transient absorption spectra obtained at 10 μs after delivering electron pulse to N_2O saturated solutions of 1×10^{-3} mol dm^{-3} 5,6-dimethyl uracil (DMU) (▲) and to N_2O saturated solutions of 1×10^{-3} mol dm^{-3} 6-methyl uracil (MU) (●) at pH 13. Inset: (a) the transient build-up and its decay at 310 nm from DMU and (b) the TMPD$^{\bullet+}$ build-up at 565 nm obtained with DMU (2×10^{-3} mol dm^{-3}) in the presence of TMPD (5×10^{-5} mol dm^{-3}).

and the decay constants ($2k/\varepsilon$) obtained for the reaction of O$^{\bullet-}$ with the selected pyrimidines are summarized in Table 1.

Reduction of the intermediates by TMPD

Reaction of TMPD, a known reductant, with the intermediates has been investigated at low doses in a solution containing the pyrimidines 2×10^{-3} mol dm^{-3} and TMPD (5×10^{-5} mol dm^{-3}). A well-defined build-up of absorption at 565 nm is observed with all the pyrimidines. A typical trace obtained in the case of DMHP is shown in Fig. 2. The G(TMPD$^{\bullet+}$) values (calculated from an ε(TMPD$^{\bullet+}$)$_{565\,nm}$ value of $12\,500$ dm^3 mol^{-1} cm^{-1} [21]), and the rate of build-up are summarized in Table 2. The percentage of oxidizing radicals has been calculated from the G(TMPD$^{\bullet+}$) by assuming a maximum G($^{\bullet}$OH/O$^{\bullet-}$) value of 6.1×10^{-7} mol J^{-1}. The pseudo first-order TMPD$^{\bullet+}$ build-up rate constants, k_{obs}, at a typical TMPD concentration of 5×10^{-5} mol dm^{-3} are 5.0–7.5×10^4 s^{-1}. Therefore, approximate second-order rate constants can be calculated for the selected compounds as 1.0–1.5×10^9 dm^3 mol^{-1} s^{-1}. These rate constants are well comparable with the rate constants obtained for the reaction of TMPD with the oxidizing radicals derived from other pyrimidines [13].

Table 2.
The $G(\text{TMPD}^{•+})$ values, the percentage of oxidizing radicals and the k_{obs} obtained for the reaction of the intermediates with TMPD

Compound	$G(\text{TMPD}^{•+})$ /10^{-7} (mol J^{-1})	Percentage of oxidizing radical[a]	$k_{obs}/10^4$ (s^{-1})[b]
DHMP	4.5	73	5.0
DMHP	4.8	78	7.5
DMU	3.9	64	7.2
MU	4.1	68	6.3

[a] This percentage is calculated based on a maximum $G(\text{intermediate})$ value of 6.1×10^{-7} mol J^{-1} which corresponds to $G(^•\text{OH}/\text{O}^{•-})$ at pH 13.

[b] k_{obs} is the pseudo first-order rate constant of TMPD$^{•+}$ for a typical [TMPD] $= 5 \times 10^{-5}$ mol dm^{-3} and therefore the second-order rate constants are approximately in the range, $1.0–1.5 \times 10^9$ dm^3 mol^{-1} s^{-1}.

The fact that $^•$OH reacts differently than its conjugate base, O$^{•-}$, is commonly accepted. For example, $^•$OH undergoes mainly addition at the C(5) and C(6) position of the pyrimidine ring [8, 9, 13, 22] but O$^{•-}$ has a tendency to either undergo an electron transfer reaction with uracil and cytosine or abstract a hydrogen if there is a methyl substituent at the pyrimidine (e.g. thymine) [6]. In the present case, the experiments were conducted at pH 13 and hence the main reagent is O$^{•-}$.

As DHMP has pK_a values at 6.35 and 12.6 [20], it exists as its dianionic form at pH 13. The likely reaction of O$^{•-}$ is, therefore, proposed as a H-abstraction from the methyl group leading to an allyl type radical (reaction (6)).


$$(6)$$
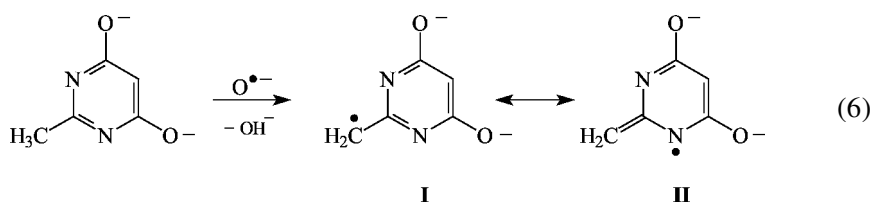
The high $G(\text{TMPD}^{•+})$ yield of 4.5×10^{-7} mol J^{-1} is in line with this interpretation as the mesomeric form of the radical dianion **I** is a nitrogen centered radical, **II**, and hence could act as an oxidizing radical with respect to TMPD. The rate of the reaction ($k_{(\text{R}^•+\text{TMPD})} \approx 1.0 \times 10^9$ dm^3 mol^{-1} s^{-1}) is of the same order as for the pyrimidine oxidizing radical reaction with TMPD [13, 15, 22]. Therefore, the spectrum obtained at 10 μs after the pulse, characterized by its maxima at 290 and 370 nm, is attributed to the radical **I**. Both EPR [11, 12, 23] and pulse radiolysis studies [6] on the reaction of O$^{•-}$ with thymine dianion where it undergoes a H-abstraction reaction, supported this assignment. The EPR study on the reaction of O$^{•-}$ with 2- and 5-methyl-4,6-dihydroxy pyrimidine clearly established the formation

of allyl type radicals [23]. The second-order kinetics of the decay of this radical is in agreement with the decay kinetics of the radical resulting from the reaction of $O^{\bullet-}$ with thymine and 1-methyl uracil where the decay constant $(2k)$ is of the order of $5-9 \times 10^8$ dm^3 mol^{-1} s^{-1} [6]. Assuming an ε value of the radical **I** is of the order of 10^3 dm^3 mol^{-1} cm^{-1} (the actual ε value could not be calculated since the exact G-value of other possible oxidizing radicals that contribute to the absorption spectra, likely to result from $^{\bullet}OH$, is not known), a $2k$ value of 1.7×10^9 dm^3 mol^{-1} s^{-1} can be obtained for DHMP from Table 1. This value is well comparable with the magnitude of any second-order decay of the radicals resulting from the reaction of $^{\bullet}OH$ or $O^{\bullet-}$ with pyrimidines [6, 9]. The second-order decay of this radical could ultimately lead to some stable products.

Since H-abstraction is the predominant reaction of $O^{\bullet-}$ with methyl substituted pyrimidines [6], the intermediate spectra obtained with DMHP, DMU and MU are also explained in terms of H-abstraction reaction from the methyl group. DMHP has a pK_a value of 9.9 and, therefore, the major reaction of $O^{\bullet-}$ is with the mono anion of DMHP. As it contains two methyl groups, the H-abstraction from both the methyl groups are equally likely (reaction (7)).

Therefore, the transient spectrum characterized by its maxima at 310 and 470 nm must be due to the combined effect of **III** and **V** or due to the one which is formed high in yield. The $G(TMPD^{\bullet+})$ of 4.8×10^{-7} $mol\,J^{-1}$ indicates only the oxidizing nature of the intermediates. The distribution of the radicals **III** and **V** cannot be obtained with TMPD as the mesomeric structures of both **III** and **V** (**IV** and **VI**, respectively) could be oxidizing in nature. The oxidizing properties of the carbon centered (C(5)-yl) radical and its mesomeric form, the oxygen-centered radical, in the case of the OH adduct of uracil derivatives are already reported [13, 15, 21].


$$(7)$$
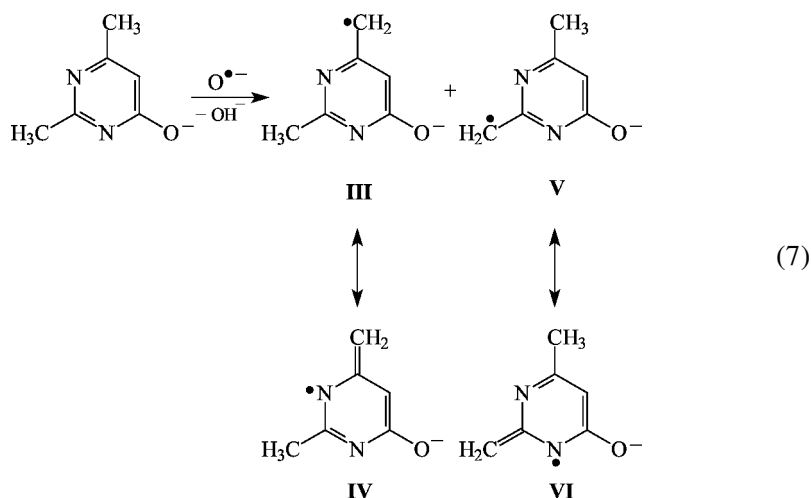
Both DMU and MU exist in their mono anionic form ($pK_{a\ (DMU)} = 9.8$ and $pK_{a\ (MU)} = 9.5$) at pH 13. Similar to DMHP, a H-abstraction from both the

−CH₃ groups is possible with DMU. However, radicals **VII/IX** and **VIII/X** must show different reactivity

$$(8)$$
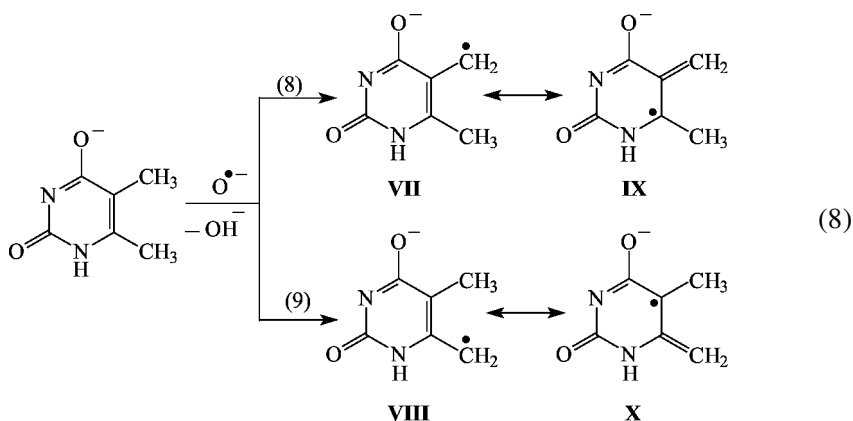
towards TMPD (reactions (8) and (9)). The radical **VII** could be a reducing radical due to its mesomeric structure, **IX**, having the spin density at the α-amino carbon (C(6)). Therefore, the intermediate spectrum with absorption maxima at 310 and 520 nm is assigned to the radical **VIII** whose oxidizing nature is demonstrated by the build-up of TMPD·⁺ absorption with a $G(\text{TMPD}^{\cdot+})$ of 3.9×10^{-7} mol J⁻¹. The comparatively lower $G(\text{TMPD}^{\cdot+})$ value obtained in this case is likely due to the simultaneous formation of radical **VII**.

$$(9)$$
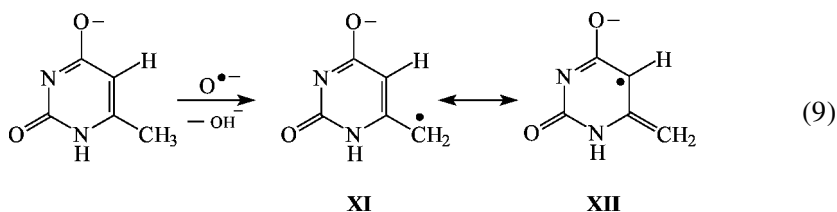
A similar assignment of the spectrum obtained with MU is made based on the formation of the radical with structure **XI** which is an oxidizing radical $(G(\text{TMPD}^{\cdot+}) = 4.1 \times 10^{-7}$ mol J⁻¹). The spectrum is, therefore, assigned to the radical **XI**.

CONCLUSION

The second-order rate constants for the reaction of O·⁻ with DHMP, DMHP, DMU and MU are in the range $2-5 \times 10^8$ dm³ mol⁻¹ s⁻¹, by an order in magnitude lower compared to that of ·OH. The transient absorption spectra obtained with all these compounds underwent a second-order decay. H-abstraction from the

methyl substituent is proposed as the major reaction that $O^{\bullet-}$ undergoes with these compounds. The resulting radicals have oxidizing properties with respect to TMPD as seen from the high yield of $TMPD^{\bullet+}$. It must be further noted that the radicals resulting from the dehydration of OH-adducts at higher pH cannot be similar to those obtained from the reaction of $O^{\bullet-}$ with pyrimidines having methyl substituent, since H-abstraction leading to the formation of allyl type radicals, is the major reaction in the latter case.

Acknowledgement

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