Reactions of hydroxyl radical (\textsuperscript{•}OH) and hydroxymethyl radical (\textsuperscript{•}CH\textsubscript{2}OH) with amino, methyl, hydroxy and methoxy substituted pyrimidines in aqueous medium: A pulse and steady state radiolysis study

Abstract: \textsuperscript{•}OH reacts with the selected pyrimidines by diffusion controlled rate (2.0-7.2 \times 10^9 \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}) at neutral pH. The difference in the spectral features of the intermediates obtained at near neutral pH and at pH 10.4 with these pyrimidines are attributed to the deprotonation of the OH-adducts. 6-16\% oxidising radicals have been determined using TMPD at pH 6.0 and at pH 10.4. However, only in the case of 2-amino-4-hydroxy-6-methylpyrimidine (AHMP) 95\% oxidising radicals have been obtained at pH 10.4. A base catalysed conversion of the initially formed non-oxidising radical to an oxidising radical is proposed for AHMP at pH 10.4. In the steady state reactions of \textsuperscript{•}CH\textsubscript{2}OH, the yield of HCHO formed has been determined and hence quantitatively estimated the extent of electron transfer reaction. It is proposed that 12-19\% of \textsuperscript{•}CH\textsubscript{2}OH reaction with the selected pyrimidines is via electron transfer mechanism at neutral pH. While investigating the reaction of (CH\textsubscript{3})\textsuperscript{2}COH with uracil derivatives using pulse radiolysis, an anomalous high reactivity was observed. Such reactions were later identified as the reactions of OH\textsuperscript{−} with uracil derivatives having hydrogen at N(1) position.

Publications from this chapter

3.1. Reactions of hydroxyl radicals (\textsuperscript{•}OH) with amino substituted pyrimidines

Investigations of the effect of primary (\textsuperscript{•}OH, e\textsubscript{aq} and \textsuperscript{'}H) and secondary radicals (CO\textsubscript{2}\textsuperscript{−}, 2-hydroxypropan-2-yl radical, SO\textsubscript{4}\textsuperscript{−} etc.) on DNA have been a topic of utmost importance due to its biological relevance.\textsuperscript{1,14,66,121} Majority of these studies were carried out using different DNA sub-units such as nucleobases, nucleosides and nucleotides.\textsuperscript{76,211-214} \textsuperscript{•}OH and e\textsubscript{aq} react with nucleobases and its derivatives with high rate (\(k \geq 10^9\text{ dm}^3\text{mol}^{-1}\text{s}^{-1}\))\textsuperscript{7,46} while alcohol derived radicals react slowly (\(k \leq 10^6\text{ dm}^3\text{mol}^{-1}\text{s}^{-1}\))\textsuperscript{215} at neutral pH. Among the primary free radicals, \textsuperscript{•}OH is the major DNA damaging agent to DNA.\textsuperscript{7,216,217} Its reaction causes base damage,\textsuperscript{218,219} strand break (single and double strand break)\textsuperscript{213,217,220,221} and cross-linking\textsuperscript{222-224} in DNA.

In the case of pyrimidines, \textsuperscript{•}OH react with C(5)-C(6) double bond to form C(5)-yl and C(6)-yl radical adducts while in the case of cytosine, a small percent of \textsuperscript{•}OH can add to N(3)-C(4) double bond in addition to the normal C(5)-C(6) addition.\textsuperscript{75,225,226} N,N,N',N'-tetramethylphenylenediamine (TMPD), tetranitromethane (TNM), methyl viologen (MV\textsuperscript{•+}), etc are used to determine the redox nature of the resulting radicals formed by the reaction of \textsuperscript{•}OH with pyrimidines.\textsuperscript{198,227} The ratio of the reducing to oxidising radical depend on the substituents present in the C(5)-C(6) position, whereas such effects are not so predominant with substituents at N(1) position. The percentage of reducing radicals reported in the case of uracil, thymine, cytosine
and 1-methyluracil are 80, 60, 87 and 65 % respectively, at neutral pH.\textsuperscript{76,228} A base catalysed dehydration reaction of the reducing radical to oxidising radical is reported for uracil and 4,6-dihydropyrimidine derivatives.\textsuperscript{75,198} In the present pulse radiolysis study, the effect of various substituents on the yield of oxidising radicals at neutral and high pH were investigated with amino, methyl, hydroxy and methoxy substituted pyrimidines. The selected pyrimidines are 2-amino-4-methyl pyrimidine (AMP), 2-amino-4,6-dimethyl pyrimidine (ADMP), 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP) and 2-amino-4-methoxy-6-methyl pyrimidine (AMMP) and their structures are given in Table 2.1.

3.1.1. Rate constant measurements

All of the selected aminopyrimidines react with \textsuperscript{1}OH by diffusion controlled rate. The bimolecular rate constants of these reactions at near neutral pH are in the range of $2.0-7.2 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ (Table 3.1), which are in agreement with other pyrimidines reported earlier.\textsuperscript{46} The bimolecular rate constant values were obtained from the plot of $k_{obs}$ \textit{versus} concentration of the substrate, where $k_{obs}$ is the pseudo first order rate constant of the reaction of \textsuperscript{1}OH with the substrate. The $k_{obs}$ values were obtained from the formation traces of the radical intermediate at their $\lambda_{max}$ at various concentration of substrates. A typical transient formation trace of AHMP at 320 nm is shown in Figure 3.1. All of the $k_{obs}$ \textit{versus} [substrate] plots were linear with good correlation coefficient (regression co-efficient $\geq 0.99$). A typical $k_{obs}$ \textit{versus} [substrate] plot obtained for AHMP at 320 nm is given in Figure 3.1.
Table 3.1. Second-order rate constants* and the absorption maxima of the intermediates obtained for the reaction of \(^*\text{OH}\) with the selected pyrimidines

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH 6</th>
<th>pH 10.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(k / 10^9) (dm(^3)mol(^{-1})s(^{-1}))</td>
<td>(\lambda_{\text{max}}) (nm)</td>
</tr>
<tr>
<td>2-Amino-4-methyl pyrimidine (AMP)</td>
<td>2.0</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td></td>
<td>550</td>
</tr>
<tr>
<td>2-Amino-4,6-dimethyl pyrimidine (ADMP)</td>
<td>7.2</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td></td>
<td>550</td>
</tr>
<tr>
<td>2-Amino-4-hydroxy-6-methyl pyrimidine (AHMP)</td>
<td>5.8</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td></td>
<td>455</td>
</tr>
<tr>
<td>2-Amino-4-methoxy-6-methyl pyrimidine (AMMP)</td>
<td>6.5</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>515</td>
</tr>
</tbody>
</table>

*These were determined from the average value of slopes of \(k_{\text{obs}}\) vs. concentration plots for intermediates having two maxima (these two values were nearly the same) and the deviation in the calculation of these values were between 5-10%.

3.1.2. Properties of the transient spectra

The transient absorption spectra obtained for all of the selected pyrimidines have two absorption maxima at near neutral pH, one around 310 nm and the second in the range 455-550 nm. In the case of AHMP, the transient spectrum obtained at 2 \(\mu\)s after the pulse gave maxima at 320 nm and 455 nm at pH 6 (Figure 3.2). The spectrum obtained at 40 \(\mu\)s after the pulse showed decay at both \(\lambda_{\text{maxes}}\). At high pH, 10.4, the spectrum gave an additional maximum at 750 nm and the lower \(\lambda_{\text{max}}\) is blue shifted to 300 nm (Figure 3.2) with a shoulder around 330 nm.

The initial spectrum undergoes a fast decay, (first order type, \(k \approx 10^6 \text{ s}^{-1}\)) with a
slow absorption builds-up around 300 nm region. This is found to continue over 300 μs and then undergoes a bimolecular decay. A typical trace obtained at 300 nm is also shown in Figure 3.1. An absorbance versus pH plot obtained at 2 μs after the pulse at 700 nm, shown in Figure 3.1, gave an inflection point at pH 9.0.

**Figure 3.1.** (a) A typical absorption build-up of the intermediate at 320 nm in N₂O saturated solution of 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP) at pH 6, [AHMP] = 0.2 × 10⁻³ mol⁻¹dm⁻³; (b) \( k_{\text{obs}} \) vs concentration plot at 320 nm obtained in N₂O saturated solution of AHMP at pH 6; (c) Absorbance vs pH of N₂O saturated solution of AHMP at 2 μs after the pulse at 700 nm and (d) decay trace of the intermediate at 300 nm obtained in N₂O saturated solution of AHMP at pH 10.5. Dose per pulse = 15 Gy.
Figure 3.2. Transient absorption spectra obtained from N₂O saturated solutions of 2-amino-4-hydroxy-6-methyl pyrimidine (AHMP) \(1 \times 10^{-3} \text{ mol dm}^{-3}\) at 2 µs after the pulse at pH 6 (Δ), at 2 (▲) and 40 µs (×) after the pulse at pH 10.5. Dose per pulse = 15 Gy.

Figure 3.3. Transient absorption spectra obtained from N₂O saturated solution of 2-amino-4-methylpyrimidine (AMP) \(1 \times 10^{-3} \text{ mol dm}^{-3}\). (a) At 2 µs (▲) and 40 µs (Δ) after the pulse at pH 6; Inset: intermediate trace obtained at pH 6. (b) At pH 6 (▲) and at pH 10.4 (Δ) obtained at 2 µs after the pulse; Inset: intermediate trace obtained at pH 10.4. Dose per pulse = 15 Gy.

The time resolved transient absorption spectra obtained for AMP at 2 µs and 40 µs after the pulse showed two absorption maxima at 315 and 550 nm at pH 6 (Figure 3.3a). The transient decay traces obtained at 315 and 550 nm showed
second order decay (Figure 3.3a, inset). At pH 10.4, the spectrum remains the same except a small shift in lower $\lambda_{\text{max}}$ to 320 nm (Figure 3.3b). At both maxima, the spectrum undergoes a second order decay. The decay traces of the intermediates at $\lambda_{\text{max}}$ are given in the inset of Figure 3.3b.

![Figure 3.4](image)

**Figure 3.4.** Transient absorption spectra obtained from N$_2$O saturated solution of 2-amino-4, 6-dimethylpyrimidine (ADMP) ($1 \times 10^{-3}$ mol dm$^{-3}$). (a) At 2 $\mu$s (▲) and 40 $\mu$s (O) after the pulse at pH 10.4; Inset: intermediate trace obtained at pH 10.4. (b) At pH 6 (▲) and at pH 10.4 (Δ) obtained at 2 $\mu$s after the pulse; Inset: intermediate trace obtained at pH 6. Dose per pulse = 15 Gy.

The transient absorption spectra obtained for ADMP at pH 6 have absorption maxima at 310 and 550 nm (Figure 3.4b). This is found to undergo a second order decay at 310 and 550 nm. The spectral properties remained the same at pH 10.4, except a shift in the lower $\lambda_{\text{max}}$ to 320 nm and the higher $\lambda_{\text{max}}$ to 565 nm. A slow increase in absorbance was observed at 320 nm at pH 10.4 about 100 $\mu$s after the pulse (Figure 3.4a) while it decayed by second order kinetics at 565 nm.
Figure 3.5. Transient absorption spectra of N\textsubscript{2}O saturated solution of 2-amino-4-methoxy-6-methylpyrimidine (AMMP) (1 x 10\textsuperscript{-3} mol dm\textsuperscript{-3}). (a) At 2 \(\mu\)s (▲) and 40 \(\mu\)s (O) after the pulse at pH 10.4; Inset: intermediate traces obtained at pH 10.4. (b) At pH 6 (▲) and at pH 10.4 (△) obtained at 2 \(\mu\)s after the pulse; Dose per pulse = 15 Gy.

In the case of AMMP, the spectrum has maxima at 300 and 515 nm at pH 6 (Figure 3.5b). At higher pH (10.4) the spectrum remains almost the same. This spectrum was found to decay by second order kinetics at neutral pH. The time resolved spectrum of AMMP at higher pH (~10.4) is shown in Figure 3.5a. The spectrum was found to decay by second order kinetics at both maxima.

3.1.3. Reduction reactions of the intermediates using TMPD

The electron transfer reaction between the radical intermediates and the reductant, TMPD, was carried out at relatively low dose rates (~5 Gy/pulse). Because of the high reducing power of TMPD, it readily gets oxidised by the oxidising radical and form TMPD\textsuperscript{+}, which can be easily monitored at 565 nm.\textsuperscript{75,196,228} In all of these electron transfer reactions, the concentrations were adjusted to minimize the direct reaction between \(\cdot\)OH and TMPD ([Pyrimidine] = 2 x 10\textsuperscript{-3}mol dm\textsuperscript{-3}, [TMPD] = 5 x 10\textsuperscript{-5}mol dm\textsuperscript{-3}). The
yield of oxidising radicals calculated based on the yield of TMPD$^+$ at pH 6 and 10.4 are tabulated in Table 3.2. The percentage of oxidising radicals were thus calculated for all the pyrimidines based on an average \( G(\text{TMPD}) \) as \( 5.7 \times 10^{-7} \text{ mol J}^{-1} \) and are also summarized in Table 3.2. The \( G(\text{OH}) \) value, \( 5.7 \times 10^{-7} \text{ mol J}^{-1} \), is derived from equation (3.1) according Schuler et al.\(^{229}\) where a solute concentration dependent increase of \( G(\text{OH}) \) is reported.

\[
G(S^+) = 5.2 + 3.0 \frac{(k_r[S]/4.7 \times 10^8 \text{ s}^{-1})^{1/2}}{1 + (k_r[S]/4.7 \times 10^8 \text{ s}^{-1})^{1/2}}
\]

(3.1)

where \( G(S^+) \) is the yield of solute radical and \( k_r[S] \) is the reactivity of \( \text{OH} \) with the solute. According to this report, \( G(\text{OH}) \) value can vary depending on the solute concentration and the second order rate constant of the reaction of \( \text{OH} \) with the solute.

The \( G(\text{TMPD}) \) values are almost the same at pH 6 and pH 10.4 for AMP, ADMP and AMMP (0.7-0.9 \( \times \) 10$^{-7}$ mol J$^{-1}$, Table 3.2). However, in the case of AHMP, a much higher yield of \( \text{TMPD}^+ \) at pH 10.4 (5.5 \( \times \) 10$^{-7}$ mol J$^{-1}$) compared to pH 6 (0.4 \( \times \) 10$^{-7}$ mol J$^{-1}$) is obtained at higher time scales. A well-defined build-up of \( \text{TMPD}^+ \) was obtained at pH 10.5 against the feeble absorbance at pH 6 in the case of AHMP and the build-up traces are shown in Figure 3.6a. In the case of other pyrimidines, the \( \text{TMPD}^+ \) build-up obtained at both pHs are almost the same with low \( G(\text{TMPD}) \) values and the typical absorption traces obtained for AHMP and AMMP are shown in Figure 3.6.
Figure 3.6. The TMPD++ build-up at 565 nm obtained with (a) AHMP (2 × 10⁻³ mol dm⁻³) in presence of TMPD (5 × 10⁻⁵ mol dm⁻³) at pH 6.4 (∆) and at pH 10.4 (●), and (b) AMMP at pH 6.4 (∆) and at pH 10.4(●). Dose per pulse = 5 Gy.

Table 3.2. The G(TMPD**) values and the percentage of oxidising radicals obtained for the selected pyrimidines at pH 6 and 10.4

<table>
<thead>
<tr>
<th>Compound</th>
<th>G(TMPD**) /10⁻⁷ mol J⁻¹ at pH</th>
<th>Percentage of oxidising radicals* at pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.0</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>10.4</td>
</tr>
<tr>
<td>AMP</td>
<td>0.72</td>
<td>0.68</td>
</tr>
<tr>
<td>ADMP</td>
<td>0.91</td>
<td>0.75</td>
</tr>
<tr>
<td>AHMP</td>
<td>0.37</td>
<td>5.49</td>
</tr>
<tr>
<td>AMMP</td>
<td>0.72</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*Percentages are calculated based on a maximum yield of the intermediates, G(intermediates) ≈ 5.7 × 10⁻⁷ mol J⁻¹.

3.1.4. OH-adducts: Their protonation/deprotonation and transformation reactions

The reaction of *OH generally takes place by the addition at C(5)-C(6) double bond of the pyrimidines to form C(5)OH-C(6)yl and C(6)OH-C(5)yl adduct radicals according to earlier reports. The resulting transient spectra are
characterized by their absorption maxima around 320 and 430 nm. The C(5)OH-C(6)yl radical is reported to be reducing in nature while C(6)OH-C(5)yl radical is oxidising with respect to known redox reagents. In the case of uracil derivatives, a comparatively high yield of the reducing radicals (C(5)OH-C(6)yl radicals) were reported based on the reactions of the OH adducts with TMPD. In the present case also a similar OH addition at the C(5) and C(6) positions of the selected pyrimidines at pH 6 as well as at pH 10.4 [reaction (3.2)] is proposed in accordance with these reports. The transient spectra obtained with AHMP have absorption maxima in the same region as mentioned above, but for AMP, ADMP and AMMP, the second \( \lambda_{\text{max}} \) is in the region 520-550 nm. This slight difference in the second \( \lambda_{\text{max}} \) could be due to the substituent effect.

The yield of oxidising radical intermediate formed in the reaction of \(^{\cdot}\text{OH}\) with the selected pyrimidines can be directly obtained from the G(TMPD\(^{++}\)) values, which are given in Table 3.2. The low yield of oxidising radicals obtained at neutral pH is in agreement with the earlier reports for thymine and uracil systems. The oxidising property of the C(6)OH-C(5)yl radical can be understood as its mesomeric structure is either an oxygen centered radical (at C(4)-O) or a nitrogen centered (at N(3)). Therefore, this gives an additional support for the assignment of the spectra to the formation of C(5)OH-C(6)yl radical in the case of all of the selected pyrimidines which could act as reducing radical as was reported in the case of uracil and thymine. Furthermore, there can be a possibility of H-abstraction reaction from the methyl group
of these compounds leading to allyl type radicals as reported in the case of thymine.\textsuperscript{228} However, these radicals cannot be reducing in the case of AHMP unlike that of thymine. Therefore, if formed, these radicals could also contribute to the TMPD\textsuperscript{+} yield. But this is expected to be a minor process.

At higher pH (10.4), the transient absorption spectra are different from those at lower pH either by their higher absorption coefficient or by the shift in their $\lambda_{\text{max}}$ (including additional peak in the case of AHMP). These differences are reported to be due to the formation of deprotonated OH adducts at higher pHs in the case of uracil, thymine and cytosine.\textsuperscript{225,230} In the present case too, such an interpretation is logical. The absorbance versus pH plot in the case of AHMP (Figure 3.1c) gave a clear pK type curve with inflection point around pH 9. The ground state pK\textsubscript{a} values of AHMP are 4.1 and 9.4. Therefore, it can be concluded that the species existing at pH 10.4 is the deprotonated form of the OH adduct of AHMP. Based on these observations and on the previous reports,\textsuperscript{225,231} the spectral differences at lower and higher pHs, are attributed to the protonation-deprotonation reaction of the OH adducts. However, in the case of AMMP, there was no observable difference between the absorption spectra at pH 6 and 10.4 (Figure 3.5). This may be due to the fact that pK\textsubscript{a} value of the OH adduct is higher than 10.5 and that the spectra corresponding to both the pHs are from the same neutral OH adduct. Furthermore, the slow build-up of absorbance after 100 $\mu$s at 320 nm at pH 10.4 in the case of ADMP may be due to the formation of some stable product even at this short time scale.
AMP : R₁ = NH₂, R₂ = CH₃, R₃ = H
ADMP: R₁ = NH₂, R₂ = CH₃, R₃ = CH₃
AHMP: R₁ = NH₂, R₂ = OH, R₃ = CH₃
AMMP: R₁ = NH₂, R₂ = OCH₃, R₃ = CH₃

The percentage of oxidising radicals obtained from the G(TMPD⁺⁺) values were almost the same for AMP, ADMP and AMMP at lower and higher pH values, but for AHMP, the calculated percentage of oxidising radicals were 95% at higher pH (Table 3.2). This high difference in the G(TMPD⁺⁺) values at lower and higher pHs gives an indication of the change in the oxidising property of the intermediate radicals at higher pH. Fujita and Steenken⁷⁵ reported a two component build-up of TMPD⁺⁺ absorption with uracil at pH > 9, where the initial component depend on the concentration of TMPD and the second component depend only on the pH (and not on the concentration).⁷⁵ They reported a base catalysed dehydration reaction of the initially formed reducing radical which ultimately transforms to an oxidising radical at longer time scale. Therefore, in the present case, we have monitored the build-up of TMPD⁺⁺ at a higher time scale with the pyrimidines at pH~10.4 and the G(TMPD⁺⁺) values were determined (Table 3.2). The high yield of G(TMPD⁺⁺) obtained at higher pH is attributed to the change in the oxidising properties of the intermediate radicals. This observed change in the oxidising properties of AHMP, is proposed as the conversion of the initially formed (deprotonated) OH adduct
(C(6)yl-C(5)OH) to an oxidising C(5)yl radical. In the case of cytosine, this conversion occurs by the dehydration of C(5)OH adducts (reducing in nature) which ultimately leads to the formation of a radical site at C(5) or oxygen at C(4) position. A possible mechanism of the similar conversion of the initially formed C(6)yl-C(5)OH adduct radical of AHMP to an oxidising radical via OH⁻ elimination at basic pH is proposed in Scheme 3.1.

![Scheme 3.1](image)

An oxyradical in the case of 4,6-dihydroxy-2-methyl pyrimidine using EPR analysis at basic pHs has been reported and this assignment is in good agreement with our observation of a high G(TMPD⁺) value at pH 10.4. The fast first-order decay of the transients at higher pH (c.f. Figure 3.1d) is proposed as the dehydration of the initially formed reducing radicals. It is also important to note that such dehydration of the reducing radicals and the transformation reactions have been observed only with AHMP. The presence of OH group at C(4) position of the pyrimidine ring is the
main structural difference between AHMP and other selected pyrimidines. It is therefore obvious that the chemical environment in the case of ADMP, AMP and AMMP is not conducive for an OH elimination reaction.

Some additional spectral features obtained with AHMP at pH 10.4 further gave some indication of the absorption spectrum of the oxidising radical. The absorption trace obtained with AHMP at 300 nm, given in Figure 3.1d, showed a slow build-up of absorption after a very fast first-order decay (corresponding to the dehydration) of the transient. As this is not due to the formation of a stable product (it started a second-order decay after about 300 μs, data not shown), this is likely due to the absorption of the oxidising radical which is formed after the dehydration reaction. The spectrum recorded at 40 μs after the pulse shown in Figure 3.2 might include a significant contribution of absorbance from the oxidising radical.

3.2. Reactions of hydroxymethyl radical and OH with substituted pyrimidines

The reactions of alcohol derived radicals have relevance in the understanding of the mechanism of DNA-DNA and DNA-Protein cross linking. Formyl radical anion and 2-hydroxypropan-2-yl radical are known for inactivating DNA.

Although there are lot of reports about the low reactivity of alcohol radicals, higher rate constants were reported in the reaction of hydroxyalkyl radicals with protonated purines and the order of reactivity is being 2-hydroxy-propyl radical > hydroxyethyl radical > hydroxymethyl radical. Two important reaction pathways-
addition and electron transfer—are reported for the reaction of hydroxyalkyl radicals with purines and pyrimidines. The addition reaction generally occurs at the C(8) position of purines and at C(6) position of pyrimidines. However, the extent of such reaction pathways on a quantitative basis is still lacking except in the case of 1,3-dimethyl uracil and 1,3-dimethyl thymine. Furthermore, most of the radiation and photochemical studies reported the preferential addition of hydroxyalkyl radicals at the C(6) position of the pyrimidine rings are due to the nucleophilic nature of these radicals. On the other hand, electron transfer from these radicals to both purines and pyrimidines is also a very important reaction pathway which has relevance in DNA radical chemistry. Such electron transfer reaction, if takes place from hydroxyalkyl radicals, will results one electron reduction of purines and pyrimidines. Therefore, in the present study, the reaction of hydroxymethyl radicals with a series of pyrimidine systems has been carried out and quantitatively assessed the extent of electron transfer at near neutral pH. This investigation is expected to provide some insight into the reaction mechanism of hydroxyalkyl radicals with nucleic acid components. An attempt to analyse the products formed by the reaction of hydroxymethyl radical with uracil has also been made using HPLC. The selected compounds are uracil, thymine, 6-methyl uracil, 5,6-dimethyl uracil, 4,6-dihydroxy-2-methyl pyrimidine, 2,4-dimethyl-6-hydroxy pyrimidine, 2-amino-4,6-dimethyl pyrimidine, 2-amino-4-methoxy-6-methyl pyrimidine and 2-amino-4-hydroxy-6-methyl pyrimidine.
2-Hydroxypropan-2-yl radical ((CH$_3$)$_2$'COH) and formyl radical (CO$_2$$^\cdot$) are known to be powerful electron donors with reduction potentials $-1.5$ and $-2.0$V (versus NHE) with $pK_a$ values 12.2 and 1.4 respectively.$^{243}$ The reason for the low reactivity of these radicals with pyrimidine bases in the pulse radiolysis scale ($k \leq 10^6$ dm$^3$ mol$^{-1}$ s$^{-1}$) was suggested to be due to the high activation barrier for electron transfer, rather than the lack of thermodynamic driving power which is expected to be sufficiently high.$^{244}$ ESR investigations of the reduction of several substituted pyrimidines by reducing radicals also elucidate the poor ability of CO$_2$$^\cdot$ to reduce the pyrimidines. However, a CO$_2$$^\cdot$ adduct at the C(2) position of a pyrimidine ring has been observed with 4,6-dihydroxy-5-methyl pyrimidine.$^{245}$ A similar adduct radical at the C(6) position has also been reported with orotic acid.$^{246}$ However, an attempt is made to look at the reaction of 2-hydroxypropan-2-yl radical with the selected pyrimidines using pulse radiolysis.

The reaction of $^\cdot$OH with methanol leads to almost quantitative formation (≥ 98%) of hydroxymethyl radicals ($k_{1,32} = 9.7 \times 10^8$ dm$^3$ mol$^{-1}$ s$^{-1}$)$^{246}$ along with a small percentage of methoxyl radicals (CH$_3$O$^\cdot$). In aqueous solutions, the methoxyl radicals rapidly get rearranged to hydroxymethyl radicals [reaction (3.4)].$^{247-249}$ Hydroxymethyl radicals can also be formed from the reaction of H atom and with methanol [reaction (1.31)] via H abstraction. Because of the competition between pyrimidine ($k_{(H+py)} = 5 \times 10^8$ dm$^3$ mol$^{-1}$ s$^{-1}$) and methanol ($k_{(H+CH$_3$OH)} = 2.6 \times 10^6$ dm$^3$ mol$^{-1}$ s$^{-1}$) for H atom, a considerable part
of H atom (~30 % in our experimental condition i.e., \([\text{CH}_3\text{OH}] = 0.5 \text{ mol dm}^{-3}\), \([\text{Py}] = 1 \times 10^{-3} \text{ mol dm}^{-3}\)) react with pyrimidine.

\[
\text{CH}_3\text{OH} + \cdot \text{OH} \rightarrow \cdot\text{CH}_2\text{OH} + \text{H}_2\text{O} \quad (1.32)
\]

\[
\text{CH}_3\text{O}^\cdot \rightarrow \cdot\text{CH}_2\text{OH} \quad (3.5)
\]

Formaldehyde, the expected product of electron transfer reaction between the pyrimidine and hydroxymethyl radical [reaction (3.6)], is measured immediately after irradiation of \(\text{N}_2\text{O}\) saturated solutions containing pyrimidine and methanol.

\[
\text{Py} + \cdot\text{CH}_2\text{OH} \rightarrow \text{Py}^\cdot + \text{HCHO} + \text{H}^+ \quad (3.6)
\]

A dose dependent increase in the concentration of \(\text{HCHO}\) is observed with all of the selected pyrimidines. A typical \([\text{HCHO}] \text{ versus } \text{dose}\) dose obtained in the case of 2-amino-4,6-dimethyl pyrimidine is shown in Figure 3.7. The \(G(\text{HCHO})\) values were calculated from such linear plots and are tabulated in Table 3.3. As can be seen from the Table 3.3, the selected pyrimidines gave \(G\) values between 0.9 - 1.4. An attempt to analyse qualitatively the end products from uracil was made using HPLC.

The HPLC analysis gave mainly 3 product peaks (Figure 3.8a), however, the exact identity of these products could not be determined due to the lack of standards. It is observed that two products at retention times 5.1 and 6.9 min increase linearly with dose. A dose dependent increase of these products is shown in Figure 3.8b.
Figure 3.7. The dose dependent formation of formaldehyde obtained from the $\gamma$-irradiation of N$_2$O saturated solution of 2-amino-4,6-dimethylpyrimidine ($1.0 \times 10^{-3} \text{ mol dm}^{-3}$) and methanol ($0.5 \text{ mol dm}^{-3}$) at pH 6.0.

In the absence of any substrates, hydroxymethyl radicals can disproportionate to form mainly ethyleneglycol [reaction (3.7)]. Formaldehyde and methanol are also formed in a lesser extent [reaction (3.8)].

Table 3.3. The yield of formaldehyde, G(HCHO), determined from the electron transfer reaction from hydroxymethyl radicals to pyrimidines at pH 6

<table>
<thead>
<tr>
<th>Substrate</th>
<th>G(HCHO) /10^{-7} \text{ mol J}^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uracil</td>
<td>1.4</td>
</tr>
<tr>
<td>Thymine</td>
<td>1.0</td>
</tr>
<tr>
<td>6-Methyl uracil</td>
<td>1.0</td>
</tr>
<tr>
<td>5,6-Dimethyl uracil</td>
<td>1.1</td>
</tr>
<tr>
<td>2-Amino-4,6-dimethyl pyrimidine</td>
<td>1.4</td>
</tr>
<tr>
<td>2-Amino-4-methoxy-6-methyl pyrimidine</td>
<td>0.9</td>
</tr>
<tr>
<td>2-Amino-4-hydroxy-6-methyl pyrimidine</td>
<td>1.4</td>
</tr>
<tr>
<td>4,6-dihydroxy-2-methyl pyrimidine</td>
<td>1.0</td>
</tr>
<tr>
<td>2,4-dimethyl-6-hydroxy pyrimidine</td>
<td>1.0</td>
</tr>
</tbody>
</table>
A $G(\text{HCHO})$ value of $0.4 \times 10^{-7}$ mol J$^{-1}$ is reported in the absence of any substrate. However, the observed $G(\text{HCHO})$ values (Table 3.3), are much higher than $0.4 \times 10^{-7}$ mol J$^{-1}$ indicating that the electron transfer does take place between $^\text{•} \text{CH}_2\text{OH}$ and Py. Expecting a $G(^\text{•} \text{CH}_2\text{OH})$ value of $7.1 \times 10^{-7}$ mol J$^{-1}$ (after correcting for the competition for $^\text{•} \text{H}$ by CH$_3$OH and Py under our experimental conditions), the $G(\text{HCHO})$ from the electron transfer constituted around 12-19% with most of the selected pyrimidines. This shows that the percentage of electron transfer with these pyrimidines are much higher compared to 1,3-dimethyl thymine reported earlier, where the $G(\text{HCHO})$ is only about $0.6 \times 10^{-7}$ mol J$^{-1}$. However, these values are in agreement with the report on 1,3-dimethyl uracil ($1.1 \times 10^{-7}$ mol J$^{-1}$).
A number of previous radiolytic and photolytic studies on the reaction of hydroxyalkyl radicals with pyrimidines have shown that these radicals add preferentially to the C-6 position of the pyrimidine ring due to their electrophilic nature.\textsuperscript{215,240-242} Such addition reactions normally lead to 6-hydroxymethyl pyrimidine derivatives along with other minor products. It is also known that photoalkylation of purines will lead to 8-hydroxyalkyl purine as the major photoproduct.\textsuperscript{238,239,250} Similar selectivity of hydroxyalkyl radicals can be observed even with pyrimidines as reported in the case of 1,3-dimethyl uracil and 1,3-dimethyl thymine.\textsuperscript{215} However, the reaction of 2-hydroxy-2-propyl radicals with inosine (a nucleoside present in t-RNA and whose structure is closely related to guanosine) gave a different reaction mechanism.\textsuperscript{236} The yield of acetone resulting from the electron transfer from 2-hydroxy-2-propyl radicals to inosine was much higher ($G_{\text{acetone}} = 3.8$) at near neutral pH and exceeded the yield of the 2-hydroxy-2-propyl radicals at pH $> 10$ indicating a chain reaction. The spectral nature of the intermediates at near neutral pH also showed an indication of the electron adduct of inosine at this condition.\textsuperscript{236} The above reports show that the addition of hydroxyalkyl radicals at the C(8) position of purines or C(6) position of pyrimidines can not be generalized with absolute certainty. However, the lower rate constant for the reaction of hydroxymethyl radicals with pyrimidines ($\leq 10^4$ dm$^3$mol$^{-1}$s$^{-1}$) will certainly evoke a competition for the bimolecular decay and their reaction with pyrimidines. Based on the present observation of the significant yield of formaldehyde and on the earlier reports that hydroxymethyl radicals can add to the C(6) position of pyrimidines,
two important reaction mechanisms can be predicted as shown in Scheme 3.2. Our present observations support the major reaction pathway as addition at the C(6) position. The 6-hydroxymethyl pyrimidine radicals can undergo disproportionation reaction and may eventually lead to 6-hydroxymethyl pyrimidine. Electron transfer reaction, on the other hand, will lead to the formation of an electron adduct of pyrimidine and a formaldehyde molecule as shown in Scheme 3.2.

\[
P_y H^+ \xrightarrow{\text{disproportionation}} \text{Products} \\
H_2O \quad \text{Addition} \\
\text{Py} + \text{CH}_2\text{OH} \quad \text{Electron transfer} \\
\left[ \text{PyCH}_2\text{OH} \right]^+ \xrightarrow{\text{disproportionation}} \text{Py-CH}_2\text{OH}
\]

Scheme 3.2

The behavior of the electron adducts of pyrimidine in aqueous solution is now clearly documented.\textsuperscript{251,252} The fast protonation and certain interesting transformation reactions were thoroughly investigated.\textsuperscript{245,251,252} Our recent results also demonstrated similar reactions of the electron adducts with some substituted pyrimidines.\textsuperscript{253} Therefore, the immediate step after the formation of electron adducts must be its protonation by water and/or $H^+$ and the formation of the corresponding protonated electron adducts. These electron adducts/protonated electron adducts may undergo disproportionation reaction to form stable products. The product analysis using HPLC only indicated the formation of at least three
products (Figure 3.8a), but these could not be identified due to the lack of standards. The major contributor of these products must be the hydroxymethyl substituted pyrimidine radical and hence a hydroxymethyl substituted pyrimidine can be assumed as one of the major products. However, a detailed product analysis resulting from the decay of electron adducts of pyrimidines or purines is still lacking and hence a better assumption on the nature of the products can not be made at this stage.

In order to investigate if the hydroxyalkyl radicals have any reaction with substituted pyrimidines in the microsecond time scale (as observed in the case of inosine and adenine), the reaction of a relatively powerful reducing radical, 2-hydroxypropan-2-yl radical \((\text{CH}_3)_2\text{COH}\), is carried out at pH 6 using pulse radiolysis. Interestingly, a fast reaction was observed in the case of uracil derivatives having H substitution at N(1) position. A fast build-up of absorption in the 280-300 nm region followed by a first order type decay \((k \approx 7 \times 10^4 \text{ s}^{-1})\) has been observed. The second order rate constants were determined by monitoring the pseudo-first order build up of the intermediate at the respective \(\lambda_{\text{max}}\) of the observed transient spectra with different concentration of the substrate. These rate constants are tabulated in Table 3.4. The transient spectrum obtained in the case of DMU, which has a \(\lambda_{\text{max}}\) at 295 nm, is shown in Figure 3.9. The fast reaction of \((\text{CH}_3)_2\text{COH}\) with only uracil and its derivatives having H at N(1) position and the absence of any measurable reaction with those without H at the N(1) position were not an easily understandable phenomena. Moreover, the highest rate constants reported with
purine were only of the order of $\sim 6 \times 10^6 \text{ dm}^3\text{ mol}^{-1}\text{s}^{-1}$ at neutral pH (c.f. Table 3.4). In order to understand this unexpected results on the reactivity, a rather rare reaction of OH but observable in the pulse radiolysis scale in the case of uracil was considered. OH formed during the radiolysis of N$_2$O saturated aqueous solution [c.f. reactions (1.2) and (1.13)] can react with compounds having H atom attached to the hetero atom of the pyrimidine by H abstraction. Greenstock et al.$^{25a}$ calculated the rate constant of the reaction of radiolytically generated OH$^-$ with purine/pyrimidine as $1-2 \times 10^{10} \text{ dm}^3\text{ mol}^{-1}\text{s}^{-1}$. They showed that pyrimidine molecules (pK $\sim 9.4$) have a different absorption maximum normalized at 287 nm ($c = 5.7 \times 10^3 \text{ dm}^3\text{ mol}^{-1}\text{cm}^{-1}$). The rate constants, compiled in Table 3.4, are quite comparable with the rate constants reported by Greenstock et al.$^{25a}$ Therefore, some additional experiments were carried out with uracil under different conditions.

The mechanism of the apparent high rate constant has been shown to be due to a reaction between the OH$^-$ ions generated intra-spur and the uracil derivatives, followed by a slow relaxation. 2-hydroxypropan-2-yl radical and formyl radical anions cannot compete with OH$^-$ for the substrates, because of the high difference in rate constants.

The absorption traces at 285 nm obtained from uracil in presence of isopropanol and N$_2$O at different pHs are given in Figure 3.10. The maximum absorbance of the transient is obtained at pH $\sim 6.8$. Similar traces, but in the presence of different concentrations of phosphate buffer, has been shown in Figure 3.10b at pH 7.
Table 3.4. Second order rate constant (dm$^3$mol$^{-1}$s$^{-1}$) for the reaction of OH$^-$ with pyrimidines/purines ($1 \times 10^{-3}$ mol dm$^{-3}$) and their absorption maxima obtained by pulse radiolysis of N$_2$O saturated solutions at pH 6

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$\lambda_{max}$/nm</th>
<th>$^*k/10^9$</th>
<th>Substrate</th>
<th>$\lambda_{max}$/nm</th>
<th>$^*k/10^9$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uracil</td>
<td>285</td>
<td>11.1</td>
<td>2,4-Dimethyl-6-hydroxy pyrimidine</td>
<td>NR</td>
<td>--</td>
</tr>
<tr>
<td>Thymine</td>
<td>295</td>
<td>8.0</td>
<td>4,6-Dihydroxy-2-methyl pyrimidine</td>
<td>NR</td>
<td>--</td>
</tr>
<tr>
<td>6-Methyluracil</td>
<td>285</td>
<td>8.4</td>
<td>Hypoxanthine$^b$</td>
<td>--</td>
<td>0.004</td>
</tr>
<tr>
<td>5,6-Dimethyluracil</td>
<td>295</td>
<td>7.8</td>
<td>Inosine$^b$</td>
<td>--</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* in the presence of (CH$_3$)$_3$COH. NR = no reaction. $^b$ from ref.236

Figure 3.9. Transient absorption spectra obtained in N$_2$O saturated aqueous solutions of 1,3-dimethyl uracil (DMU) ($1 \times 10^{-3}$ mol dm$^{-3}$) and isopropanol ($1 \times 10^{-1}$ mol dm$^{-3}$) at 2 $\mu$s after the pulse at pH 7. Inset: intermediate trace obtained at pH 6.0. (Dose per pulse $\sim$ 15 Gy)
Figure 3.10. Pulse radiolysis traces of uracil ($1 \times 10^{-3}$ mol dm$^{-3}$) obtained by the reaction with OH$^-$ at 285 nm and 15 Gy dose; (a) pH effect of transient in absence (-----) and in presence of $1 \times 10^{-3}$ mol dm$^{-3}$ buffer (——) at pH 6.8 and 9.0 and $20 \times 10^{-3}$ mol dm$^{-3}$ buffer at pH 6.8 (---) in N$_2$O saturated solution containing $1 \times 10^{-1}$ mol dm$^{-3}$ isopropanol. (b) Decay traces of the intermediates (A) in the absence of buffer; in the presence of (B) $10^{-3}$ mol dm$^{-3}$ and (C) $10^{-1}$ mol dm$^{-3}$ buffer in N$_2$O saturated solution containing $10^{-1}$ mol dm$^{-3}$ isopropanol at pH 7. (c) pH effect of transient in presence of $1 \times 10^{-3}$ mol dm$^{-3}$ (—) and $20 \times 10^{-3}$ mol dm$^{-3}$ buffer (----) buffer at pH 6.8 and 9.0 in N$_2$O saturated solution containing $1 \times 10^{-1}$ mol dm$^{-3}$ isopropanol, (d) pH effect on the build-up of transient in N$_2$O saturated solution containing tert-butylalcohol (0.5 mol dm$^{-3}$) in the absence of buffer.

The presence of buffer leads to a reduction in the absorbance signal maximum ‘spikes’, by the intra-spur-generated OH$^-$. To confirm this, the absorption spectra of the parent uracil was taken at pH 5 and 12 (Figure 3.11), wherein the alkaline solution
clearly gave an absorption maximum at 285 nm, compared to 260 nm for the neutral form. Experiments were also carried out to investigate the decay of the intermediates in the absence of $(\text{CH}_3)_2\text{COH}$ at different pHs using $\text{N}_2\text{O}$ saturated solution in presence of tert-butyl alcohol (0.5 mol dm$^{-3}$). Under this conditions, all of the primary free radicals are eliminated [c.f. reactions (1.2) and (1.13)]. The results obtained are shown in Figure 3.11.

This clearly shows that as the proton concentration is high, the decay of the intermediate becomes faster. The effect of pH in the absence of buffer (Figure 3.10d), the effect of buffer at pH 6.8 and 9 (Figure 3.10c) and the pH effect on the decay of traces in the absence of buffer (Figure 3.11) displays interesting differences in the initial and final absorbance of the transient. These results indicate a proton transfer equilibrium [reaction (3.9)].

![Figure 3.11](image-url)

**Figure 3.11.** (i) Absorption spectra of uracil ($1 \times 10^{-3}$ mol dm$^{-3}$) at pH 5 (---) and pH 12 (---). (ii) pH effect on the transient decay traces obtained by the pulse radiolysis of uracil ($1 \times 10^{-3}$ mol dm$^{-3}$) at 285 nm in presence of $\text{N}_2\text{O}$ saturated solution containing 0.5 mol dm$^{-3}$ tert-butyl alcohol in the absence of buffer. Dose = 15 Gy/pulse
\[ \text{OH}^- + \text{UH} \rightleftharpoons \text{U}^- + \text{H}_2\text{O} \]  
\[ (3.9) \]

In acidic conditions, the \text{U}^- immediately gets protonated and the fast decay of the transient at pH 4.3 (Figure 3.11) clearly indicate this (re)protonation reaction. This phenomenon is further supported by the results obtained in the presence of phosphate buffer (Figure 3.10b) at pH 7.0. The absorbance of the transient within 1000 ns was highly reduced in the presence of buffer (Figure 3.10a) and the corresponding decay of the transient observed within 40 \( \mu \)s was practically impossible to detect in the presence of buffer (0.1 mol dm\(^{-3}\)). At higher pHs (> 9), such an absorption was not observable (Figure 3.10c). This is not measurable due to the fact that uracil is already in its deprotonated form \((pK_a = 9.5)\) and that any ground state absorption will be taken into account by the detection set-up as the absorbance of the transient is the difference in the ground state absorbance and the absorbance due to the intermediate species. Therefore, the entire reaction in the case of pyrimidines having H at N(1) position can be summarized as follows.

\[ \text{PyH} + \text{OH}^- \longrightarrow \text{Py}^- + \text{H}_2\text{O} \quad (k \sim 1 \times 10^{10} \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}) \]  
\[ (3.10) \]

\[ \text{OH}^- + \text{H}_3\text{O}^+ \longrightarrow 2 \text{H}_2\text{O} \quad (k = 1.4 \times 10^{11} \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}) \]  
\[ (3.11) \]

\[ \text{OH}^- + \text{H}_2\text{PO}_4^- \longrightarrow \text{HPO}_4^{2-} + \text{H}_2\text{O} \]  
\[ (3.12) \]

\[ \text{OH}^- + \text{HPO}_4^{2-} \longrightarrow \text{PO}_4^{3-} + \text{H}_2\text{O} \]  
\[ (3.13) \]

The ionic yield of \text{OH}^- is approximately equal to ionic yield of \text{H}_3\text{O}^+ \((5 \times 10^6 \text{ mol dm}^{-3} \text{ at a dose of } 15 \text{ Gy})\). The initial strong ‘spike’ in the pulse radiolysis traces may thus be attributed to the formation of the uracil anion, followed by a slower reaction with \text{H}^+ to attain a pseudo-equilibrium condition.
Based on these results, the transient absorption spectrum obtained in the case of DMU (Figure 3.9) is undoubtfully attributed to the deprotonated DMU (at N(1) position) and the fast decay of the transient at its $\lambda_{\text{max}}$ is due to the (re)protonation of the DMU$^-$ [reaction (3.15)]. The neutral form of DMU does not absorb in the region $> 280$ nm.

$$\text{UH} \rightarrow^{\text{H}^-, \text{fast}} \text{U}^{-} \rightarrow^{+\text{H}^-, \text{slow}} \text{UH}$$ (3.14)

It must be further noted that substitution of H at N(1) position is a pre-requisite for this kind of reaction and the corresponding formation of a relatively long lived pyrimidine anion in other selected pyrimidines which do not posses a H in the N(1) position have not shown such a phenomenon. The observed reaction of OH$^-$ with pyrimidine and the detection of the corresponding ionic form in the case of pulse radiolysis is rather a rare phenomenon.$^{64}$

3.3. Summary and conclusions

The reactions of OH$^*$ and CH$_2$OH with pyrimidine derivatives using pulse and steady state radiolysis have been presented in this chapter. The second-order rate constants of the reaction of OH$^*$ with these systems are in the range $2.0 - 7.2 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ at near neutral pH. Transient intermediates of all of the selected
pyrimidines showed two absorption maxima, one around 310 nm and the second in the region 515-550 nm at pH 6 and pH 10.4 except in the case of AHMP. In the case of AHMP, it showed absorption maxima at 320 nm and 455 nm at pH 6 while at pH 10.4, it gave an additional absorption maximum at 750 nm. The yield of oxidising radicals was calculated from the absorption build-up of the oxidising intermediates resulted from the electron transfer to the reductant, TMPD. The yield, G(TMPD⁺⁺), obtained at pH~6 are in the range 0.4-0.9 × 10⁻⁶ mol J⁻¹ which constituted about 6-16% oxidising radicals. In basic solutions (pH 10.4), the G(TMPD⁺⁺) was found to increase up to 5.5 in the case of AHMP (~95% oxidising radical).

*OH adds to the C(5)-C(6) double bond of pyrimidines to form the OH adducts. The difference in the spectral features of the intermediates at near neutral pH and at higher pH (10.4) obtained with AHMP is attributed to the deprotonation of the OH-adducts in basic solutions. Based on the G(TMPD⁺⁺) values at lower and higher pHs, it is proposed that the initially formed C(5)OH-C(6)yl adduct radical (non-oxidising) undergoes a base catalysed conversion (via a dehydration reaction) to C(6)OH-C(5)yl adduct radical (oxidising). Such a phenomenon was not observed with other selected pyrimidines. The major structural difference in AHMP compared to other selected pyrimidines is that it has an OH group at the C(4) position. It is therefore, obvious that the chemical environment in the case of ADMP, AMP and AMMP is not conducive for OH⁻ elimination. This clearly points
to the profound effect of the nature of substituents at the C(4) position of pyrimidine ring, on the redox behaviour of the OH adducts.

The reactions of hydroxymethyl radicals with nucleobase derivatives and other substituted pyrimidines have been carried out at near neutral pH using steady state radiolysis. A quantitative assessment of the electron transfer from hydroxymethyl radicals to pyrimidines is made from the yield of formaldehyde, which is the main product of electron transfer reaction. The yield of formaldehyde, expressed in terms of G-values, with the selected pyrimidines were in the range 0.9 - 1.4 x 10^{-7} \text{ mol J}^{-1} which constituted about 12-19% of the total reaction. It is shown that the electron transfer from hydroxymethyl radicals to uracil, thymine and a variety of substituted pyrimidines is an important reaction pathway, though not a major process. Addition is assumed to be the major reaction. Electron transfer process leading to the formation of electron adducts/protonated electron adducts of nucleobases is one among the base damages that has significance in DNA radical chemistry. However, the role of hydroxyalkyl radicals in the DNA-protein cross linking is still debatable due to their low reactivity with purines and pyrimidines.226 Our observation on the low reactivity of the hydroxyalkyl radicals with the selected pyrimidines in the pulse radiolysis scale rules out such a possibility. The anomalous high reactivity observed in the presence of 2-hydroxypropan-2-yl radical was later understood as the reaction of radiolytically formed OH\textsuperscript{-} with the pyrimidines (ruling out any measurable reactivity of 2-hydroxypropan-2-yl radical).