Properties of the Radical Cation and Radical Anion of 2-Aminopurine and its Charge Transfer Reactions in the Presence of Nucleobases in Aqueous Medium

Abstract: The properties of the radical anion and radical cation of 2-aminopurine (2AP), and their fate in the presence of natural nucleobases have been investigated using the pulse radiolysis technique. The radical anion was produced by the reaction of radiolytically produced hydrated electron (eaq) with 2AP. In the reaction of eaq with 2AP the second order rate constant was calculated as $1.73 \times 10^{10}$ dm$^3$ mol$^{-1}$ s$^{-1}$ at neutral pH. The transient absorption spectrum obtained for the reaction of eaq at neutral pH has an absorption maximum around 350 nm and is assigned to a protonated electron adduct (2AP(NH$_3^+$)). The reducing nature of this radical was demonstrated using methyl viologen (MV$^2$). The radical cation of 2AP was produced by the reaction of sulfate radical anion (SO$_4'^-$). In the reaction of SO$_4'^-$ with 2AP the second order rate constant was calculated as $4.7 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ at pH 7. The time resolved absorption spectra obtained by the reaction of SO$_4'^-$ with 2AP at neutral pH have two distinct maxima at 380 and 470 nm and are assigned to the formation of a neutral radical (2AP-N(9)-H$^+$). The second order rate constant for the reaction of 'OH with 2AP was determined as $3 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$. The transient absorption spectrum obtained from this reaction at pH 7 has absorption maxima at 370 and 470 nm. The spectrum undergoes a time dependent transformation at higher time scale. The transient intermediate species at pH 7 are assigned to the formation of 2AP-4-OH$^+$ (54%), 2AP-5-OH$^+$ (7%) and 2AP-8-OH$^+$ (39%) based on the spectral evidence and TMPD$^2$ build up. The second order rate constant for the reaction of O$_2^-$ with 2AP was determined as $7.1 \times 10^8$ dm$^3$ mol$^{-1}$ s$^{-1}$. In the reaction of O$_2^-$ with 2AP, it is proposed that a nitrogen centered 2AP-N(9)$^+$ radical is formed by an electron transfer reaction at N(9). In the reaction of 'OH, at pH 10, and in the reaction of SO$_4'^-$ at pH 10.2 a similar N-centered radical is proposed (2AP-N(9)$^+$). As the hole transport from 2AP to guanine is a highly probable process, the reaction of SO$_4'^-$ is carried out in the presence of guanosine, adenosine and inosine. The spectrum obtained in the presence of guanosine was significantly different from that in the absence and it showed prominent absorption maxima at 380 and 470 nm, and a weak broad maximum centered around 625 nm which match well with the reported spectrum of a neutral guanine radical (G(-H)$^+$). The electron transfer reaction from the radical anion of 2AP to thymine (T), cytidine (Cyd) and uridine (Urd), was also investigated at neutral pH. Among the three pyrimidines, only the transient spectrum in the presence of T gave a significant difference from the spectral features of the electron adduct of 2AP, which showed a prominent absorption maximum at 340 nm and this spectrum is similar to the electron adduct spectrum of T. The preferential reduction of thymine by 2AP$^-$ and the oxidation of guanosine by 2AP$^+$ clearly follow the oxidation/reduction potentials of the purines and pyrimidines.
Publications from this chapter

1. P. Manoj, H. Mohan, V. M. Manoj, J. P. Mittal, and C. T. Aravindakumar
   “Reactions of \(^{\cdot}\text{OH}\) and \(^{\cdot}\text{O}^-\) with 2-Aminopurine in Aqueous Medium”, Res.

2. P. Manoj, H. Mohan, V. M. Manoj, J. P. Mittal, and C. T. Aravindakumar
   “Charge Transfer from 2-Aminopurine Radical Cation and Radical Anion to Nucleobases: A Pulse Radiolysis Study”, (Under preparation).

3. P. Manoj, H. Mohan, V. M. Manoj, J. P. Mittal, and C. T. Aravindakumar,
   “Reactions of Hydroxyl Radical (\(^{\cdot}\text{OH}\)) and Oxide Radical Anion (\(^{\cdot}\text{O}^-\)) with
   2-Aminopurine in Aqueous Medium” Proc. National Symposium on

4. P. Manoj, V. M. Manoj, H. Mohan, J. P. Mittal and C. T. Aravindakumar,
   “Properties of the Radical Anion and Radical Cation of 2-Aminopurine in
   Aqueous Medium” Proc. Trombay Symposium on Radiation and

5. P. Manoj, V. M. Manoj, H. Mohan, J. P. Mittal and C. T. Aravindakumar,
   “Properties of the Radical Anion of 2-Aminopurine in Aqueous Medium”,
   Proc. National Symposium on Radiation and Photochemistry, IIT-

   Aravindakumar, “Reactions of oxide radical ion (\(^{\cdot}\text{O}^-\)) with substituted
   pyrimidines”, Proc. Third Asian Photochemistry Conference, Mumbai,
   India 2002.
3.1 Charge Transfer in Supramolecular Assemblies of DNA

The DNA charge transport reactions are extremely sensitive to DNA base pair stacking. Hence, the charge transfer (CT) studies will provide a measure of the sequence dependent conformational dynamics of DNA. More precisely it will provide an insight to how the electron donor and acceptor bind DNA, as well as the DNA base sequence; conformational dynamics and local flexibility, all contribute to coupling within the base pair π-stacked array and therefore, to the efficiency of the DNA-mediated charge transport reaction. It is also noteworthy that the sensitivity of the DNA CT to base stacking provides the basis for sensor applications.\textsuperscript{1-4}

The quenching of 2AP fluorescence in DNA is a well-documented phenomenon.\textsuperscript{5} Various mechanisms have been proposed to account for quenching of 2AP\textsuperscript{*} in DNA, including stacking interactions, hydrogen bonding, collisional deactivation,\textsuperscript{6-8} electron transfer\textsuperscript{5,6,9-14} and enhanced population of a nonfluorescent dark state.\textsuperscript{15} This kind of quenching is often attributed to stacking of 2AP with DNA. Although the charge transfer between 2AP\textsuperscript{*} and DNA bases is a feasible process, very few studies demonstrated the chemical evidence for such a CT. An important study by O'Neill \textit{et. al.} however, clearly established a hole transfer from 2AP\textsuperscript{*} to guanine in duplex DNA, which leads to oxidation.\textsuperscript{5} In the process
of hole injection where the donor (photoexcited 2AP) and acceptor (guanine) are connected by a bridge molecule (adenine), a radical anion of 2AP is reported to be formed. In the case of an electron injection where the bridge molecule is either thymine or cytosine, a radical cation of 2AP is formed. Similarly, in the interpretation of a superexchange/tunneling mechanism where there is a direct charge transfer from 2AP* to G (this also requires the involvement of a bridge molecule) the formation of a radical anion of 2AP is predicted. It is, therefore, necessary to understand the fate of the radical cation as well as the radical anion of 2AP in solution state, and to explore the possible charge transfer from their radical ions to different natural nucleobases. The advantage of using radiation chemical technique is that one can very selectively produce the radical anion and radical cation of 2AP under appropriate conditions in solution state.

Despite the importance of 2AP as a fluorophore, their free radical chemistry remains poorly understood. In the photoinduced electron transfer reaction using 2AP incorporated in DNA duplexes the possibility of 2AP radical formation is well predicted. Consequently, its free radical chemistry becomes important in elucidating the mechanistic aspect of the charge transfer process. A detailed investigation of the properties of radical cation and radical anion of 2AP, its free radical reactions and the probable charge transfer reactions to various nucleobases in aqueous medium has
been carried out using pulse radiolysis technique. The structure of 2-aminopurine is shown in figure 3.1.

![Chemical structure of 2-aminopurine](image)

**Figure 3.1** Chemical structure of 2-aminopurine

### 3.2 Reactions of Hydrated Electron (e$_{aq}^-$) with 2-Aminopurine and the Properties of the Electron Adduct

The radical anion of 2AP is produced by the reaction of e$_{aq}^-$ with 2AP and its properties were studied at neutral pH and at pH 12. The second order rate constant was determined by following the decay of the hydrated electron at 700 nm \([k_{obs}]\) as a function of substrate concentration. This plot gave a straight line graph with a good correlation coefficient (0.97). A plot of \(k_{obs}\) versus concentration of 2AP obtained with e$_{aq}^-$ is shown in figure 3.2. The second order rate constant of the reaction of e$_{aq}^-$ with 2AP was calculated as \(1.73 \times 10^{-10}\) dm$^3$ mol$^{-1}$ s$^{-1}$ at neutral pH. Generally, purines have high intrinsic reaction with e$_{aq}^-$ (\(k = 10^{-10}\) dm$^3$ mol$^{-1}$ s$^{-1}$) due to the presence of electron deficient pyrimidine ring.$^{21}$ This value is also well comparable to a reported rate constant of the electron with 2AP in a photoionization study using a 308 nm laser pulse from XeCl excimer laser at neutral pH.$^{18}$
Figure 3.2 Kinetics of the Reaction of $e_{aq}^-$ with 2AP: Plot of pseudo first order rate constant obtained from the decay of $e_{aq}^-$ at 700 nm ($k_{obs}$) versus concentration of 2AP at pH 7. Inset: Decay trace of hydrated electron at 700 nm (i) in the presence and (ii) in the absence of 2AP.

The transient absorption spectrum in the reaction of $e_{aq}^-$ with 2AP at neutral pH and at pH 12 was recorded from the pulse radiolysis of a mixture of N$_2$ saturated aqueous solutions of 2AP containing 2-methyl-2-propanol. Figure 3.3 presents the time resolved absorption spectrum obtained in this reaction. The transient spectrum recorded at 5 µs after the pulse showed a major absorption maximum at 350 nm and a broad absorption centered around 440 nm at pH 7 (figure 3.3). A similar spectrum is obtained when the pH is raised to 12 (however with a broad
absorption band around 480 nm region) where 2AP (pK_a = 11.2) exists in its deprotonated form. In both the pHs, the intermediate species were found to undergo a second order decay.

Figure 3.3 Reaction of e_{aq}^- with 2AP at pH 7 and 12: Transient absorption spectra obtained from the pulse radiolysis of N_2 saturated aqueous solutions of 2AP (1 \times 10^{-3} \text{ mol dm}^{-3}) containing 2-methyl-2-propanol (0.2 mol dm^{-3}) at pH 7 (\triangle) and at pH 12 (\bullet) Inset: MV^{2+} buildup at 605 nm obtained from the reaction of the intermediate with MV^{2+} at pH 7.

The oxidant, methyl viologen (MV^{2+}), was used to investigate the reducing nature of the electron adducts and the formation of methyl viologen radical cation (MV^{+}) was monitored at 605 nm (figure 3.3). The calculated G
value of MV$^{2+}$ were $2.4 \times 10^{-7}$ mol J$^{-1}$ at pH 7 and $2.6 \times 10^{-7}$ mol J$^{-1}$ at pH 12 which constitute about 89% and 79%, respectively, of the total reaction. The percentage is calculated by taking a quantitative yield of the electron as $2.7 \times 10^{-7}$ mol J$^{-1}$ at pH 7 and as $3.3 \times 10^{-7}$ mol J$^{-1}$ at pH 12. The higher yield at pH 12 is from the reaction of H$^+$ with OH$^-$ which yields e$_{aq}^-$. The high G value of MV$^{2+}$ clearly indicates the reducing nature of the intermediate as MV$^{2+}$ is a one electron oxidant. Based on the similarities of the spectral properties as well as the reactions of the intermediate with MV$^{2+}$ with adenine and its substituted derivatives, it is proposed that an electron adduct is initially formed which on fast protonation gives rise to a protonated electron adduct.

The reactions of e$_{aq}^-$ with adenine, its nucleoside and many of its substituted derivatives are well established. The electron adducts undergo a fast protonation (being much stronger base compared to the pyrimidine bases) at the heteroatom ($k$(protonation) = $1.4 \times 10^8$ s$^{-1}$) to form the protonated electron adducts. The spectral features observed in this case is similar to the adenine, hypoxanthine and their derivatives. In a similar way in the present case, the radical anion has a negative charge at nitrogen due to its greater electron affinity. Being a Bronsted base (very similar to adenine radical anion), the radical anion of 2AP gets rapidly protonated to form N-protonated radical (2AP(NH$^+$)). However, there are
three similar sites of electron attack and hence the electron adducts must be represented by their mesomeric structures as shown in the scheme 3.1. Correspondingly, the protonated counterparts would exist in their tautomeric forms (scheme 3.1). Unlike the protonation at nitrogen, the protonation at carbon is much slower, though carbon-protonated species having higher $pK_a$ and would be thermodynamically more favored. Such carbon protonation reactions catalyzed by $\text{OH}^-$, $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ etc, are common in adenine and hypoxanthine derivatives having substitution at their N-9 position\textsuperscript{25,28} (however absent in adenine and hypoxanthine). Therefore the aim of the experiments at basic medium (pH 12), was to look at similar transformation reaction of the N-protonated to C-protonated species. However, the spectral similarities (figure 3.3) as well as the oxidation reaction with $\text{MV}^{2+}$ clearly ruled out this kind of transformation reactions, but fully support the existence of a nitrogen protonated carbon centered radical even at higher pH. The nearly quantitative reaction of the intermediate with $\text{MV}^{2+}$ is an indication of the presence of carbon centered radical (reaction 3.5) as such radical can easily transfer an electron to $\text{MV}^{2+}$.\textsuperscript{23} The absence of such a transformation in the case of adenine and hypoxanthine is explained based on the assumption that the electron density at C-2 and C-8 positions (these are the two potential sites of carbon-protonation) is low due to the deprotonation at higher pH and hence results a slower rate of protonation at these sites.\textsuperscript{25,28}
Scheme 3.1 The proposed mechanism of the reaction of $e_{aq}^-$ with 2 AP.

Similar explanation is equally valid in the case of 2AP, where the only one probable carbon site available for protonation might have low electron density due to the deprotonation at N-9 position as 2AP has a $pK_a$ value at around 11.2. Therefore based on the spectral characteristics...
and the yield of MV$^{2+}$ it is proposed that the radical species responsible for the absorption maxima at 350 nm is the N-protonated radical of 2AP which has three tautomeric structures as shown in scheme 3.1. The existence of neutral electron adducts (singly protonated) at higher pH has been reported in adenine and hypoxanthine derivatives.$^{25,29,30}$ Assuming very similar optical properties of the N-protonated radical of 2AP, the molar extinction coefficient of 2AP(NH$^+$) is approximately calculated as $4130 \pm 5$ dm$^3$ mol$^{-1}$ cm$^{-1}$ at 350 nm. This value match very well with the reported molar extinction coefficient value of the protonated electron adduct of adenosine.$^{31,32}$ However the noticeable difference in the 480 nm region of the spectrum recorded at pH 12 (figure 3.3) compared to that at pH 7 can be attributed to the existence of a negatively charged electron adduct since 2AP has a pK around 11.2.

The transient absorption spectrum obtained in the reaction of hydrogen atom with 2AP is shown in figure 3.4. This spectrum is characterized with its absorption maximum at 350 nm similar to the reaction of hydrated electron with 2AP.
Figure 3.4 Reaction of $H^+$ with 2AP: Transient absorption spectra obtained from the pulse radiolysis of $N_2$ saturated aqueous solutions of 2AP ($1 \times 10^{-3}$ mol dm$^{-3}$) containing 2-methyl-2-propanol (0.2 mol dm$^{-3}$) at 6 $\mu$s at pH 1.

In the case of adenine in solid state, the most probable sites of hydrogen attack are C2 and C8 positions. In the aqueous phase, similar sites were reported in a number of structural analogues of adenine. Since the yield of H-atoms in neutral pH is only about $0.6 \times 10^{-7}$ mol J$^{-1}$, it is convenient to perform the investigation of this reaction at acidic medium where the $e_{\text{aq}}^-$ is quantitatively converted to $H^+$. Therefore, the reaction mechanism may not necessarily be the same as that at neutral pH. However, in the case of adenine as well as of hypoxanthine the $H^+$ undergoes addition at C2 and C8 positions even at strongly acidic medium where these two are in their protonated forms.
In the present case, however, the most probable site of attack is proposed as the C8. At C2 position the attack of H may not be feasible because of the presence of the -NH2 group. Hence a nitrogen-centered radical of the type 2AP-C8(H)N(7) is expected. The observed spectrum is therefore attributed to the formation of this radical.

3.3 Reactions of Sulfate Radical Anion (SO_4^{2-}) with 2-Aminopurine and the Properties of the Radical Cation

Sulfate radical anion (SO_4^{2-}) is a powerful oxidizing radical with an oxidation potential of 2.5-3.1 versus NHE. This radical is frequently used in the study of DNA damage as it can produce a DNA radical cation and an electron in aqueous medium, which is analogous to the direct effect of ionizing radiation. Since direct energy deposition within DNA results in its ionization to form a radical cation and an electron, the use of a powerful one-electron oxidizing agent to produce one-electron oxidized radicals of nucleobases in aqueous medium may provide a means to mimic the direct energy transfer deposition within DNA bases.

The reaction of SO_4^{2-} was carried out at neutral as well as at basic pH. The second order rate constant was determined at pH 7 from the plot of the pseudo first-order formation of the intermediates (k_{obs}) versus concentration of the substrate at 380 nm. Figure 3.5 shows a plot of k_{obs} versus concentration of the substrate at 380 nm at pH 7. This plot gave a
straight line graph with a good correlation coefficient (0.98) and the second order rate constant was calculated as $4.7 \times 10^9 \text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$.

![Figure 3.5 Kinetics of the Reaction of SO$_4^{2-}$ with 2AP: Plot of pseudc first-order formation of the intermediates in the reaction of SO$_4^{2-}$ with 2AP ($k_{obs}$) versus concentration of the substrate at 380 nm at pH 7. Inset: Absorption buildup of the transient intermediate at 380 nm.](image)

The rate constant observed is similar to that in the case of adenine ($k_2 = 4.6 \times 10^9 \text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$) and close to the diffusion controlled level. Therefore, in the case of 2AP also it shows a high reactivity because of the presence of imidazole ring and the electron-donating amino group.

The transient absorption spectra in the reaction of SO$_4^{2-}$ with 2AP at neutral pH and at basic pH (pH 10.2) were recorded from the pulse
radiolysis of a mixture of N₂ saturated aqueous solutions of 2AP, K₂S₂O₅ and 2-methyl-2-propanol. Figure 3.6 shows the time resolved absorption spectra obtained by the reaction of SO₄²⁻ with 2AP at neutral pH, which is characterized by two distinct maxima at 380 and 470 nm. These spectra were appeared to undergo a second order decay at higher timescales. The transient absorption spectra were also recorded at pH 10.2 and have two distinct absorption maxima at 400 and 480 nm (figure 3.6).

![Figure 3.6](image)

**Figure 3.6 Reaction of SO₄²⁻ with 2AP at pH 7 and 10.2:** Transient absorption spectra obtained from the pulse radiolysis of N₂ saturated aqueous solutions of 2AP (1 × 10⁻³ mol dm⁻³) containing 2-methyl-2-propanol (0.2 mol dm⁻³) and S₂O₅²⁻ (5 × 10⁻² mol dm⁻³) at pH 7 (△) and at pH 10.2 (●) 4 μs after the pulse. **Inset: Reaction of O₂⁻ with 2AP:** Transient absorption spectrum obtained from the pulse radiolysis of N₂O saturated aqueous solutions of 2AP (pH ~14) (1 × 10⁻³ mol dm⁻³) at 5 μs (▲).
One of the general reactions of the radical cations of purines is the reaction with water or OH\(^{-}\) to form the corresponding OH-adducts.\(^{39}\) Therefore, a comparison of the spectral properties from the reaction of SO\(_4\)\(^{2-}\) and of *OH with 2AP will be necessary. Hence, the spectral properties of the hydroxyl radical adducts (OH-adducts) were also studied at neutral pH (see section 3.4). However, the transient absorption spectrum obtained from the reaction of *OH with 2AP (see section 3.4, figure 3.7) at neutral pH was very different compared to that from SO\(_4\)\(^{2-}\). However, when the reaction of the oxyradical anion (O\(^{\cdot}\)) was carried out at pH = 14, a high degree of spectral similarity was observed with that from the reaction of SO\(_4\)\(^{2-}\) at pH 10.2 (see figure 3.6 inset and section 3.5 figure 3.12).

Being a very powerful oxidant, SO\(_4\)\(^{2-}\) undergoes one-electron oxidation with purines either by outer-sphere electron transfer or by inner-sphere electron transfer mechanism\(^{39,40}\) leading to the formation of a radical cation which has a very short life time in aqueous solutions in the case of purine systems due to their high Bronsted acidity.\(^{23}\) Therefore, they deprotonate very fast (k > 10\(^7\) s\(^{-1}\)), resulting a neutral radical.\(^{40}\) Generally the stability of the radical cation depends on the nature of the substituents and their position in the aromatic ring. In the reaction of adenine with SO\(_4\)\(^{2-}\), in the initial step, it adds to the ring leading to the formation of a radical cation and then undergoes deprotonation reaction.
leading to the formation of a one electron oxidized and deprotonated species.\textsuperscript{23} In a similar way in the case of 2AP, it is proposed that SO\textsubscript{4}\textsuperscript{2-} undergoes an addition/elimination reaction leading to the formation of a radical cation. The SO\textsubscript{4}\textsuperscript{2-} adducts of adenine and similar purine derivatives were reported to be very short lived and much below the detection limit of the pulse radiolysis setup.\textsuperscript{23} No major change in the lifetime of the SO\textsubscript{4}\textsuperscript{2-} adduct of 2AP in the present case in comparison with that of adenine is expected. In the later stages, this radical cation may undergo deprotonation leading to the formation of a neutral species.

The radical cation of 2AP formed in the first place, may undergo, in principle, a number of reactions in aqueous state. The most probable among those reactions are the deprotonation of the radical cation to form a neutral radical and/or its reaction with water or OH\textsuperscript{-} (at basic pH) to form the corresponding OH-adducts. If latter is the case, then the spectral features at pH 7 should match with the reaction of *OH at the same pH. However, this was not the case as the spectral properties of the OH adduct were different. Then the most likely mechanism is the deprotonation of the radical cation to form a neutral radical of the form 2AP\textsuperscript{-}\textsubscript{N\textsuperscript{\ddagger}(-H)}\textsuperscript{-} (scheme 3.2). Therefore, the spectrum having absorption maxima at 380 nm and 470 nm is attributed to the formation of a neutral radical of the form I. In order to further confirm this assignment, (i.e. the deprotonation from the N\textsuperscript{\ddagger}H\textsubscript{2}) the transient spectrum of
the neutral radical is compared with that from the reaction of $O^-$ (the conjugate base of $^·OH$) at pH $=14$. This comparison is shown in figure 3.6 inset where the absorption spectrum obtained from the reaction of $O^-$ with 2AP (which exists in the deprotonated form) has maximum at 400 and 480 nm. In the latter case, the deprotonated 2AP ($pK = 11.2$) can undergo an electron transfer reaction at $N(9)^-$ to form the corresponding 2APN(9)$^*$ similar to the case with deprotonated purines and pyrimidines.$^{41-49}$ The spectral comparison, thus gave a very clear indication that 2APN(9)$^*$ has different features compared to the spectrum obtained in the case of the reaction of $SO_4^{2-}$ with 2AP at neutral pH. Therefore, the assignment of the neutral radical, 2AP-N$^\prime$(-H)$^*$ (scheme 3.2) is well supported. A molar extinction coefficient of $4725 \pm 5$ dm$^3$ mol$^{-1}$ cm$^{-1}$ at 380 nm (pH 7) is calculated for this neutral radical.

Scheme 3.2 The proposed mechanism of the reaction of $SO_4^{2-}$ with 2AP
In an earlier photoionization study of 2AP, Shafirovich et al.\textsuperscript{18} has demonstrated the formation of 2AP\textsuperscript{**} from the bleaching of 2AP absorption at 310 nm. An indication of the formation of a neutral radical (2AP(-H)\textsuperscript{*}) which absorbs in the region 380-500 nm is also demonstrated in that study.\textsuperscript{18} However the molar extinction coefficient was higher (\(\sim 8400 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}\) at 385 nm) compared to the present value. It is probable that the electron adduct of 2AP also contributes to the absorbance of the transient species within the time scale mentioned in that study.\textsuperscript{18} In the case of photoionization of purine nucleosides a similarly high molar extinction coefficient in the region 320-340 nm (\(\epsilon \sim 8000-9000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}\)) was reported for the neutral radical compared to that from the reaction of radiolytically produced SO\textsubscript{4}\textsuperscript{2-} (\(\epsilon \sim 4000-5000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}\)).\textsuperscript{50} This discrepancy was attributed to the presence of both oxidized and reduced species in the case of photoionization studies. On the other hand, in the case of the reaction of radiolytically produced SO\textsubscript{4}\textsuperscript{-} in the reported study\textsuperscript{50,51}, as well as in the present study, any absorption interference due to other intermediate species is not expected and hence the value of the molar extinction coefficient can be fully reliable. Furthermore, the structural identity of the neutral radical (2AP(-H)\textsuperscript{*}) was also not specified in the early report.\textsuperscript{18}
The transient absorption spectrum recorded at pH 10.2 showed two distinct maxima at 400 and 480 nm (figure 3.6). This spectrum is well comparable to the spectrum obtained in the reaction of O' (see inset figure 3.6 and section 3.5 figure 3.12). The similarity of these spectral characteristics favors a mechanism where the initially formed radical cation reacts with OH\(^{-}\) leading to the formation of 2AP-4OH\(^{+}\) which on dehydration reaction gives rise to a nitrogen centered radical of the form 2AP-N(9)\(^{+}\) as shown in scheme 3.2. Therefore it is proposed that the transient species formed at pH 10.2 having absorption maxima at 400 and 480 nm is due to the formation of a nitrogen centered radical (2AP-N(9)\(^{+}\)).

3.4. Reactions of Hydroxyl Radical (\(^{\ast}\)OH) with 2-Aminopurine

The absorption spectra of the intermediates obtained from the reaction of \(^{\ast}\)OH were recorded at pH 7 and 10. Figure 3.7 shows the time resolved absorption spectrum recorded in the reaction of \(^{\ast}\)OH with 2AP at neutral pH.
Figure 3.7 Reaction of \textsuperscript{·}OH with 2AP at pH 7: Transient absorption spectra obtained from the pulse radiolysis of N\textsubscript{2}O saturated aqueous solutions of 2AP (1 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}) at 4 µs (●) and 40 µs (△) at pH 7. Inset: TMPD\textsuperscript{+} build up at 565 nm obtained from the reaction of the intermediate with TMPD.

The transient absorption spectrum recorded at 4 µs after the pulse is characterized by its maximum at 370 and 470 nm at pH 7. The rate of initial absorption build up of the transients (k\textsubscript{obs}) was found to depend linearly on the concentration of 2AP and from this dependence at 370 nm, a bimolecular rate constant of 3 × 10\textsuperscript{9} dm\textsuperscript{3} mol\textsuperscript{-1} s\textsuperscript{-1} was determined at pH 7. This rate constant is in the same order as that of adenine (6.4 × 10\textsuperscript{9} dm\textsuperscript{3} mol\textsuperscript{-1} s\textsuperscript{-1}).\textsuperscript{39,52,53} Figure 3.8 depicts the plot of pseudo first order build up versus concentration of 2AP at 370 nm at pH 7.
Figure 3.8 Kinetics of the Reaction of •OH with 2AP: The plot of $k_{obs}$ versus concentration obtained for the reaction of •OH with 2AP at 370 nm (pH 7). Inset: Absorption build up of the transient intermediate at 370 nm.

The absorption spectrum obtained at 40 µs after the pulse has also shown maxima at 370 and 470 nm (pH 7), however this spectrum indicated a time dependent change characterized by a decrease in the absorbance in the region of 370 nm and an increase at 450 nm (figure 3.7). This transformation phenomenon was similar to the case of adenine derivatives\textsuperscript{39,52,53} however, the wavelength regions were different. For example, in the case of adenine and its derivatives, the spectra undergo time dependent changes characterized by an increase in optical density in the region of 320-350 nm and a decrease at 390-420 nm.\textsuperscript{53} The spectral features at higher pH (pH 10) with 2AP were different compared to that at pH 7. The transient absorption
spectrum obtained in the reaction of \( \cdot \)OH with 2AP at pH 10 is shown in figure 3.9. This spectrum has absorption maxima at 400 and 480 nm at 3 \( \mu \)s after the pulse (figure 3.9). No visible spectral transformation was observed at this pH unlike at pH 7.

\[ \text{Figure 3.9 Reaction of } \cdot \text{OH with 2AP at pH 10: Transient absorption spectra obtained from the pulse radiolysis of N}_2\text{O saturated aqueous solutions of 2AP (1 \times 10^{-3} \text{ mol dm}^{-3}) (pH 10) at 3 } \mu \text{s (●). Insert: TMPD}^+ \text{ build up at 565 nm obtained from the reaction of the intermediate with TMPD.} \]

One of the helpful methods to understand the redox nature of the transient intermediates, is the use of known oxidants/reductants at sufficiently low concentrations so that there will not be any direct reaction of \( \cdot \)OH with the oxidants/reductants, while the intermediates would react with the oxidants/reductants, making use of their slight differences in the oxidation/
reduction potentials. Therefore, in order to understand the nature of the transient intermediate, the electron transfer reaction is carried out by using the well known reductant N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD). The concentration of the reductant was kept low (5 \times 10^{-5} \text{ dm}^3 \text{ mol}^{-1}) in order to avoid direct reaction of \textsuperscript{1}OH with the reductant. A strong absorption build up of the radical cation of TMPD (TMPD\textsuperscript{+}) which is typical of similar electron transfer from the oxidizing intermediate to TMPD has been observed at 565 nm at pH 7. The yield of TMPD\textsuperscript{+} was calculated in terms of G(TMPD\textsuperscript{+}) and was found to be 3.3 \times 10^{-7} \text{ mol} \text{ J}^{-1}. The total yield of \textsuperscript{1}OH (G(\textsuperscript{1}OH)) is calculated as 5.4 \times 10^{-7} \text{ mol} \text{ J}^{-1} which is based on the concentration of 2AP and its second order rate constant. Considering this G value, the observed G(TMPD\textsuperscript{+}) constituted about 61\% of the total reaction. A similarly high G(TMPD\textsuperscript{+}) of 3.9 \times 10^{-7} \text{ mol} \text{ J}^{-1} was obtained at pH 10 as well, which constitutes about 72\% of the total \textsuperscript{1}OH reaction. Generally \textsuperscript{1}OH radicals react by addition to a double bond. It is reported that in the case of adenine and its nucleosides the potential site for the attack of OH is C(4), C(5) and C(8). In a similar way in the case of 2AP, it is proposed that OH radical adds to C(4), C(5) and C(8) to form the corresponding OH adducts. The presence of \textsuperscript{-NH\textsubscript{2}} group at C(2) position in 2AP instead of C(6) is not likely to alter the electron density distribution significantly at C(4), C(5) or C(8).
positions. The addition at the C4 position may lead to the formation of C4-OHC5-yl radical (2AP-4-OH'), at the C5 to the formation of C5-OHC4-yl radical (2AP-5-OH') and at C(8) to the formation of 2AP-8-OH' (scheme 3.3). The addition of OH in the case of adenine leads to the formation of A-4-OH', A-5-OH' and A-8-OH'\(^\text{39,52,53}\). The A-8-OH' undergoes the ring opening reaction and forms 5-formamido-6-aminopyrimidine as the final product.\(^\text{30,52,53,56,57}\)

It is reported that in the case of OH-adducts of purines with substituents at C-6 other than -NH\(_2\), the optical density changes at \(-330\) nm and at \(-400\) nm are due to different radicals.\(^\text{53}\) It is recognized that the OD changes are due to the dehydration reaction of A-4-OH' and the ring opening (imidazole) reaction of A-8-OH'. These two reactions were distinguished by monitoring the effect of substituents on the rate of reaction, their pH dependencies and their activation parameters. It is also identified that in the case of adenine and its nucleosides, the spectral transformation is due to two distinct reactions.\(^\text{39,52,53}\) With the help of conductance measurements and optical detection techniques, it is demonstrated that, in the case of fully alkylated adenines, "OH mainly adds at C4/C5 double bond of the purine leads to the formation of OH- adduct and these undergo unimolecular elimination of OH' at later stages. It is documented that
the decrease of optical density at 400 nm is due to the dehydration reaction of the initially formed OH-adducts and the increase in OD at ~330 nm is assigned to the ring opening reaction of A-8-OH\textsuperscript{+}.\textsuperscript{53} It is also noteworthy that the ring opening reaction of A-8-OH\textsuperscript{+} has low enthalpy and a negative entropy of activation (-21 cal), whereas for the dehydration of A-4-OH\textsuperscript{+}, the values are higher (~-10 cal).\textsuperscript{53}

In a similar way in the present case too the time dependent transformation of the spectrum is proposed as due to two different reactions of the initially formed OH adducts. The decrease in the absorbance at 370 nm is due to the OH\textsuperscript{-} elimination from 2AP-4-OH\textsuperscript{+} and 2AP-5-OH\textsuperscript{+} which leads to the formation of 2AP\textsuperscript{**} and this may ultimately be transformed to a nitrogen centered radical (2AP-N(9)\textsuperscript{+}). The increase in the absorbance at 450 nm may be due to the ring opening reaction of 2AP-8-OH\textsuperscript{+}. However, no other evidence is obtained for the ring opening reaction.
Scheme 3.3 Proposed reaction mechanism of the reaction of \(^*\)OH with 2AP

The formation of 2AP-4-OH\(^*\) can be supported from its reaction with TMPD. The results show that about 61% of the intermediates are oxidizing in nature. Among the three proposed radicals, the oxidizing property of 2AP-4-OH\(^*\) can well be explained due to the presence of unpaired spin density on N1 or N3 in all the possible mesomeric structures (scheme 3.4). But for 2AP-5-OH\(^*\), the unpaired spin density is on carbon in all its possible mesomeric structures (scheme 3.4). So this radical should be reducing in nature. Similarly 2AP-8-OH\(^*\) can be understood as reducing in nature. It was reported that A-8-OH\(^*\) undergoes oxidation to give rise to 8-hydroxy purines which subsequently undergoes ring opening reaction to
yield 5-formamido-6-aminopyrimidines as the final product.\textsuperscript{39,52,53} In the case of 2AP also it is probable that the 2AP-8-OH\(^{\bullet}\) can undergo a ring opening reaction similar to adenine.

\textbf{Scheme 3.4} Mesomeric structures of 2AP-4-OH\(^{\bullet}\) and 2AP-5-OH\(^{\bullet}\)

On a close observation of the build up of TMPD\(^{\bullet\bullet}\) at pH 7, it can be understood that the fast initial build up is followed by a slow build up which extends up to about 1 millisecond. Figure 3.10 shows the TMPD\(^{\bullet\bullet}\) build up observed at different timescale. The initial fast build up is well within the time scale of the formation of 2AP-4-OH\(^{\bullet}\) and therefore this initial build up is assigned to the reaction of 2AP-4-OH\(^{\bullet}\) with TMPD. The later slow build up is assigned to the reaction of the radical cation of 2AP (scheme 3.3), which could be formed by the OH\(^{-}\) elimination of 2AP-5-OH\(^{\bullet}\). The formation of such a radical cation which is a better oxidant compared to A-4-OH\(^{\bullet}\) is reported in the case of adenine.\textsuperscript{39,52,53}
In the present case, 2AP-5-OH' is not expected to have any reactivity with TMPD, however the product of OH\(^-\) elimination, the radical cation, could have a high reactivity to TMPD. The yield of the radical cation resulting from 2AP-5-OH' is expected to be very low. This is the reason why even after several hundreds of microseconds, the G(TMPD\(^{**}\)) has been increased only marginally (figure 3.10). The calculated G(TMPD\(^{**}\)) for the fast initial build up was \(2.9 \times 10^{-7}\) mol J\(^{-1}\) and from the later slow build up was \(3.3 \times 10^{-7}\) mol J\(^{-1}\). As the only one oxidizing species in the initial stage is the 2AP-4-OH', the difference between the G values for the fast build up and the slow build up is assigned to the yield of 2AP-5-OH' (i.e. \(0.4 \times 10^{-7}\) mol J\(^{-1}\)). The difference between the G value after the slow build up and the total yield of \(^{*}\)OH (5.4 \(\times 10^{-7}\) mol J\(^{-1}\)) is assigned to the yield of 2AP-8-OH'. Hence, a G value of \(2.1 \times 10^{-7}\) mol J\(^{-1}\) is calculated for 2AP-8-OH'.
Figure 3.10 **TMPD**\(^{**}\) buildup: TMPD\(^{**}\) buildup at 565 nm obtained from the reaction of the intermediate (formed in the reaction of \(\cdot\)OH with 2AP) with TMPD at pH 7.

The spectral behavior at pH 10 is very different compared to that at pH 7 (figures 3.7 and 3.9). The absorption spectrum with maxima at 400 and 480 nm is attributed to an N-centered radical of the form 2AP-N(9)\(^{\ast}\) (scheme 3.3) which is formed by the dehydration of the OH-adducts. This result is, thus, very different from the reaction of \(\cdot\)OH with adenine and its derivatives\(^{39,52,53}\) at higher pH where an OH\(^{-}\) induced inhibition of OH\(^{-}\) elimination of A-4-OH\(^{\ast}\) leading to a deprotonated radical A-4O\(^{\ast+}\) is reported. Because of this reason, the yield of TMPD\(^{**}\) is seen highly reduced at higher pH.\(^{39,52,53}\) The existence of 2AP-N(9)\(^{\ast}\) in the
present case is further confirmed by the formation of a transient species from the reaction \( \text{Cl}^- \) with 2AP which has very similar spectral characteristics (see section 3.5). The reaction of \( \text{O}^- \) with nucleobases proceeds via an electron transfer reaction with the deprotonated base at pH =14 leading to a N(9)-centered radical.\(^{41,58}\)

The electron transfer reaction of the intermediate with TMPD is also carried out at pH 10 and a strong absorption build up of \( \text{TMPD}^{''} \) is obtained, and the yield of \( \text{TMPD}^{''} \) was calculated in terms of \( G(\text{TMPD}^{''}) \) as \( 3.9 \times 10^{-7} \text{ mol J}^{-1} \). Therefore from the similarity of the spectral characteristics with \( \text{O}^- \) and also on the basis of \( G(\text{TMPD}^{''}) \), it can be concluded that the transient species formed in the reaction of \( \cdot \text{OH} \) at pH 10 is 2AP-N(9)'. The dehydration of both 2AP-4-OH' and 2AP-5-OH' is expected to be enhanced in the basic medium. As the \( G(\text{TMPD}^{''}) \) is not quantitative (only 71%), it is proposed that the remaining 29% corresponds to the formation of 2AP-8-OH'. This is understandable since the pK of 2AP is at 11.2, the attack at the C(8) is highly likely similar to the case at pH 7.

3.5 Reactions of Oxide Radical Anion (\( \text{O}^- \)) with 2-Aminopurine

Reports on the reaction of oxide radical anion (\( \text{O}^- \)) with purine and pyrimidine bases and their nucleosides are fewer in number compared to the reaction of hydroxyl radical (\( \cdot \text{OH} \)). In strongly basic medium \( \cdot \text{OH} \) is rapidly converted into its conjugate base \( \text{O}^- \).\(^{21}\)
Oxide radical anion is an oxidant \( \left( E^\circ(O^+\cdot/H^+/OH^-) = 1.77 \text{ V} \right) \). The reaction of \( O^+ \) with organic molecules is different compared to the reaction of \( \cdot\text{OH} \). Oxide radical anion has a little tendency to add to double bond or aromatic ring, whereas its hydrogen abstracting ability is not significantly different from that of hydroxyl radical. It is known that \( O^+ \) reacts with molecular oxygen \( \left( k_1 = 3.6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \right) \) and reacts with the phenoxide anion by electron transfer to produce the phenoxy radical. It is also reported that oxide radical anion undergoes an electron transfer oxidation with unsubstituted substrates, whereas with methyl substituted derivatives it abstracts a hydrogen atom from the methyl moiety. Therefore, electron transfer and H-abstraction was identified as the major reaction pathways of oxide radical anion. The reactions of \( O^+ \) with nucleosides are important, because a strong increase in the base release was reported during the radiolysis of \( \text{N}_2\text{O} \) saturated aqueous solution of deoxyribonucleoside series at high pH.

In the reaction of oxide radical anion with 2AP, a second order rate constant of \( 7.1 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) is determined from the pseudo first order build up of the intermediates with respect to the concentration of 2AP at 400 nm. Figure 3.11 presents the plot \( k_{\text{obs}} \) versus concentration of the substrate obtained at 400 nm.
Figure 3.11 *Kinetics of the Reaction of O*\textsuperscript{\textminus} with 2AP:* The plot of $k_{\text{obs}}$ *versus* concentration obtained for the reaction of O*\textsuperscript{\textminus} with 2AP at 400 nm (pH = 14). *Inset:* Absorption build up of the transient intermediate at 400 nm.

It is reported that the rate of the reaction of O*\textsuperscript{\textminus} with purines and pyrimidines is less than that of the reaction of *OH with purines and pyrimidines because of the difference in the reaction mechanism of these two radicals.\textsuperscript{41}

Figure 3.12 is the transient absorption spectrum recorded from the pulse radiolysis of N\textsubscript{2}O saturated solution of 2AP at pH \approx 14. The transient spectrum recorded at 5 \textmu s after the pulse has $\lambda_{\text{max}}$ at 400 and 480 nm similar to the reaction of *OH at pH 10. At higher time scales it showed only a second order decay. In order to investigate the redox nature of the
intermediate, electron transfer reaction was carried out with TMPD, and obtained a very strong absorption build up of TMPD" at 565 nm. The yield of TMPD" was calculated in terms of \( G(\text{TMPD}^\cdot) \) and was found to be \( 4.01 \times 10^{-7} \text{ mol J}^{-1} \).

**Figure 3.12** Reaction of \( \text{O}^\cdot \) with 2AP at \( pH = 14 \): Transient absorption spectra obtained from the pulse radiolysis of \( N_2O \) saturated aqueous solutions of 2AP (\( pH \approx 14 \)) \( (1 \times 10^{-3} \text{ mol dm}^{-3}) \) at 5 \( \mu s \) (\( \bullet \)). **Inset:** (a) Absorption build up of the transient intermediate at 400 nm (b) TMPD" build up at 565 nm obtained from the reaction of the intermediate with TMPD.

At \( pH \approx 14 \), 2AP exists in the deprotonated form since 2AP has a \( pK_a \) at 11.2. \( \text{O}^\cdot \) has a low tendency to add to the double bond or to aromatic ring. Generally \( \text{O}^\cdot \) undergoes electron transfer and H-abstraction with purines and pyrimidines.\(^{43,44,46,47}\) Hence based on the previous reports and
on the spectral characteristics it is proposed that $O^-$ undergoes an electron transfer reaction with 2AP (reaction 3.13) at N(9) leading to the formation of a nitrogen centered radical $2AP\cdot N(9)^*$.

$$\text{H}_2\text{N}$$

$\text{N}$

$\text{O}^\cdot-$(3.16)

$\text{N}^\cdot-\text{N}$

$\text{O}^\cdot-$(3.16)

$\text{N}^\cdot-\text{N}$

$\text{H}_2\text{N}$

The oxidizing nature of this radical can well be explained due to the formation of TMPD$^*$. The nitrogen centered neutral $(2AP\cdot N(9)^*)$ radical spectrum is characterized by its maxima at 400 and 480 nm. In the case of adenine and its derivatives it is reported that $O^-$ undergoes an electron transfer reaction to form a nitrogen centered radical $(A\cdot N(9)^*)$. The similarity of the spectrum obtained from the reaction of $^*\text{OH}$ at pH 10 and of $O^-$ at pH =14 gave a clear indication of the formation of $2AP\cdot N(9)^*$ in both the cases.

3.6 Charge Transfer to Thymine and Guanosine

Double helical DNA is the most excellent structurally characterized molecular $\pi$-stacked array for electron transfer. Radiation induced electron migration along DNA is a mechanism by which randomly produced stochastic energy deposition events which can lead to non-random types of damage in DNA. Electrons can migrate to bases of highest electron affinity and lowest ionization potential to produce the non-random distribution of radicals. In DNA, the purine bases, guanine
(E₀ = 1.29 versus NHE) and adenine (E₀' = 1.42 versus NHE) are the most
easily oxidizable moieties and the pyrimidine bases cytosine (E₀ = 1.6 versus
NHE) and thymine (E₀ = 1.7 versus NHE) are the most difficult to be
oxidized.⁵⁴,⁵⁸,⁹⁹ The reduction potentials of guanine, adenine, cytosine and
thymine are -2.7, -2.52, -2.35 and -2.18 respectively.⁶⁹ This implies that
cytosine and thymine are much easier to get reduced than adenine and
guanine. Hence the electrons are mainly trapped at thymine and the electron
hole is localized at guanine during the charge transport.⁶⁸,⁷⁰ Hence,
photioxidants bound to DNA can promote oxidative damage at a remote
guanine site through DNA charge transfer. Since guanine exhibit lowest
oxidation potential⁶⁸ among the four bases of DNA, the electron-transfer
reactions of guanine are central to understanding both the hole transfer along
DNA and biological damage to nucleic acids.⁷¹,⁷⁴ It is also of great importance
to investigate the kinetic and mechanistic aspect of electron transfer from 2AP
to thymine in order to understand the photoinduced charge transport in 2AP
mismatched DNA, since thymine has the highest oxidation potential among
the four DNA bases.

3.6.1 Electron Transfer from the Radical Anion of 2-Aminopurine
to Pyrimidines

In order to investigate the electron transfer from the radical anion of
2AP to the most reducible nucleobases (i.e. the pyrimidines), pulse
radiolysis experiments were carried out in N₂ saturated aqueous solutions
containing 2AP \((1 \times 10^{-3} \text{ mol dm}^{-3})\), 2-methyl-2-propanol \((0.2 \text{ mol dm}^{-3})\) and low concentrations \((5 \times 10^{-5} \text{ mol dm}^{-3})\) of pyrimidines \((\text{thymine (T), cytidine (Cyd) and uridine (Urd)})\) at neutral pH. Under this condition the hydrated electron will react with 2AP to form the electron adduct and the reaction of \(e_{an}^-\) with pyrimidine will be negligible \((< 5 \% \text{ based on the concentrations and rate constants})\). Among the three pyrimidines \((\text{T, Cyd and Urd})\), only the transient spectrum in the presence of T gave a significant difference from the spectral features of the electron adduct of 2AP. In the other cases with Cyd and Urd, the transient spectra were very similar to that of the electron adduct spectrum of 2AP \((\text{figure 3.13})\). Figure 3.14 presents a comparison of the transient absorption spectra of the protonated electron adduct of 2AP and the spectra obtained in the presence of thymine at 40 \(\mu\text{s}\) after the pulse. The latter spectrum measured 40 \(\mu\text{s}\) after the pulse has a prominent absorption maximum at 340 nm and a weak broad maximum around 480 nm regions. The reported spectrum of electron adduct of thymine has an absorption maximum at 340 nm.\(^{24,75}\) We have also recorded the electron adduct spectrum of T at neutral pH and similar spectral features were obtained. As the pK value of the thymine electron adduct is 7.2,\(^{24}\) the observed spectrum at neutral pH must correspond to the existence of both neutral and ionic form of the electron adduct of thymine. This unambiguously demonstrates the electron transfer from the electron adduct of 2AP to T. \((\text{reaction 3.17})\).
Figure 3.13  Reaction of $e_{aq}^-$ with 2AP in the presence of cytidine and uridine: Transient absorption spectra obtained from the pulse radiolysis of N$_2$ saturated aqueous solutions of 2AP ($1 \times 10^{-3}$ mol dm$^{-3}$) containing cytidine ($5 \times 10^{-6}$ mol dm$^{-3}$) and 2-methyl-2-propanol ($\triangle$) at 4 $\mu$s and uridine ($5 \times 10^{-5}$ mol dm$^{-3}$) (○) at pH 7.

But in the case of cytidine and uridine, because of the similarities of the spectral characteristics of the electron adduct of 2AP (figure 3.13), no electron transfer reaction is taking place from radical anion of 2AP to these bases. It can be well explained on the basis of the reduction potentials of the free bases.

$$2AP\cdot^- \rightarrow 2APNH\cdot \xrightarrow{T} T\cdot^- + 2AP$$
Properties of the Radical Cation and Radical.... 133

Figure 3.14 Reaction of $e_{aq}^-$ with 2AP in the absence and in the presence of thymine: Transient absorption spectra obtained from the pulse radiolysis of N$_2$ saturated aqueous solutions of 2AP ($1 \times 10^{-3}$ mol dm$^{-3}$) containing thymine ($5 \times 10^{-5}$ mol dm$^{-3}$) and 2-methyl-2-propanol (0.2 mol dm$^{-3}$) (pH 7) (●) at 40 µs and N$_2$ saturated aqueous solutions of 2AP ($1 \times 10^{-3}$ mol dm$^{-3}$) containing 2-methyl propan 2-ol (0.2 mol dm$^{-3}$) (○) at pH 7.

As mentioned in section 3.2 the protonation of the electron adduct is quite fast and the reported rate constant for the protonation (at nitrogen) of the radical anion of adenine derivative is in the order of $10^6$ s$^{-1}$.26-30,70,76 Since both 2AP$^-$ and 2AP(NH$^+$) are strong one electron reductant, as shown by its ability to reduce MV$^{2+}$, there will not be any hindrance of the electron transfer to T due to the protonation process. Moreover, the rate of electron transfer to T is expected to be comparatively slower to the
protonation of the electron adduct which is reported to be very fast in the case of purines \( k(\text{protonation}) \sim 10^8 \text{ s}^{-1} \).\textsuperscript{25-30} This is justified due to the fact that the rate of electron transfer from the (protonated) electron adduct to \( \text{MV}^{2+} (E^0 (\text{MV}^{2+}) = 0.44 \text{ V}) \) is only \( 6.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) and the expected rate of the electron transfer to \( T \) is relatively lower as the oxidation potential of \( T \) is \( E^0 = 1.7 \text{ versus NHE} \). It is therefore concluded that the electron transfer is from the protonated electron adduct of 2AP to \( T \). However, the actual rate of this electron transfer could not be calculated due to the overlap of the absorption spectrum of the protonated electron adduct of 2AP and the thymine radical anion or its protonated form. Furthermore, it can be understood that the lack of electron transfer to other pyrimidines such as Cyd and Urd is due to their higher reduction potential (compared to \( T \)). This means, the electron transfer process strictly follows the redox potentials of the base moiety.

### 3.6.2 Hole Transfer from Radical Cation of 2-Aminopurine to Purines

It is reported that a positive charge injected into DNA base triggers electron transport over long distances in a multi-step hopping process.\textsuperscript{10} As the hole transfer from 2AP to guanine is a highly probable process, experiments were carried out to study the oxidation of purines by 2AP\textsuperscript{2+} at neutral pH. The reaction of \( \text{SO}_4^{2-} \) with 2AP was carried out in the presence of low concentrations of purine nucleosides (guanosine,
adenosine and inosine) \((5 \times 10^{-5} \text{ mol dm}^{-3})\). Under this condition, it is calculated that only about less than 5% of \(\text{SO}_4^{2-}\) will directly react with the purine nucleosides, and hence the prominent reaction will be between \(\text{SO}_4^{2-}\) and 2AP. The spectral properties obtained from the reaction of \(\text{SO}_4^{2-}\) with 2AP remained the same in the presence of adenosine and inosine. However, the transient spectrum obtained in the presence of guanosine was significantly different compared to that in its absence. In figure 3.15, a comparison of the spectrum obtained in the reaction of \(\text{SO}_4^{2-}\) with 2AP and the spectrum recorded in the presence of guanosine is shown. The inset of the figure shows a comparison of the absorption trace obtained at 625 nm in the presence and in the absence of guanosine. The transient absorption spectrum showed two prominent absorption maxima at 380 and 470 nm, and a weak broad maximum centered around 625 nm (figure 3.15).
Figure 3.15 *Reaction of SO₄²⁻ with 2AP in the absence and in the presence of guanosine*: Transient absorption spectra obtained from the pulse radiolysis of N₂ saturated aqueous solutions of 2AP (1 × 10⁻³ mol dm⁻³) containing S₂O₅²⁻ (5 × 10⁻² mol dm⁻³), guanosine (5 × 10⁻⁵ mol dm⁻³) and 2-methyl-2-propanol (0.2 mol dm⁻³) (pH 7) at 4 μs (●) and N₂ saturated aqueous solutions of 2AP (1 × 10⁻³ mol dm⁻³), S₂O₅²⁻ (5 × 10⁻² mol dm⁻³) and 2-methyl-2-propanol (0.2 mol dm⁻³) at 4 μs (△) at pH 7. Inset: Absorption buildup of the intermediate at 625 nm (i) in the presence of guanosine and (ii) in the absence of guanosine.

The reported transient spectrum obtained from the direct reaction of SO₄⁻ with guanine has absorption maxima at 380 and 470 nm along with a weak broad band centered around 625 nm.⁷⁷,⁷⁸ Since the prominent maxima of the transient spectrum obtained from the reaction of SO₄²⁻ with 2AP (380 and 470nm) overlap with that obtained in the presence of
guanosine, the spectral difference can be best observed in the wavelength region 600-680 nm (figure 3.15). The absorption trace obtained at 625 nm (figure 3.15), clearly demonstrate the formation of a neutral guanosine radical of the type G(-H)' since G'' has a pK at 3.9 as reported earlier. 

![Graph showing transient absorption spectra](image)

**Figure 3.16 Reaction of SO₄²⁻ with 2AP in the presence of inosine, adenosine and cytidine:** Transient absorption spectra obtained from the pulse radiolysis of N₂ saturated aqueous solutions of 2AP (1 × 10⁻³ mol dm⁻³) containing S₂O₆²⁻ (5 × 10⁻² mol dm⁻³), 2-methyl-2-propanol (0.2 mol dm⁻³) and adenosine (5 × 10⁻⁵ mol dm⁻³) (△) and inosine (5 × 10⁻⁵ mol dm⁻³) (●) at 4 µs and at pH 7.

A rate constant of 2 × 10⁵ s⁻¹ is also calculated from this trace. It is therefore, evident that the initially formed radical cation of 2AP undergoes
an electron transfer reaction with guanosine to form the corresponding radical cation. At neutral pH, the most feasible reaction is the deprotonation of the radical cation, which results the formation of a neutral guanosine radical. The neutral guanosine radical thus has absorption maxima at 380 and 470 nm with a broad absorption band around 625 nm and the observed spectrum in the present case (figure 3.15) match very well with this reported spectrum. Thus the spectral features clearly support the mechanism proposed in reaction 3.15. In the case of adenosine and inosine the spectral features are similar to that that of 2-aminopurine neutral radical hence practically no charge transfer is observed (figure. 3.16). It can be explained on the basis of the oxidation potential of these bases (G (1.29 V) < A (1.42 V) < I (1.5 V) < C (1.6 V) < T (1.7 V) vs NHE at pH 7).

\[
2\text{AP}^{++} + G \xrightarrow{(3.18)} 2\text{AP} + \text{G}^{++} \xrightarrow{(3.19)} \text{G}(-\text{H})^* \text{ (pH 7)}
\]

In a DFT calculation by Reynisson et al., it is reported that 2AP\textsuperscript{++} has sufficient energy to ionize guanine. The clear spectral evidence presented in this study supports this theoretical demonstration. In an earlier report by Shafirovich et al. it is demonstrated that neutral guanine radicals are formed during photo excitation of 2AP in a solution containing GMP and our observation is in line with this report.
3.7 Conclusion

The general radical chemistry of (i.e. reactions of $e_{aq}^-$, $SO_4^{2-}$, 'OH and O') of 2-aminopurine has close similarity with that of adenine. In the reaction of hydrated electron with 2AP it is identified that at both the pHs (pH 7 and 12) an N-protonated radical ($2AP(NH^+)$) is formed similar to the reaction with adenine. A neutral radical, $2AP-N^2(-H)^*$ is proposed to be formed in the reaction of $SO_4^{2-}$ with 2AP at neutral pH similar to that of adenine. However, at higher pH (pH 10) the radical cation of 2AP reacts with OH$^-$ leading to the formation of $2AP-4-OH^*$ which on dehydration reaction gives rise to a nitrogen centered radical ($2AP-N(9)^*$). This is similar to the reaction of oxide radical anion with 2AP. In the reaction of 'OH it is observed that 2AP undergoes a similar reaction as that of adenine at pH 7, which leads to the formation of $2AP-4-OH^*$, $2AP-5-OH^*$ and $2AP-8-OH^*$. The spectrum at pH 7 undergo a time dependent change which is attributed to the OH$^-$ elimination from $2AP-4-OH^*$ and $2AP-5-OH^*$. However, the reaction pattern was very different compared to adenine at basic pH. At basic pH the initially formed OH-adducts undergo a dehydration reaction leading to the formation a transient species, which is similar to the one formed from the reaction with O$^-$. The similarity of the radical $2AP-N(9)^*$, formed from the reaction of 'OH at basic pH and of O$^-$ at pH \( \approx 14 \) is an interesting observation. The absorption spectrum of
the identified radical species of 2AP would probably help in interpreting
the fate of 2AP after its electron transfer reaction in the excited state with
guanine in the photo induced charge transport in DNA.\textsuperscript{18,20}

The preferential reduction of thymine by 2AP\textsuperscript{+} and the oxidation of
guanosine by 2AP\textsuperscript{++} clearly follow the redox potentials of the neutral bases.
The relative potentials of the nucleobases in DNA may follow a similar trend
as that of the reported potentials of the free bases (G = 1.29, A = 1.42,
C = 1.6 and T = 1.7 V versus NHE). In the much highlighted photoinduced
electron transfer between 2AP\textsuperscript{+} and guanine in DNA duplexes, the
intermediate product would be 2AP\textsuperscript{+} and G\textsuperscript{++}\.\textsuperscript{18} The present results give
clear experimental support to the hole transfer from the radical cation of
2AP to guanine. On the other hand, the fate of 2AP\textsuperscript{++} in the presence of
other DNA bases is not yet studied. The results presented in this study
clearly demonstrate the reduction of thymine by 2AP\textsuperscript{+}. It is therefore,
probable that during the photoinduced charge transport in 2AP
mismatched DNA, selective reduction of thymine can take place in addition
to the oxidation of the guanine moiety.
References