Chapter 3

Reactions of *OH and O*− with 5-azacytosine: A Theoretical study
Part A

Reactions of \(^{\bullet}\)OH with 5-azacytosine

DFT calculations at B3LYP/6-31+G(d,p) level have been carried out to study the reactions of \(^{\bullet}\)OH with 5-azacytosine (5AC). The \(^{\bullet}\)OH additions occur in direct manner, without the intervention of any precursor complex formation. The H-abstraction reactions were assumed to occur from precursor complexes (H-bonded complexes represented as S1, S2, S3, and S4) resulted from the electrostatic interactions of the lone pairs on the N3, N5, and O8 atoms of 5AC with the incoming \(^{\bullet}\)OH. It was found that, the conversion of these precursor complexes to their respective transition states has ample barrier heights, and it persists even when the effect of solvent is considered. It was also found that the formation of precursor complexes itself is highly endergonic in solution phase. Hence, the possibilities of H-abstraction reactions are ruled out. TDDFT calculations predicted a \(\lambda_{\text{max}}\) of 292 nm for the 5AC_N3OH*, which is close to the earlier pulse radiolytically observed spectral maxima at 290 nm. It is assumed that the addition to the most reactive center N3, which results the 5AC_N3OH*, occurs via a kinetically driven process.
Publications from this section


3.1 Introduction

5-azacytosine (5AC) is the 1,3,5-triazine analogue of the nucleic acid base cytosine (C). The structures of these two compounds along with their atomic numbering scheme are depicted in Figure 3.1. In contrast to C, there is no report for the natural origin of 5AC in normal DNA or RNA.

5-azacytidine (under the trade mark VIDAZA) was approved by the US Food and Drug Administration for the treatment of all subtypes of myelodysplastic syndromes¹. Myelodysplastic syndromes represent a group of diseases in which the bone marrow does not make enough healthy blood cells. 5-azacytidine is also used in the treatment acute myeloid leukemia (a cancer that starts inside bone marrow)².³. It is known that 5AC (when deliberately incorporated into DNA in the form 5-aza-2’-deoxycytidine) containing DNA can inhibit the activity of the bacterial DNA (cytosine-5)–methyltransferase enzyme, which catalyses the transfer of a methyl group from S-adenosylmethionine to the C5 position of cytosine⁴.⁵. Moreover, 5-azacytidine and 5-5-aza-2’-
Chapter 3

deoxyctydine were proved as effective inhibitors of HIV-1 replication in CEM cells\(^6\). Considering the therapeutic applications such as anti-tumor properties, the radiation chemistry of 5AC with water derived radicals is very important as tumor treatments involves both chemo and radio treatments.

An exhaustive experimental study of the free radical chemistry (especially with *OH) of nucleic acid components using the pulse radiolysis technique with optical absorption detection has been reported\(^7\). The results of the pulse radiolysis studies of *OH reactions with C is reported by different groups\(^8\text{-}^\text{10}\) and that of 5AC done by our group\(^\text{11}\) are compiled in Table 3.1.

**Table 3.1:** Pulse radiolysis experimental results of *OH reactions with cytosine and 5-azacytosine.

<table>
<thead>
<tr>
<th>Properties</th>
<th>*OH reaction at pH~6</th>
<th>pH~10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>5AC</td>
</tr>
<tr>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>335, 440</td>
<td>290</td>
</tr>
<tr>
<td>(k_2 \times 10^9) (M(^{-1})s(^{-1}))</td>
<td>6.1</td>
<td>5.1</td>
</tr>
<tr>
<td>% reducing</td>
<td>87</td>
<td>70</td>
</tr>
<tr>
<td>% oxidizing</td>
<td>10</td>
<td>27</td>
</tr>
</tbody>
</table>

nd = no data. *These are approximate values evaluated from the graphs in references and may have slight variations (±5) from actual experimental values

From the table it can be seen that, there are marked differences between *OH reactions of C and 5AC in terms of \(\lambda_{\text{max}}\) and percentage yield of oxidizing/reducing transients produced at pH 6 and 10. The
percentage yield denotes the yield of a particular transient produced with respect to the total yield of hydroxyl radicals produced in the pulse radiolysis experiment. While the $\lambda_{\text{max}}$ (290 nm) for $^\cdot$OH reaction with 5AC remains constant at pH 6 and 10; the values are 340 nm and 440 nm at pH 6 and 390 nm at pH 10 for C. Moreover, if we explicitly make a comparison of the yield of say oxidizing transients, the G(oxidizing) for C increases on going from pH 6 to 10. OH$^-$ catalyzed conversion of reducing transients to oxidizing transients was quoted as responsible for the higher yield of G(oxidizing) in C$^{12}$. Similar types of base catalyzed conversion of reducing transients to oxidizing transients were reported for other pyrimidine systems$^{13-16}$. However, there is no base catalyzed conversion of reducing intermediates to oxidizing intermediates is observed in the case of 5AC as compared to C or other pyrimidines. The oxidizing radicals in the case of 5AC are suggested as nitrogen centered while the non-oxidizing or reducing transient(s) as carbon centered$^{11}$. Generally $^\cdot$OH addition reactions with pyrimidine nucleic acid bases occur at or near diffusion controlled rates$^7$ with bimolecular rate constants $10^9$-$10^{10}$ M$^{-1}$ s$^{-1}$. It can be seen that, the $^\cdot$OH reaction with 5AC also follow the same trend in bimolecular rate constant with that of other nucleic acid bases. Pulse radiolysis studies have revealed that the reactions of $^\cdot$OH with C are quite selective with addition as the major reaction and postulated that additions mainly occur at the C5 and C6 atoms$^{10,13,14}$.

Obviously, it is difficult to obtain the structural information, energetics, spin localization etc. of the transients experimentally via the pulse radiolysis or laser photolysis experiments which could provide the quantitative details such as absorption maximum, kinetics, redox properties etc. of the transients. Thus, theoretical studies are helpful for the interpretation and structural identification of experimental results. In
line with these, there are many reports of the application of theoretical studies especially using DFT methods to study the energetics of the reaction of primary radicals from water radiolysis, the base pairing abilities, electron affinities and proton affinities etc. of nucleic acid components and their analogues.\textsuperscript{17-29} The addition reactions and H-abstraction reactions that \(^{1}\text{OH}\) can induce in cytosine have been studied by using B3LYP/6-31++G(d,p)//B3LYP/6-31G(d,p) DFT method by Ji, Y. J. et al.\textsuperscript{25} They have considered the \(^{1}\text{OH}\) additions to the N3, C4, C5 and C6 ring atoms and the H-abstractions from the N7 and C5 atoms. Their studies revealed that, \(^{1}\text{OH}\) addition to the C5 and C6 sites are thermodynamically and kinetically favorable over the additions at the N3 and C4 atoms. However the \(^{1}\text{OH}\) addition to the C5 and C6 sites constitute the most probable reactions than the H-abstraction reactions. However, our approach to study the addition and H-abstraction reactions of \(^{1}\text{OH}\) with 5AC is quite distinct from the methods used by Ji et al.\textsuperscript{25} At the initial phase of our study, we will make a comparison of the structural and electronic features of C with 5AC, but we are not interested in an exhaustive comparison at later stages.

3.2 Computational details

The density functional theory calculations were performed with Gaussian 03 suite of program\textsuperscript{30}. The Becke’s three parameter hybrid functional using the correlation functional of Lee, Yang and Parr (B3LYP)\textsuperscript{31,32} have been proven effective for determining the optimal geometries, harmonic frequencies, and electronic properties of nucleic acid bases\textsuperscript{33,34}, and it was selected for the current study for the same reason. The geometries were completely optimized without any symmetry constrains. Spin unrestricted calculations were performed for open shell systems. The 6-31+G(d,p) basis set is selected for all calculations, which includes the necessary diffuse and polarization functions for modeling.
radical systems. Harmonic vibrational frequencies were estimated at this level to classify the stationary points as local minima (all real frequencies) or saddle points (one imaginary frequency). The BSSE corrections were done by using the counterpoise method\textsuperscript{35,36}. The transition state geometries were obtained using the QST3 search method\textsuperscript{37,38}. The gas phase geometries were reoptimized including the effect of aqueous environment using PCM\textsuperscript{39}. Excited-state energies were calculated using the TDDFT method\textsuperscript{40} with the same basis set.

We know that, purine and pyrimidine bases exist in several different tautomeric forms which differ from each other mainly in the position of one of the hydrogens which may be bound to the exocyclic nitrogen or oxygen atom or one of the ring nitrogen atoms. Numerous computational studies have discussed the tautomerism of the cytosine molecule which has provided a reliable picture of the relative stability of its tautomers, both in the gas phase and in solution\textsuperscript{41-45}. It appears that the canonical form (i.e. the amino-oxo form, schematically shown in Figure 3.1) is the local minimum at almost all theoretical levels. Similarly in the case of 5AC several different tautomeric forms can be considered\textsuperscript{46}, however the most stable one is the amino-oxo form (schematically shown in Figure 3.1) as in the case of C. So in modeling the experimental results we have considered only the canonical form.

3.3 Results and discussion

Geometry optimization

The gas phase optimized geometry of 5AC at B3LYP/6-31+G(d,p) level of theory is depicted in Figure 3.2 together with selected bond lengths. It is sensible to have a comparison between the structural features of 5AC with C, so that we have determined the
The dihedral angle \( \tau(\text{H11-N7-C4-N5}) \) is 180.0° for 5AC suggesting a planar geometry, whereas the corresponding H11-N7-C4-C5 dihedral angle for C is 174.7°, showing a slight pyramidalization of the amino group. We can see that, the shortest bond found in the ring for 5AC is between N5 and C6 (1.300 Å) which is shorter than the C5C6 (1.361 Å) bond length of C. Obviously, the N3C4 bond of length 1.322 Å and 1.321 Å respectively for 5AC and C are also largely localized as a double bond. The bond between the amino nitrogen N7 and ring C4 is significantly shorter than a typical CN single bond (1.472-1.479 Å) for both systems, which may be attributed to the strong resonance interaction between the nitrogen lone pair and the ring \( \pi \)-system. However the C4N7 bond in 5AC of length 1.349 Å is 0.012 Å shorter than that of C. It implies that 5AC has stronger resonance interaction between the nitrogen lone pair and the ring \( \pi \)-system when compared to C which is evident from the more planar character of the amino group in 5AC. The above cited structural features of 5AC and C was found to be
Reactions of *OH and O" with 5-azacytosine

in reasonable agreement with the results of MP2/6-31G(d,p) level study on these systems reported by Podolyan et al. The N3C4 and C5C6 ring bonds are more prone to the attack of *OH in the case of C. Similarly, as more double bond character is found in N3C4 and N5C6 bonds of 5AC, the constituent atoms of these bonds are expected to be more reactive than the rest for electrophilic addition reactions with *OH.

**Molecular electrostatic potential**

It is better to have a clear idea about the electron rich centers in the ring for electrophilic addition reactions of hydroxyl radical. With this notion in mind we have analyzed the molecular electrostatic potentials (MESP) of both 5AC and C at B3LYP/6-31+G (d, p) level of theory. The MESP plotted on the van der Waals’ surface is given in Figure 3.3 (part (a) and part (b) for 5AC and C respectively) suggests quite similar electron distribution at the π-face of both the systems. The π-faces of both the systems are more electron rich (blue in color) around the region showing maximum double bond character and this feature is more dominant in the 5AC molecule (red regions have more single bond character). A more quantitative picture regarding the lone pair strength of N and O atoms is obtained by locating the most negative valued MESP point ($V_{\text{min}}$) in these systems. In the case of 5AC, the $V_{\text{min}}$ points are located near the O8, N3, and N5 atoms (Figure 3.3(c)) while in the case of C these values are located near O8, and N3 atoms (Figure 3.3(d)).

**Molecular orbital scheme**

We have also calculated the HOMOs of 5AC and C to get further insight to the electron rich centers for an electrophilic addition reaction of OH radical with these systems. An inspection of the HOMO shown in Figure 3.4 (part (a)) of 5AC, suggests that the principal site for an
electrophilic addition of *OH is N3 atom of the N3C4 double bond as it shows the highest orbital coefficient towards the HOMO.

**Figure 3.3:** The MESP plots of 5-azacytosine and cytosine. (a) and (b) are the MESP plotted on the van der Waal’s surface, (c) and (d) $V_{\text{min}}$ points located near the O8, N3, and N5 atoms, an isosurface of value -35.75 kcal/mol is also depicted.

**Figure 3.4:** HOMO of (a) 5-azacytosine and (b) cytosine. Isocontour value = 22 kcal/mol
Reactions of \*OH and O\* with 5-azacytosine

The HOMO of C is also depicted in Figure 3.4 (part (b)), wherein the C5 atom of the C5C6 double bond has the largest MO coefficient. In fact, the C is known for its addition reaction with \*OH at the C5 atom giving rise to the C6-centerd radical (with 87\% quantitative yield)\(^7\). As we have a detailed knowledge about the structural properties and electron rich centers of 5AC in hand we could able to analyze the various reaction possibilities that \*OH may induce on 5AC. In the present case, we have considered two reaction possibilities viz \*OH and H-abstractions synchronously for 5AC. The structural features of 5AC favor N3, C4, N5, and C6 as the addition sites. There are four abstractable hydrogen atoms viz. the H9 attached to the N1 atom, the H10 attached to the C6 atom, and the H11 and H12 attached to N7.

**The \*OH - addition reactions**

Initially we have considered the possibility of the formation of \(\pi\)-complex for adduct formation reactions. Two situations were considered, one in which the hydrogen of \*OH pointed towards the \(\pi\)-face, and the other in which the oxygen of \*OH is pointed towards the \(\pi\)-face. In both cases, the \*OH radical moved out from the \(\pi\)-face to the molecular plane and eventually led to the formation of hydrogen bonded (H-bonded) complex S1 as depicted in Figure 3.8 (see H-abstraction reactions section). As we have realized that, the formation of a \(\pi\)-complex is an unlikely process, the adduct formation reactions are expected to be spontaneous processes leading to the ‘direct’ addition of \*OH to 5AC. We have perceived earlier in the HOMO picture of 5AC that (Figure 3.4.a) the most dominant site for an electrophilic addition of \*OH radical is the N3 atom. Apparently, N3 atom is expected to be the most reactive, we have also considered the possibility of the addition of \*OH with the constituent atoms of the double bonds, viz. N3, C4, N5, and C6. The additions of \*OH at N3, C4, N5, and C6 yields the adduct
systems $5\text{AC}_\text{N3OH}^\bullet$, $5\text{AC}_\text{C4OH}^\bullet$, $5\text{AC}_\text{N5OH}^\bullet$ and $5\text{AC}_\text{C6OH}^\bullet$. The optimized geometries of these adducts are depicted in Figure 3.5 along with their relative energy values (with respect to the sum of the energies of the reactants viz. $5\text{AC}$ and *OH).

![Figure 3.5: The optimized structures of possible *OH adducts of 5-azacytosine. The relative energy values in kcal/mol are given in parenthesis. All bond lengths are in Å units.](image)

For $5\text{AC}_\text{N3OH}^\bullet$ and $5\text{AC}_\text{N5OH}^\bullet$ adducts the Mulliken spin population was found to maximum on C6 atom. The spin values found on C6 was 0.70 a.u. and 0.78 a.u. respectively for $5\text{AC}_\text{N3OH}^\bullet$ and $5\text{AC}_\text{N5OH}^\bullet$ adduct systems. The high spin value on C6 for $5\text{AC}_\text{N3OH}^\bullet$ implies more delocalization of the unpaired electron in this
Reactions of *OH and O" with 5-azacytosine

radical. However for the 5AC_C4OH* adduct, the spin density value is maximum on the N3 (0.74 a.u.) and for the 5AC_C6OH*, the spin maximums was found on the N3 (0.45 a.u.) and N5 (0.63 a.u.) atoms.

The stability order of adduct systems are, 5AC_C6OH* > 5AC_C4OH* > 5AC_N3OH* > 5AC_N5OH* based on the relative energy values. The relative enthalpies as well as the relative free energy values of addition reactions are shown in Figure 3.6, the pattern show the same trend as that of relative energy values. The formations of the 5AC_N3OH* and the 5AC_N5OH* adducts are 9.24 and 18.65 kcal/mol endothermic, whereas the formations of the 5AC_C4OH* and the 5AC_C6OH* adducts are exothermic by 1.13 and 13.01 kcal/mol respectively. Therefore, the observed N-centered radical system in the

Figure 3.6: The relative enthalpy change (ΔH) and free energy change (ΔG) for the *OH addition reactions
experiment can be considered as 5AC_C6OH* while the C-centered radical system in the experiment may be 5AC_N3OH*.

Nevertheless, it may be noted that the amount of C-centered radical (73 %) formed in the experiment was much higher than the amount of N-centered radical (27 %), which cannot be explained by the thermodynamic stability of adducts alone. Since the reactivity of the HOMO is in favor of the addition of *OH at the N3 atom leading to the formation of the C-centered radical 5AC_N3OH*, we can assume that a higher yield of this product observed in the experiment is due to the kinetic control of the reaction.

It may be noted that the calculated stability order of the different adduct systems of cytosine obtained by Ji et.al25 follows the order C_C6OH* > C_C5OH* > C_C4OH* > C_N3OH*. Their results suggested that, the formation of the experimentally observed product C_C5OH* was mainly derived via kinetically controlled process. Similarly the theoretical study on the *OH reaction with thymine by Wu et.al51 also pointed out the kinetic control of *OH addition at C5 over the thermodynamic factors. In their studies, the free energy changes (ΔG) associated with the addition of *OH to the C5 and C6 atoms of thymine as -62.6 kJ mol⁻¹ and -85.5 kJ mol⁻¹ whereas for the H-abstraction from the methyl group the ΔG value was found to be more exergonic with -117.2 kJ mol⁻¹. However, experimentally the H-abstraction reaction occurs only about 10% of the total *OH reaction with thymine13.

The 1,2-Hydrogen shift of initial *OH adduct

We have seen that the formation of a reducing radical (C-centered) is derived in a kinetically driven process, an alternate possibility for the formation of a C-centered radical is considered by the 1,2-hydrogen migration from C6 to N5 of the initially formed
Reactions of *OH and O• with 5-azacytosine

5AC_C6OH* adduct (an N-centered radical) leading to the isomeric 5AC_C6OH_N5H* (a C-centered radical). This possibility is considered because the energetics of addition reactions discussed in the previous section suggested a high stability for 5AC_C6OH* adduct. The hydrogen transfer can be envisaged to occur from C6 to N5 via the transition state TS1. Selected geometrical features of the optimized structures are shown in Figure 3.7.

![Diagram showing transition state TS1](image)

**Figure 3.7:** Schematic representation of the 1,2-hydrogen migration from C6 to N5 in 5AC_C6OH*. Bond lengths are in Å.

In TS1, the labile hydrogen atom is acting as a bridge between N5 and C6 atoms. The calculated activation energy for this transformation at B3LYP/6-31+G(d,p) level of theory is 32.0 kcal/mol and the product system (5AC_C6OH_N5H*) showed 8.06 kcal/mol higher stability than the reactant (5AC_C6OH*). Although the reaction is exothermic, the activation barrier is considerably above the reaction enthalpies of a feasible reaction, and it seems safe to assume that such a process may not occur at the conditions used in the experiment. We have also computed the activation energy at the MP2/6-31+G(d,p) level of theory and the result turned out to 31.6 kcal/mol. Thus, the results calculated at the B3LYP level are in good consistency with the results of the ab initio MP2 calculations.
H-abstraction reactions

While considering the possibility of π-complex formation between *OH and 5AC for hydroxyl addition reactions we have seen that the *OH move out of the π phase and forms a hydrogen bonded complex (S1). In this complex (see Figure 3.8), the hydrogen of *OH form an H-bond with the carbonyl oxygen at a distance of 1.781 Å, while oxygen of *OH is in H- bond with H9 at a distance of 2.071 Å.

Figure 3.8: The optimized geometries of the H-bonded complexes for H-abstractions. BSSE corrected interaction energies (in kcal/mol) are shown in parenthesis.

As the H-bonded complex formation is mainly aroused from the electrostatic interaction of *OH and the lone pair on the carbonyl
oxygen, the interactions of the lone pairs on the N3, and N5 atoms (see the MESP plot in Figure 3.3c) with the hydrogen of *OH radical can also lead to the formation of H-bond complexes S2, S3 and S4. The optimized geometries of these systems are shown in Figure 3.8. The BSSE corrected interaction energies (i.e. binding energy of *OH) of the H-bonded complexes are also shown in Figure 3.8. The strength of the interaction is in the order S3 > S4 > S1 > S2.

**Figure 3.9:** Transition states corresponding to the H-abstractions. H-bond lengths are in Å units.

The H-bonded complexes are converted into the product systems (product system means, the H-abstracted radical and a water molecule) represented as P1+H$_2$O, P2+H$_2$O, P3+H$_2$O, and P4+H$_2$O respectively
resulted from the abstractions of H9, H10, H11 and H12 hydrogens through the transition states represented as TS2, TS3, TS4, and TS5. All the transition states are characterized by one imaginary frequency in their vibrational frequency analysis. The optimized structures of the transition states are presented in Figure 3.9 with selected geometrical parameters. In all the TSs, the abstractable hydrogen atom is located closer to *OH.

![Figure 3.10: Optimized geometries of H-abstracted radicals. Bond lengths are in Å units.](image)

The structures of the H-abstracted radicals P1, P2, P3, and P4 are presented in Figure 3.10. The spin density values of these radical systems are observed mainly on two atoms, viz. (O8 = 0.57, N5 = 0.32)
Reactions of 'OH and O\(^-\) with 5-azacytosine

in P1, (C6 = 0.74, N5 = 0.11) in P2, (N7 = 0.76, N3 = 0.39) in P3, and (N7 = 0.76, N3 = 0.36) in P4. It may be noted that except P1, all the systems showed the maximum spin density on the atom from which the hydrogen has been abstracted. In the case of P1, the spin density distribution suggests more delocalization of the odd electron as compared to others.

The relative enthalpy and free energy changes of reactions (in kcal/mol) [5AC + 'OH] \(\rightarrow\) S1 \(\rightarrow\) TS2 \(\rightarrow\) [P1 + H\(_2\)O], [5AC + 'OH] \(\rightarrow\) S2 \(\rightarrow\) TS3 \(\rightarrow\) [P2 + H\(_2\)O], [5AC + 'OH] \(\rightarrow\) S3 \(\rightarrow\) TS4 \(\rightarrow\) [P3 + H\(_2\)O], and [5AC + 'OH] \(\rightarrow\) S4 \(\rightarrow\) TS5 \(\rightarrow\) [P4 + H\(_2\)O] are depicted in Figure 3.11 and 3.12, respectively. It can be seen from the enthalpy profile that, all the H-bonded complexes are more stable than the separated systems [5AC + 'OH]. Among these complexes, S3 showed the highest stability of 8.24 kcal/mol while the S2 showed the least stability of 3.11 kcal/mol. The abstraction from S2 led to the most stable product system [P2 + H\(_2\)O]. The H-abstraction from S1 and S2 were nearly barrier-less.

Figure 3.11: The relative enthalpy profile of H-abstractions

89
processes. In the case of S3 and S4, the hydrogen abstraction reaction required activation barrier of 9.71 and 8.62 kcal/mol respectively.

As can be seen from the relative free energy plot that, the formation of the H-bonded complexes are endergonic processes, as the entropy of the system decreases on going from separated systems to the associated systems. However, the formations of the product systems are exergonic processes as the entropy for these processes are positive. The activation free energy barriers for the formation of the product molecules are 1.99, 0.92, 11.44 and 5.48 kcal/mol respectively for the abstractions of H9, H10, H11, and H12 atoms. When the effect of solvation is taken into account using the PCM model, it was observed that the solvation considerably influences the relative free energies of the H-bonded complexes and the product molecules as it is shown in Figure 3.13. The total free energy values were calculated by adding the ΔG solvation correction to the gas phase relative free energy values. It

![Relative Energy Profile](image-url)
can be seen that, free energies of the H-bonded complexes increases on comparing with the gas phase data. The stability of the complexes S1, S2, S3, and S4 decreases by 15.08, 4.32, 8.79 and 9.71 kcal/mol respectively in the presence of solvent. All these reactions show activation free energy barrier in the range of 3-12 kcal/mol, which is within the acceptable limit of a facile reaction. However, as the formation H-bonded complexes S1, S2, S3, and S4 are highly endergonic processes in the solution phase, we can assume that formation of the H-bonded complexes is unlikely to occur in the solution phase and hence the hydrogen abstraction reactions may not occur.

Figure 3.13: The relative total free energy change for the H-abstractions in solution phase.

**TDDFT calculations**

From the previous sections we have seen that, the $^*\text{OH}$ addition reactions are more feasible than the H-abstraction reactions. The
assignments of precise adduct species responsible for the experimental spectrum can be deduced from the TDDFT calculations. The $\lambda_{\text{max}}$ along with their oscillator strengths ($f$) calculated for adducts in solution phase are presented in Table 3.2.

**Table 3.2:** The $\lambda_{\text{max}}$ and corresponding oscillator strengths of the $^\cdot$OH adducts of cytosine and 5-azacytosine

<table>
<thead>
<tr>
<th>Adduct</th>
<th>Cytosine</th>
<th>5-azacytosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>$f$</td>
</tr>
<tr>
<td>N3OH$^\cdot$</td>
<td>272</td>
<td>0.095</td>
</tr>
<tr>
<td>C4OH$^\cdot$</td>
<td>463</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>227</td>
<td>0.083</td>
</tr>
<tr>
<td>C5(N5)OH$^\cdot$</td>
<td>339</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>426</td>
<td>0.041</td>
</tr>
<tr>
<td>C6OH$^\cdot$</td>
<td>336</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>476</td>
<td>0.019</td>
</tr>
</tbody>
</table>

The calculated $\lambda_{\text{max}}$ for the C-centered radical species 5AC_N3OH$^\cdot$ (310 nm) is found to be very close to the experimental maximum of 290 nm. On the other hand, the higher intensity transition computed for other probable transient systems such as 5AC_N5OH$^\cdot$ (350 nm), 5AC_C6OH$^\cdot$ (440 nm) and 5AC_C4OH$^\cdot$ (460 nm) doesn’t give a value close to experimental maximum. The TDDFT calculations point out that, the intense transition in the experimental spectrum is solely as a result of C-centered adduct namely 5AC_N3OH$^\cdot$. We have also determined the $\lambda_{\text{max}}$ values of possible transients in the reaction of $^\cdot$OH with C and the values are listed in Table 3.2. The transient spectrum obtained from the pulse radiolysis studies on the reactions of C with $^\cdot$OH was characterized by $\lambda_{\text{max}}$ at 335 nm and 440 nm$^8$, from our
calculations it can be seen that, the C_C5OH* adduct reproduces the experimental values. Nevertheless, the C_C6OH* may also contribute to the absorption at 340 nm.

3.4 Conclusions

The present theoretical studies established that the formations of C-centered radicals in the pulse experiments were due to the addition of \(^{\cdot}\text{OH}\) to the N3 atom of 5AC. The formation of 5AC_N3OH* radical is due to kinetic control of the \(^{\cdot}\text{OH}\) reaction similar to the addition of \(^{\cdot}\text{OH}\) to C5 in cytosine. Also the addition reactions are more feasible compared to the H-abstraction reactions. The thermodynamically most stable addition product in the present case is 5AC_C6OH* radical while P2 is the stable H-abstracted radical. The addition reactions are assumed to occur in a direct fashion without the involvement of any pre-complex formation. Subsequently, the possibility of hydrogen abstraction reaction of \(^{\cdot}\text{OH}\) is ruled out as the formations of pre-reactant complexes are highly endergonic in solution phase. The TDDFT calculations suggest that the experimental spectrum is due to kinetically driven transient species viz. 5AC_N3OH*. Similarly, the assignment of the dominant radical (C_C5OH*) in the experimental spectrum of C is further supported by TDDFT calculations. Thus, the theoretical calculations fully substantiate earlier pulse radiolysis experiments.
Part B
Reactions of $\text{O}^\cdot$ with 5-azacytosine

The reactions of $\text{O}^\cdot$ with 5AC in aqueous medium were studied by using B3LYP/6-31+G(d,p) level DFT calculations. The mechanism is evoked by studying the energetics of possible reaction channels, pKa determinations of transients and the prediction of $\lambda_{\text{max}}$ values of intermediates. It is demonstrated that the major reaction pathway is the addition of $\text{O}^\cdot$ to the ring nitrogen of 5AC, followed by fast protonation of adducts by water molecules. Direct electron transfer followed by deprotonation constitutes the minor reaction pathway. The similarity in the reaction mechanism of $\text{O}^\cdot$ (addition to 5AC) and $^\cdot\text{OH}$ (which generally participates in addition reaction) is an interesting observation and is quite unusual in the case of heterocyclic compounds.
Publications from this section


Reactions of *OH and O" with 5-azacytosine

3.5 Introduction

In strongly alkaline solutions the hydroxyl radical (pKa (*OH) = 11.9) produced during the radiolysis of water gets rapidly converted to its conjugate base namely the oxide radical ion (O")\textsuperscript{52,53}. O" is an oxidant with \(E^\circ(O\textsuperscript{−}, H^+/\textsuperscript{•}OH)) = 1.77\text{ V}^{54,55}. The modes of reactions of O" are quite different compared to *OH; it has fewer tendencies to undergo addition reactions with double bonds and to aromatic rings (2–3 orders of magnitude less than *OH), but its H-abstraction ability is comparable to that of *OH. It combines with molecular oxygen\textsuperscript{7} at a rate of \(3.6 \times 10^9\text{ M}^{-1}\text{ s}^{-1}\).

With pyrimidine bases of nucleic acids, an addition of O" to cytosine (C) and uracil (U) while H-abstraction from the methyl group of thymine (T) has been reported in a much earlier pulse radiolysis/EPR studies\textsuperscript{9,52,56}. Later, Ioele, M. et.al\textsuperscript{57} has extensively studied the reactions of O" with nucleic acid bases in aqueous solutions at pH 13.7. Their studies demonstrated that, O" causes one electron oxidation in the case of U (it is the di-anionic form of uracil that react with O") leads to the formation of uracil radical anion, whereas the transients observed with T is assigned as carbon-centered radical derived by H-abstraction from the methyl group. In the case of C (cf. Figure 3.1), they proposed that, O" undergoes a hydrogen abstraction (abstraction of one hydrogen from the amino group) giving rise to a radical anion, which could also be produced pulse radiolytically from Br\textsuperscript{−} reactions at similar pHs. The bimolecular rate constants for O" and Br\textsuperscript{−} reactions with C were cited as \(7.8 \times 10^8\) and \(1.6 \times 10^8\text{ M}^{-1}\text{ s}^{-1}\) respectively. However, in contrast to C, the pulse radiolysis studies\textsuperscript{11} on the reactions of O" with 5AC have some interesting observations (concisely, similarity between the *OH and O" reactions) and the results are presented in Table 3.3. For comparisons we have included in Table 3.3 the spectral, kinetic, and redox properties of the transients derived from the *OH reactions of C\textsuperscript{8-10}, T\textsuperscript{13,14}, 6-methyl
Chapter 3

Table 3.3: Absorption maxima, rate constant and yield of oxidizing/reducing transients obtained for the reaction of \(^\cdot\)OH and O\(^-\) with cytosine, 5-azacytosine, thymine, 6-methyluracil and 4,6-dihydroxy-2-methyl pyrimidine.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>5AC</th>
<th>T</th>
<th>6MU</th>
<th>DHMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>345</td>
<td>290</td>
<td>&lt;300</td>
<td>410</td>
<td>420</td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>350(^\dagger)</td>
<td>380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k_2 (10^9 \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}) )</td>
<td>6.1</td>
<td>5.1</td>
<td>6.4</td>
<td>9.0</td>
<td>5.6</td>
</tr>
<tr>
<td>% reducing</td>
<td>87</td>
<td>70</td>
<td>60</td>
<td>97</td>
<td>92</td>
</tr>
<tr>
<td>% oxidizing</td>
<td>10</td>
<td>27</td>
<td>30</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>5AC</th>
<th>T</th>
<th>6MU</th>
<th>DHMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>400</td>
<td>280</td>
<td>&lt;325</td>
<td>425</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>700</td>
<td>350(^\dagger)</td>
<td>525</td>
<td>370</td>
<td></td>
</tr>
<tr>
<td>( k_2 (10^9 \text{ dm}^3\text{mol}^{-1}\text{s}^{-1}) )</td>
<td>0.78</td>
<td>1.9</td>
<td>0.63</td>
<td>0.36</td>
<td>0.24</td>
</tr>
<tr>
<td>% reducing</td>
<td>~13(^*)</td>
<td>92</td>
<td>nd</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>% oxidizing</td>
<td>87</td>
<td>8</td>
<td>nd</td>
<td>68</td>
<td>73</td>
</tr>
</tbody>
</table>

\(^\dagger\)Minor peak, nd = no data. \(^*\) These are approximate values evaluated from the graphs in references and may have slight variations (±5) from actual experimental results.
Reactions of \( ^{\cdot} \text{OH} \) and \( ^{-} \text{O} \) with 5-azacytosine

It can be seen from Table 3.3 that the \( \lambda_{\text{max}} \) of the intermediate radicals resulting from the reactions of \( ^{\cdot} \text{OH} \) with C, T, 6MU, and DHMP are quite different compared to their corresponding \( ^{\cdot} \text{OH} \) reactions. Interestingly, in the present case with 5AC, the absorption maxima resulting from both \( ^{\cdot} \text{OH} \) and \( ^{-} \text{O} \) radicals are almost comparable. Generally, the rate constants for the reaction of \( ^{-} \text{O} \) with organic molecules are lower by an order of magnitude compared with that of \( ^{\cdot} \text{OH} \) reactions. The reaction of \( ^{\cdot} \text{OH} \) is interpreted as an addition at the carbon positions of the pyrimidines ring, which has rate constant of the order of \( 10^{9} \text{ M}^{-1} \text{ s}^{-1} \). On the other hand, the hydrogen abstraction reaction of \( ^{-} \text{O} \) with these compounds has a rate constant of the order of \( 10^{8} \text{ M}^{-1} \text{ s}^{-1} \). However, in the present case, the rate constants of the reactions of both \( ^{\cdot} \text{OH} \) and \( ^{-} \text{O} \) with 5AC are on the order of \( 10^{9} \text{ M}^{-1} \text{ s}^{-1} \). Thus, we can note that, except 5AC, all other systems follow the general trend in \( ^{\cdot} \text{OH} \) and \( ^{-} \text{O} \) kinetics. Such resemblances of both kinetic and spectral data have led to the assumption that \( ^{-} \text{O} \) undergoes an addition reaction with 5AC.

In order to explain the unusual pattern of reaction of \( ^{-} \text{O} \) with 5AC we have performed the theoretical calculations. As the pKa value of 5AC determined spectrophotometrically was 11.9, in the subsequent sections we will consider the anionic form of 5AC (i.e. 5AC\(^{-}\), cf. Figure 3.16) for modeling the \( ^{-} \text{O} \) reactions. The calculation includes, (a) locating the electrophilic centers in target molecule (b) spin densities as obtained from Mulliken atomic spin densities to locate the radical centers, (c) determination of the pKa values of the transients (pKa determination of transients under consideration is a difficult task experimentally - desirable conditions are experimentally inaccessible) and (d) the related vertical optical absorptions of the transients.
3.6 Computational details

All the electronic structure calculations in this section were performed with the Gaussian 03 program\(^{30}\). The Becke’s three parameter hybrid functional with the correlation functional of Lee, Yang and Parr (B3LYP)\(^{31,32}\) density functional theory method has been used for geometry optimizations along with the 6-31+G(d,p) basis set. The minimum energy geometries were determined by the absence of imaginary frequencies in the vibrational frequency calculation. The PCM\(^{39}\) solvation models have been utilized for the solution phase geometry optimizations starting with the gas phase optimized structures.

The pKa value of 5AC and some of its important radical systems derived from the \(\text{O}^-\) reaction have been determined theoretically. The theoretical determination of pKa values are based on the thermodynamic cycle shown in Figure 3.14 (wherein, the subscripts “g” and “aq” represent gas and aqueous phases respectively for the dissociation of an acid AH (AH → A\(^-\) + H\(^+\)) in water. Solvation free energies are denoted with the “sol” subscript).

\[
\begin{align*}
\Delta G_{\text{sol}}(\text{AH}) &\quad \Delta G_{\text{sol}}(\text{A}^-) &\quad \Delta G_{\text{sol}}(\text{H}^+) \\
\text{AH}_{\text{(g)}} &\quad \text{A}^-_{\text{(g)}} &\quad \text{H}^+_{\text{(g)}}
\end{align*}
\]

\[
\begin{align*}
\text{AH}_{\text{(aq)}} &\quad \Delta G_{\text{aq}} &\quad \Delta G_{\text{aq}}(\text{A}^-) &\quad \Delta G_{\text{aq}}(\text{H}^+) \\
\text{AH}_{\text{(aq)}} &\quad \text{A}^-_{\text{(aq)}} &\quad \text{H}^+_{\text{(aq)}}
\end{align*}
\]

Figure 3.14: Schematic of the thermodynamic cycle considered for pKa calculation.

The pKa value at 298.15K (25°C) can be obtained from the value of \(\Delta G_{\text{(aq)}}\) as,

\[
pKa = \frac{\Delta G_{\text{(aq)}}}{2.303RT} \tag{3. 1}
\]
where R is the gas constant (1.98 cal K\(^{-1}\) mol\(^{-1}\))

The \(\Delta G_{(aq)}\) value can be calculated from the thermodynamic cycle in terms of the gas phase and solvation free energy terms,

\[
\Delta G_{(aq)} = \Delta G_{(g)} + \Delta \Delta G_{(sol)} \quad (3.2)
\]

where the values of \(\Delta G_{(g)}\) and \(\Delta \Delta G_{(sol)}\) are respectively,

\[
\Delta G_{(g)} = [G_{(g)}(A^-) + G_{(g)}(H^+) - G_{(g)}(AH)] \quad (3.3)
\]

\[
\Delta \Delta G_{(sol)} = [\Delta G_{(sol)}(A^-) + \Delta G_{(sol)}(H^+) - \Delta G_{(sol)}(AH)] \quad (3.4)
\]

Since the calculation of \(\Delta G_{(g)}\) uses a reference state of 1 atm (24.46 L at 298.15 K) and the calculation of \(\Delta G_{(sol)}\) uses a reference state of 1 M, we need to convert the \(\Delta G_{(g)}\) (1 atm) \([\Delta G_{(g)}\) (1 atm) synonymous to \(\Delta G_{(g)}\)]

\[
\Delta G_{(g)}(1 \text{ M}) = \Delta G_{(g)}(1 \text{ atm}) + RT \ln[22.46] = \Delta G_{(g)}(1 \text{ atm}) + 1.89 \quad (3.5)
\]

We have used the gas phase free energy and solvation free energy of proton, \(G_{(g)}(H^+) = -6.28\) kcal/mol and \(\Delta G_{(sol)}(H^+) = -264.61\) kcal/mol, as used by Liptak et. al.\(^{59}\). Then,

\[
\Delta G_{(aq)} = \Delta G_{(g)}(1 \text{ M}) + 1.89 + \Delta \Delta G_{(sol)}
\]

\[
= [G_{(g)}(A^-) + G_{(g)}(H^+) - G_{(g)}(AH) \quad +1.89] + [\Delta G_{(sol)}(A^-) + \Delta \Delta G_{(sol)}(H^+) - \Delta G_{(sol)}(AH)]
\]

\[
= [G_{(g)}(A^-) - (6.28) - G_{(g)}(AH) \quad + (1.89) + \Delta G_{(sol)}(A^-) - (264.61) - \Delta G_{(sol)}(AH)]
\]

\[
= [G_{(g)}(A^-) - G_{(g)}(AH) + \Delta G_{(sol)}(A^-) - \Delta G_{(sol)}(AH) - (269)]
\]

Therefore,

\[
pK\alpha = [G_{(g)}(A^-) - G_{(g)}(AH) + \Delta G_{(sol)}(A^-) - \Delta G_{(sol)}(AH) - 269]/1.3644 \quad (3.6)
\]
The theoretical strategy that we have adopted for obtaining the gas phase and aqueous phase free energies are as follows. Since the pKa determination requires more accurate energy values, we have used B3LYP calculations with the more extended basis set AUG-cc-pVDZ starting with B3LYP/6-31+G(d,p) level optimized geometries. The solvation free energies then calculated by performing single point calculations at the Hartree-Fock (HF) level using 6-31G(d) basis set for the protonated systems and HF/6-31+G(d) level for the deprotonated systems, as those considered by da Silva et al. The “scfvac” keyword has been used with the solvent phase calculations; so that the free-energy of solvation (ΔG(sol)) can be directly read from the Gaussian output files and have employed the “bondi” atomic radii for constructing the solvent cavity.

3.7 Results and discussion

One electron oxidation/H-abstraction

Initially, we have considered the one electron oxidation/H-abstractions of 5AC−, since O− mainly causes one electron oxidation or H-abstraction reactions on its reactions with heterocyclic systems. The one electron oxidation induced by O− can lead to the formation of radical 1 (see Figure 3.15). The radical 1 is unambiguously oxidizing in nature as the odd electron spin should be on the O8 or one of the ring nitrogen. The Mulliken spin densities are found to be maximum on O8 (0.57 a.u.) and N5 (0.32 a.u.) atoms for the radical 1, obtained at B3LYP/6-31+G(d,p) level of theory. The direct H-abstraction reactions similar to that reported in the case of cytosine from the amino group (abstraction of either H11 or H12) leads to the formation of radical anions represented as 2 and 3 in Figure 3.15.
For the sake of explanations we have considered the independent existence of species 2 and 3, however, the barrier for rotation of 2 to 3 is only 1.1 kcal mol\(^{-1}\) calculated at B3LYP/6-31+G(d,p) in vacuum. Also it is not possible to have a time resolved look at the two species separately under the experimental conditions. The spin densities are found to be maximum on two atoms (N7 = 0.77 a.u. and N3 = 0.30 a.u.) for the radical anion 2, and (N7 = 0.78 a.u. and N3 = 0.26 a.u.) for radical anion 3. The radical anions 2 and 3 can also be derived obliquely from radical 1 by deprotonation reactions. This possibility is in fact supported by the pKa value calculations. To testify the acceptability of our pKa calculation procedure, we have first determined the pKa value of 5AC, theoretical value is 11.9, match well with the experimental
value of 11.9. In order to check whether the current procedure is applicable to radical systems, we have considered the protonation–deprotonation of uracil radical system (UH$^\cdot$ ↔ U$^\cdot$– + H$^+$) whose pulse radiolytically determined pKa value is 7.3$^{63}$ and our theoretical calculations predicted a value of 6.7. The theoretically calculated pKa values of the H11 and H12 protons of radical 1 are respectively -1.3 and -2.1. The very low pKa values imply that the instantaneous deprotonation might hide the presence of 1 during the experimental data acquisition.

We have compared the reaction possibilities by taking the energetics into account (using the equations 3.7 and 3.8 below) for the one electron transfer (leads to radical 1) and direct H-abstraction reactions (leads to radical anions 2 and 3).

\[
\begin{align*}
5\text{AC}^- + \text{O}^\cdot & \rightarrow \text{1} + \text{O}^{2-} & (3.7) \\
5\text{AC}^- + \text{O}^\cdot & \rightarrow \text{2} + \text{OH}^- & (3.8a) \\
5\text{AC}^- + \text{O}^\cdot & \rightarrow \text{3} + \text{OH}^- & (3.8b)
\end{align*}
\]

The free energy changes associated with reaction 3.7 is 251.70 kcal/mol, whereas the corresponding values for reactions 3.8a and 3.8b respectively are -9.57 kcal/mol and -10.82 kcal/mol calculated at B3LYP/6-31+G(d,p) level in vacuum. So the direct H-abstraction reactions are exergonic with almost equal probability for H11 and H12 abstraction, while the electron transfer reaction is highly endergonic. However when the effect of solvation is considered, it was found that the $\Delta\Delta G_{\text{sol}}$ greatly favorable for the direct one electron reaction, the solvation of the O$^{2-}$ anion constituted a considerable driving force. The $\Delta\Delta G_{\text{sol}}$ values associated with reaction 3.7 is -193.0 kcal/mol and for reactions 3.8a and 3.8b are respectively 2.36 kcal/mol and 3.25 kcal/mol. However, as the theoretically calculated pKa values of the H1
and H2 protons of radical 1 are respectively -1.3 and -2.1, the radical 1 get converted into 2 or 3 by deprotonation immediately after its formation.

The above theoretical calculations predicted (of course support the experiment) the possibility for the formation of oxidizing radical, the formation of non-oxidizing radical (this constitutes about 82% for 5AC) is still in question. Although there is an unprecedented mention about an nucleophilic addition reaction of O\textsuperscript{−} to cytosine moiety in 2\textsuperscript{′}-deoxycytidine in a much earlier pulse radiolysis study\textsuperscript{9} due to the spectral similarity at pH 7 and pH 13, such a reaction is fully ruled out in later studies\textsuperscript{37} with a clear demonstration of the attack of O\textsuperscript{−} to the sugar moiety.

![Figure 3.16](image)

**Figure 3.16:** (a) Optimized geometry of 5AC\textsuperscript{−} with selected bond lengths in Å units. (b) The HOMO of 5AC\textsuperscript{−}.

If the addition of O\textsuperscript{−} to 5AC\textsuperscript{−} is nucleophilic in nature (means O\textsuperscript{−} is behaving as an electrophile), then addition likely to occur on N1, N3 or N5 atoms as these are the electron rich centers found on 5AC\textsuperscript{−} based on HOMO analysis (Figure 3.16). However, the possibility of addition to N1 can be ruled out since, the N1 substituted 5-azacytidine (substituent is ribose sugar) system also exhibited similar spectral,
kinetic and redox properties\textsuperscript{11} as that of 5AC. Thus, the addition of $O^-$ to the ring moiety is limited to the N3 and N5 atoms.

The addition of $O^-$ to N3 and N5 ring atoms results in the formation of radical di-anions 4 or 6; we assumed that such radical di-anions would get protonated readily by water molecule to give the radical mono anions 5 or 7 (see Figure 3.17). This assumption is supported by the pKa calculations of the radical anions 5 and 7. Theoretically determined pKa value of radical anion 5 is 25.4 and that of radical anion 7 is 19.3; signifies the possibility of protonation of radical di-anions 4 and 6 which could be formed by the addition of $O^-$ to 5AC$^-$ under the pulse radiolysis conditions. The Mulliken atomic spin densities are found to be maximum on C4 (0.27 a.u.) and C6 (0.59 a.u.) atoms in the case of 5, and on C4 (0.94 a.u.) in the case of 7 calculated

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{figure3.17.png}
\caption{Schematic of $O^-$ additions to N3 and N5 and subsequent protonation by water.}
\end{figure}
Reactions of *OH and O\textsuperscript{−} with 5-azacytosine

at B3LYP/6-31+G(d,p) level of theory, the unpaired spin is more delocalized in the case of 5 compared to 7.

The possibility of the addition of O\textsuperscript{−} to N3 and N5 positions are compared by considering the energetics of reactions represented by equations 3.9 and 3.10.

\[
5\text{AC}^- + O\text{^−} \rightarrow 4 \quad (3.9)
\]

\[
5\text{AC}^- + O\text{^−} \rightarrow 6 \quad (3.10)
\]

The free energy change associated with equation 3.9 is 73.19 kcal/mol, whereas that for equation 3.10 is 74.89 kcal/mol. Thus, the additions of O\textsuperscript{−} to N3 or N5 are thermodynamically not facile in vacuum. But when the effect of solvation is considered, it was found the \(\Delta\Delta G_{\text{sol}}\) value for reactions 3.9 and 3.10 respectively are -49.09 kcal/mol and -50.77 kcal/mol. The free energy values in solution phase are very conducive for the addition feasibility.

**TDDFT calculations**

In order to differentiate the formation of the exact transient species observed experimentally, the TDDFT calculations at the B3LYP/6-31+G(d,p) level have been performed in aqueous solution. The calculated \(\lambda_{\text{max}}\) values of the transients with corresponding oscillator strengths \((f)\) are depicted in Table 3.4.

It can be seen from the table that, the experimental values of 280 nm (intense one) and a shoulder at 350 nm can be considered due to the transients 5, 2, and 3. So it can be ascertained that, the experimentally observed spectrum corresponds to the formation of 5 and the radical anions 2 and 3.
3.8 Conclusions

The present study demonstrated that the reaction of O$^\bullet$ with 5AC proceeds via a quite different mechanism. Addition of O$^\bullet$ to ring nitrogen followed by the rapid protonation of the addition product is considered as the major reaction pathway which corresponds to non-oxidizing transient produced in earlier pulse radiolysis experiments. The presence of oxidizing radical intermediates is invoked by considering the formation of radical anions generated by rapid deprotonation of one electron oxidized species. The pKa determinations and TDDFT calculations predicted that, these two reaction channels i.e. (i) the addition of O$^\bullet$ followed by protonation and (ii) one electron oxidation of 5AC$^-$ and instantaneous deprotonation of oxidized species, are occurring alongside. The 280 nm peak is contributed by 5 and the 350 nm shoulder by H11 abstracted radical 2 or H12 abstracted radical 3. Energetically the direct hydrogen abstractions are not facile reactions. To the best of our knowledge, the addition of O$^\bullet$ to the ring unit of any heterocyclic system is rather unusual and is the first demonstration.

Table 3.4: Optical absorption maximum and oscillator strengths (f) of the transients derived from O$^\bullet$ reaction with 5-azacytosine calculated at B3LYP/6-31+G(d,p) level in aqueous phase.

<table>
<thead>
<tr>
<th>Transient</th>
<th>$\lambda_{max}$ (nm)</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>419</td>
<td>0.061</td>
</tr>
<tr>
<td>2</td>
<td>349</td>
<td>0.047</td>
</tr>
<tr>
<td>3</td>
<td>350</td>
<td>0.047</td>
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<tr>
<td>5</td>
<td>273</td>
<td>0.065</td>
</tr>
<tr>
<td>7</td>
<td>307</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Reactions of 'OH and O\textsuperscript{2-} with 5-azacytosine

References


Reactions of \( ^\cdot \text{OH} \) and \( ^\cdot \text{O}^- \) with 5-azacytosine


Reactions of \( \cdot \text{OH} \) and \( \cdot \text{O}^- \) with 5-azacytosine


