Summary
Pulse radiolysis technique has been useful in the evaluation of kinetics and spectral characterization of the transient radicals while steady state radiolysis is used for product analysis and together they enable in the understanding of the reaction mechanism. In the recent past, the ease of using quantum chemical methods in the determination of the stabilities and thermodynamic properties such as reaction enthalpies, free energies, molecular properties like bond lengths and bond angles of the reaction intermediates has provided complementary information to accurately predict the site of radical attack. Thus, the pulse and steady state radiolysis combined with theoretical calculations have become a powerful tool in the formulation of detailed reaction mechanism even in the case of relatively large molecules and this thesis incorporates a detailed investigation on the reactions of oxidising radicals (\(\cdot\text{OH}, \text{N}_3^\cdot, \text{Br}_2^\cdot, \text{SO}_4^\cdot\)) with several indole and chalcone derivatives using all the above methods. Also investigated briefly are the fluorescence lifetime measurements of hydroxy indole derivatives to complement the pulse radiolysis work.

*The thesis is divided into 5 chapters, each describing a particular aspect of the work.*

**Chapter 1: Introduction**

This chapter deals with general aspects of radiation chemistry, different modes of interaction of ionising radiation with matter and radiation sources and the techniques for obtaining the transient absorption spectra and kinetics. A description of the study of water radiolysis, time scale for early events, formation of primary radicals and their properties and generation of few specific secondary
radicals relevant to the thesis is given in detail. The scope of the present work is incorporated at the end of the chapter.

Chapter 2: Experimental

The details of chemicals, preparation of solutions and various experimental techniques used are described in this chapter. These include the description of pulse radiolysis experiments carried out using the LINAC facilities at NCFRR, Pune, BARC, Mumbai and NDRL, Notre Dame, USA. Product analysis has been performed by HPLC and ground state spectra by UV-VIS spectrophotometry. The computational methods for DFT calculations used are described in detail.

Fluorescence lifetime measurements were conducted by time correlated single photon counting technique using an ultrafast Ti-sapphire femtosecond laser (Tsunami, Spectra-Physics) with 78 MHz repetition rate delivering 30 fs pulses at 750 nm at Pohang Institute of Science and Technology, Pohang, South Korea.

Chapter 3: Radiation Induced Oxidation of Indole Derivatives

This chapter deals with the reactions of oxidising radicals \( ^\cdot \text{OH}, \text{N}_3^\cdot, \text{Br}_2^-\) and \( \text{NO}_2^\cdot \) with indole derivatives tryptophan (Tpn), tryptamine (Tpe), \( 5\)-hydroxyindole (HIn), \( 5\)-hydroxytryptophan (HTpn), \( 5\)-hydroxytryptamine (HTpe) and \( 5\)-hydroxytryptophol (HTpl) by both pulse and steady state radiolyses and analysed by quantum chemical calculations.

i) Kinetics and spectra

Barring \( \text{NO}_2^\cdot \) radical, the rate constants for reactions of other oxidising radicals with indole derivatives used in this work were found to be diffusion-
controlled and remained unaffected by pH in the range 5 – 9. The rate constants in
the NO$_2^-$ radical reaction with hydroxy indole derivatives are lower by two orders
of magnitude and increase from $10^5$ to $10^7$ dm$^3$ mol$^{-1}$ s$^{-1}$ on going from pH 5 to 9.
This gradual increase in reactivity with pH is due to the decrease in the reduction
potentials of indoloxyl radicals formed on oxidation.

The transient spectra recorded on radiation induced oxidation of
tryptophan and tryptamine exhibited peaks at 330 and 530 nm (indolyl radical)
whereas hydroxy derivatives absorbed at 420 nm (indoloxyl radical) which is in
reasonable agreement with the calculated value (407 nm). Only was the indoloxyl
radical found to react further with the solute forming the radical adduct and its
decay was observed to be pH dependent in derivatives containing an amino group
in the side chain. At pH 5, no decay of the radical adducts was seen in all
derivatives up to 5 ms whereas those with the amino group decayed faster at pH 9.
Further, both ABTS$^{2-}$ and MV$^{2+}$ were found to react with the indoloxyl radical
with all hydroxy indole derivatives and the combined yields of oxidised and
reduced intermediates generated from their reactions nearly accounted for the
total. Our results suggest that NO$_2^-$ radical is inefficient in oxidising hydroxy
indoles under physiological conditions but escapes by self dimerisation and other
radical reactions.

ii) Quantum Chemical Calculations

The energetics of the *OH radical addition and the water elimination for
the *OH adducts of both Tpe and HTpe systems were determined using B3LYP/6-31+G(d,p) level of theory. Among the possible *OH adducts, the more
energetically favourable positions were calculated to be C2 and C4. The overall enthalpies of \( ^\cdot \text{OH} \) addition and water elimination for various \( ^\cdot \text{OH} \) adducts of both systems were calculated.

The energetics for the \( ^\cdot \text{OH} \) addition have shown dependence on the site of activation with (HTpe-OH)\(^\cdot \) adducts at C2 and C4 and the (Tpe-OH)\(^\cdot \) adduct at C2 being thermodynamically more stable and fast water elimination from (HTpe-OH)\(^\cdot \) adduct at C4 to give the indoloxyl radical occurs due to favourable geometry.

Density functional theory calculations of the optimised geometries of indoloxyl radical in water revealed the formation of two distinct types of radical adducts, one through the H – O bond and the other by C – C linkage. The H – O bonded radical adduct is found to be exothermic with a reaction enthalpy -4 kcal mol\(^{-1} \) and bond length 0.1819 nm and the C – C bonded radical adducts are endothermic and rate determining but are finally driven by exothermic processes involving intermolecular H transfer followed by intramolecular reorganisation through H shift resulting in stable C4 – C4’ and C2 – C4’ dimers with reaction enthalpies of -39 and -44 kcal mol\(^{-1} \) respectively and this process is thermodynamically as efficient as direct recombination of indoloxyl radicals.

The formation of the two dimer products is also seen in steady-state radiolysis. Radical adduct formation from subsequent reaction of the indolyl radical with Tpe or Tpn was not noticed by pulse radiolysis and is in accord with the positive free energy change (\( \Delta G = 10 \) kcal mol\(^{-1} \)) calculated by quantum
chemical methods. Thus, the indolyl radical is less reactive with the substrate but it is far more reactive with itself than is the indoloxy radical.

Chapter 4: Fluorescence Studies of Hydroxy Indole Derivatives

Multiphoton excitation of 5-hydroxytryptamine (serotonin) is known to exhibit green fluorescence with maximum at about 500 nm but its origin is still not clearly understood and needs further investigation of fluorescence lifetime measurements. We have been involved in pulse radiolysis studies on oxidation of serotonin and other hydroxy indoles where the indoloxy radical absorbing at 420 nm is formed as an intermediate. Therefore, it is interesting to extend the pulse radiolysis work to fluorescence studies of these derivatives.

We have chosen three derivatives of 5-hydroxy indoles: HTpe, HTpn and HIn and employed multiphoton excitation by time correlated single photon counting method using ultrafast femtosecond laser. This study is undertaken to examine whether the indoloxy radical is responsible for the emission.

The emission in neutral solutions is characterized by a major component (95%) with a time constant of 460 ps in unsubstituted indole which increased to 850 ps in substituted HTpn. Significant fluorescence quenching was seen on going from neutral to highly acidic solutions (pH 1). Neither fluorescence intensities nor lifetimes were affected on addition of reductant ABTS$^{2-}$. The green fluorescence was attributed to the indoloxy radical formed on multiphoton excitation and the underlying mechanism consistent with the results is discussed.
Chapter 5: Redox Chemistry of Chalcones

This chapter is concerned with radiation chemical studies of reactions of oxidising radicals with two classes of substituted chalcones. The first group has an indole ring with the functional groups such as –OH (p-HIC), –CH$_3$ (p-MIC) or –NH$_2$ (p-AIC) group at the para position of the phenyl ring. The other group has both benzenoid rings with an -OH group (4/4′-HBC) on one of the rings. Specifically, the study involves the reactions of ’OH, Br$_2$•− and SO$_4$•− radicals with both classes of compounds using pulse radiolysis, product analysis and DFT calculations. The ’OH radical reacts at diffusion controlled rates forming the ’OH adduct and the rate constants were found to be pH independent in the entire pH range 4 – 10.

The transient absorption spectra measured in the reaction of ’OH radical with p-HIC at pH 7 exhibited a peak at 390 nm. In basic media (pH 9 – 10), though the spectra are similar to that measured in neutral solutions, bleaching to a small extent around 320 nm was observed in all the three para isomers. Besides the bleaching, an additional low intense peak around 460 nm was seen in the case of p-MIC and p-AIC. Furthermore, the spectra in acidic solutions of p-MIC exhibited an additional peak of low intensity at the higher wavelength (570 nm). In contrast, Br$_2$•− and SO$_4$•− radicals showed different spectral behaviour with two distinct peaks around ~330 and 560-580 nm which is attributed to the N centered indolyl type radical.

On the other hand, the spectral features observed in the reactions of ’OH radical with both 4/4′-HBC are different from those measured in the case of
indole chalcones with an intense bleaching around 360 nm and weak peaks at 280 and around 420 nm. The spectrum measured in the Br$_2^•−$ radical reaction exhibited a peak at 280 nm and a shoulder at 380 nm which is assigned to phenoxyl type radical and is different from that observed with indole chalcones.

Stability calculations of •OH radical addition to para isomers of indole chalcones revealed that the adducts at C2 position of the indole ring and α, β-unsaturated bond are more stable than at all other positions. Strong frontier orbital interaction and negligible coulombic repulsion on chalcone result in higher stability of these •OH adducts. Furthermore, the calculated spectra of the indolyl type radical in the gas phase formed by electron transfer and deprotonation of chalcone derivatives have shown two peaks at 415 and 540 nm. A marked red shift by 35 nm from the experimental (380 nm) spectra at the lower wavelength but an excellent agreement in the higher wavelength region is observed.

In conclusion, the work incorporated in the thesis is a comprehensive study involving both radiation and photochemical techniques and theoretical methods in the elucidation of the oxidation mechanisms of indole and chalcone derivatives which are biologically important compounds.