CHAPTER 1

Introduction and Objectives

This chapter presents a general introduction to the relevant topics mentioned in the thesis followed by the scope and objectives of the work under the following titles.

1. **Nitric oxide**: Historical developments, chemistry, biology, and physiological and pathological details of Nitric Oxide.

2. **S-Nitrosothiols**: Earlier developments, chemistry and biology of S-nitrosothiols.

3. **Aqueous radiation chemistry**: Production of free radicals, radiation sources, techniques, radiolysis of water, primary radicals and their properties, secondary radicals and their properties.

4. **Photochemistry**: General introduction to photochemistry, quantum yield, interaction of light with matter and various types of photochemical reactions.

5. **Scope and Objectives.**
1.1 Nitric Oxide: A Newly Discovered Messenger Molecule

Nitric Oxide (NO), a diatomic free radical, was first synthesised by the Belgian scientist, Jan Baptista van Helmont in 1624 [Helmont, 1624]. Joseph Preistley, in 1772, observed that NO did not support plant life [Priestley, 1772]. He also noticed that meat can be preserved if exposed to NO. He christianised it as 'nitrous air'. Mitchell, in 1916, found that oxides of nitrogen were produced in mammals [Mitchell et. al., 1916]. The NO production in mammals was first confirmed by Tannenbaum in 1928 [Tannenbaum et al., 1928]. Till late 1980's, the scientific world has almost ignored NO's biological involvement as NO was proved to be a constituent of environmental pollutant found mainly in the exhausts of automobiles and factories, in cigarette smoke and a by-product of microbial metabolism. It was placed in the black list of the scientific community due to its involvement in ozone destruction, carcinogenesis and acid rain. Furchgott and Zawadzki, in 1980, discovered that acetyl choline acted upon the endothelium which leads to the production of a secondary messenger called Endothelium Derived Relaxation Factor (EDRF) [Furchgott et al., 1980]. Ignarro et al. reported in 1987 that NO and EDRF behaved alike [Ignarro et al., 1987]. Murad showed that nitroglycerine and the related nitrovasodilator drugs releases NO and the Guanylate Cyclase (GC) is subsequently
activated [Murad, 1988]. Palmer et al. reported in 1987 that NO is synthesised from L-arginine [Palmer et al., 1988]. The mutation of DNA by NO was experimentally proved by Wink et al. in 1991 [Wink et al., 1991]. After these discoveries, the scientific world has witnessed an exponential rise in the NO research. The drug Sildenafil (Viagra®) was launched in 1998 for the treatment of impotency. Thus a wide range of studies on NO slowly revealed both its merits and demerits. Even though the initial investigations were concentrated on its role in the regulation of vascular tone, a wide and diverging direction of research of both physiological and pathological actions of NO are now under the thrust areas in this field. It has been proven that free radical species with oxygen and nitrogen based unpaired electrons are having a diverse role in many aspects of physiological and pathological events [Nitric Oxide, 1996].

It is now understood that NO has been preserved throughout evolution as a mediator in many biological systems. An example for this is the shoe crab, a species that has not evolved significantly in 500 million years. Aggregation of its single type of blood cell is modulated by NO in the same way as that of modern mammalian platelets [Bernnan et al., 2003]. The role of NO has been discovered in a wide range of biological activities. NO's role starts from the fusion of gamete with egg, its activity
is required for the activation of egg immediately after insemination [Kuo et al., 2000]. NO is involved in the developmental processes and governs an immense number of physiological and pathological reactions. NO is emerging as an important chemical mediator of neuroendocrine function and behaviours [Nelson et al., 1997]. Considering its wide biological significance, Science magazine named NO as the molecule of the year in 1992 [Koshland, 1992]. The discovery of the biological role of NO is regarded as one of the greatest discoveries of the 20th century. The Chemical and Engineering News highlighted it as "Biochemistry's unexpected new superstar" in 1993 [Feldman et al., 1993]. The 1998 Nobel Prize in the field of Physiology or Medicine have been awarded to Furchgott, Igarra and Murad for their pioneering works on the biological role of nitric oxide.

1.2 Physical and Chemical Properties of NO

NO is a diatomic gas which is paramagnetic in nature. As per IUPAC, NO is termed as "oxidonitrogen(*)" [Koppenol, 2002]. This molecule composes of 7 electrons from nitrogen and 8 electrons from oxygen. The canonical forms of NO can be represented as follows

\[ \overset{+}\text{N} - \overset{-}\text{O} \leftrightarrow \overset{+}\text{N} = \overset{-}\text{O} \leftrightarrow \overset{+}\text{N} \equiv \overset{-}\text{O} \]  

(1.1)
At STP, the colourless NO is having a boiling point of 121 K and a melting point of 110 K. The N-O bond length is of 1.154 Å and it has a \( ^2 \pi \) ground state. Simple Molecular Orbital Theory (MOT) predicts a bond order (BO) of 2.5. The unpaired electron in NO exists in an antibonding \( \pi \) orbital, polarized towards nitrogen, which weaken the triple bond by 0.5 resulting in an overall BO of 2.5. If NO dimerises, then the bond order is 5 i.e., the overall bonding does not increase when two NO molecules interact [Butler et al., 1993]. NO absorbs significantly in the deep UV with sharp bands at 224, 217 and 203 nm. NO stretches, \((v_{NO})\) at 1875 cm\(^{-1}\). It exhibits \( P, Q, \) and \( R \) rotational branches in its gas phase vibrational spectrum [Laane et al., 1980].

Solubility and transport of NO are similar to those of oxygen. Aqueous solution solubility is \( 1.9 \times 10^{-3} \) mol dm\(^{-3}\) at 25\(^{\circ}\)C. NO is readily diffusible and has a diffusion constant of 3300 \( \mu \) m\(^{-1}\) s\(^{-1}\) under physiological conditions. NO can diffuse across the cell membrane because of its neutral charge [Davis et al., 2001]. The half-life of NO is 3.5 s in biological tissues [Koppenol, 1998]. The concentration of NO decreases and the half-life increases as the distance from the site of production increases [Wink et al., 1998]. At lower concentrations (<1 \( \mu \) mol dm\(^{-3}\)) the
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direct effects of NO predominates while for large amounts of NO (>1 μ mol dm⁻³), the indirect effects predominate (Fig. 1.1).

**Fig. 1.1: Chemistry of NO [Davis et al., 2001]**

1.3 Biosynthesis of NO

NO is generated *in vivo* by a group of evolutionarily conserved cytosolic or membrane bound isoenzymes called Nitric Oxide Synthase (NOS). NOS are characterized as cytochrome P450 like heme protein and are having bi-domain structure. The C terminus, NOS reductase domain, is the binding site for Calmodulin, Flavin adenine dinucleotide (FAD), Flavin mononucleotide (FMN) and Nicotinamide adenine dinucleotide phosphate (NADPH) while the NOS oxidase domain at the N terminus contain heme and is the binding site for Tetrahydrobioptenin (BH₄) [Sessa et al., 1992]. NO can also be formed non-enzymatically by the direct reduction of nitrite under low pH. For example,
during digestive processes in the stomach, the pH is between 2.5 and 4.5 which means dietary sources of nitrate and nitrite can also possess a role in the production of NO independent of NOS activation [Duncan et al., 1995].

1.3.1 Production of NO

A two step mechanism is proposed for the production of NO [Stuehr et al., 1991]. The first step is the formation of N[^5]-hydroxy L-arginine (2) from L-arginine (1) by the hydroxylation of the guanidino nitrogen using one mole of NADPH. Electrons are shifted from NADPH to FAD forming FADH\(_2\). Disproportionation with FMN would lead to FADH/FMNH. Electron flow from FMNH to Fe\(^{3+}\) gives Fe\(^{2+}\) and FADH\(^+/FMNH^+\). The sixth ligand position is then occupied by oxygen forming Fe\(^{3+}\)O\(_2\) which is further reduced by FADH\(^+/FMNH^+\) to Fe\(^{2+}\)-O\(_2\)H. Then an electron rich thiolate promotes the formation of [Fe\(^{5+}=O\)]\(^{3+}\) with the release of water.

In the second step, 0.5 mole of NADPH is consumed to convert 2 to NO and L-citruline (3). This involves a 3 electron oxidation.

![Scheme 1.1](image-url)
Thus NO is generated via a 5 electron oxidation of terminal guanidinium nitrogen on L-arginine [Rosen et al., 2002].

1.4 The fate of NO

NO does not rapidly react with most of the biological molecules since majority of them contain bonds filled with two electrons. NO, however, reacts with oxygen, superoxide (O$_2^-$), thiols (RSH), transition metals and non-heme iron [Dawson et al., 1996; Huie et al., 1993; Ignarro, 1990; Kharitonov et al., 1995].

NO can interact with oxygen to form $\cdot$NO$_2$. In oxygenated aqueous solution, $\cdot$NO$_2$ results nitrite ion. The autoxidation of NO in aerated aqueous solution is very fast. The reaction is second order with respect to NO and first order with respect to oxygen. The concentration of NO is relatively low during bioregulatory activities, and therefore, under in vivo conditions, even at high concentration of oxygen the life time of NO is high [Ignarro et al., 1993; Kharitonov et al., 1994; Williams, 2003].

$$2NO + O_2 \rightarrow 2\cdotNO_2 \quad (1.2)$$

$$2NO + 2\cdotNO_2 \rightarrow 2N_2O_3 \quad (1.3)$$

$$2N_2O_3 + 2H_2O \rightarrow 4NO_2^- + 4H^+ \quad (1.4)$$
NO interacts with transition metals to form metal nitroxy1 complexes which is a reversible reaction. The electron pair in nitrogen atom forms a $\sigma$ bond and through the antibonding $2p\pi^*$ unpaired electron it forms a $\pi$ bond with transition metal $d$ electron. Thus NO can act as a 3 electron donor. NO-iron interaction in guanylyl cyclase (GC) activates the enzyme, which cause the increase in cyclic guanosine monophosphate (cGMP) levels. The NO-metal interaction also activates cyclo oxygenase while in some cases this leads to the inhibition of the function of proteins [Salvemini et al., 1993]. NO binds to the heme of deoxy hemoglobin very rapidly. NO also reacts with hemoglobin to form met hemoglobin and nitrate [Meyer et al., 1989; Stamler et al., 1992]. Stopped flow spectroscopy showed that the intermediate of this reaction is peroxynitrite [Herold, 1998]. This reaction is used in the identification and quantitative analysis of NO [Fan et al., 2000]. Binding of NO to the non-heme iron of ribonucleotide reductase leads to the inhibition of DNA synthesis. Desolution of the clusters takes place in non-heme interaction. In cytochrome P450, the formation of Fe-NO takes place and this prevents the binding of oxygen [Radi, 1996]. Various metal-oxygen and metal oxo complexes rapidly react with NO. NO helps in lipid peroxidation chain termination and thus protects the cells against peroxide induced cytotoxicity [Rubbo et al., 1995].
NO reacts with radicals at diffusion controlled rate. Nitrite is the end product of the reaction of NO with \( ^*\text{OH} \) while with \( ^*\text{NO}_2 \) the end product is \( \text{N}_2\text{O}_3 \) \([\text{Mohankumar et al., 1998; Wink et al., 1993}]\). With superoxide radical anion \( (\text{O}_2^{*-}) \) the end product is peroxynitrite, which is a potent oxidant. The rate of this reaction is three times faster than the rate of reaction of the enzyme superoxide dismutase (SOD), which means NO can effectively compete with SOD for \( \text{O}_2^{*-} \) \([\text{Goldstein et al., 1995}]\). The cytotoxicity of NO is believed to be due to the production of peroxynitrite. Peroxynitrite can oxidize lipids, proteins and DNA \([\text{Beckman et al., 1996; Fukuto et al., 1997}]\). NO yields singlet oxygen with hydrogen peroxide \([\text{Noronha-Dutra et al., 1993}]\). Interaction of NO with thiol leads to the formation of nitrosothiols, which are now considered as the transporting and storage agents for NO \textit{in vivo} \([\text{Keshive et al., 1996}]\). Under \textit{in vivo} conditions glutathione (GSH) and L-cysteine (CySH) are the naturally occurring thiols while N-acetyl cysteine (ACySH) is the effective precursor and stimulator of GSH \([\text{Shishido et al., 2000}]\). Penicilamine helps to chelate excess \( \text{Cu}^{2+} \) \textit{in vivo} in the treatment of Wilson's disease \([\text{Williams, 1999}]\). The reaction is first order with respect to thiol and second order with respect to NO.

Two types of mechanisms are proposed for the formation of RSNOs, through an electrophilic nitrosation (reaction 1.5) and by a radical mechanism.
where RS\(^*\), formed from the reaction between \(^\cdot\)NO\(_2\) and RS\(^-\), reacts with NO (reaction 1.8) \([\text{Keshive et al., 1996; Kharitonov et al., 1995}]\). In the former case, under aerated condition N\(_2\)O\(_3\) directly nitrosate thiols while under unaerated condition electrophilic attack of NO on thiolate anion takes place \([\text{Aravindakumar et al., 2002}]\).

\[
N_2O_3 + RSH \rightarrow RSNO + NO_2^- + H^+ \quad (1.5)
\]

\[
RS^- + \cdot NO \rightarrow RSN^*O^- \quad (1.6)
\]

\[
RS^- + \cdot NO_2 \rightarrow NO_2^- + RS^* \quad (1.7)
\]

\[
RS^* + \cdot NO \rightarrow RSNO \quad (1.8)
\]

As in the case of reactive oxygen species (ROS) formed from oxygen, reactive nitrogen intermediates (RNI) are formed from NO. Their production can be represented as shown in the Scheme 1.2. These RNIs then undergo nitrosation, nitration and oxidation reactions and produce different compounds, which are both physiologically and pathologically important.

\[
\begin{array}{cc}
\text{NO}^+ & \xrightarrow{\text{Oxidation}} \text{NO}^* & \xrightarrow{\text{Reduction}} \text{NO}^- \\
\text{O}_2^- & \xrightarrow{\text{O}_2^-} \text{ONO}_2^-
\end{array}
\]

**Scheme 1.2**: Formation of reactive nitrogen intermediate (RNI) species.
NO$^+$ and NO$^-$ are highly reactive and they do not play any significant role in biological systems. Under in vivo conditions NO cannot be oxidized to NO$^+$ [Butler et al., 1995].

1.5 Biological Implication of NO

The effect of NO can be protective, regulatory or deleterious as shown below.

![Scheme 1.3: Protection, regulation and deleterious effects of NO](image)

The chemical biology of NO can be divided into direct and indirect effects. In direct effect, the fast reactions of NO under physiological conditions, like reaction with metal complexes and with high energy radicals, are important. In indirect effects, the Reactive Nitrogen Oxide Species (RNOS), produced during the reaction with superoxide or oxygen, are important [Wink et al., 1998].
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The biological importance of NO is now reasonably known. The most important one is its ability to act as EDRF. NO inhibits platelet activation and adhesion. In central nervous system (CNS), the important role of NO is in regulating male and female sex behaviour [Benelli et al., 1995; Zeran et al., 1996], learning and memory [Muller, 1996], and motor coordination and pain [Nelson et al., 1997]. While in the case of peripheral nervous system, the L-arginine-NO system account for the nonadrenergic noncholinergic neurotransmission (NANC transmission) in several anatomic sites [Garthwaite, 1991; Sanders, 1992]. NO functions as an important mediator in the action of hormone and neuro transmitters, which are vital for the regulation of reproduction [Dixit et al., 2001]. NO is the NANC neurotransmitter which regulates penile erection. In females, the sexual arouse is regulated in the same manner. Viagra® (Sildenafil) inhibits the breakdown of cGMP after NO-mediated activation of GC evoked by para sympathetic nerves in the genital tract during sexual stimulation. The essential role of NO and cGMP as mediators of corpus cavernosum smooth muscle relaxation and their erectile function is already demonstrated [Zanzinger, 1999].
**Scheme 1.4: Various biological functions of NO**

NO is shown to be an antimicrobial molecule in humans. NO derived from iNOS has been involved in immune response while reports are available for the involvement of nNOS and eNOS [Stuehr, 1987]. NO induces mutation resulting from the nitrosative deamination of DNA [Bogdan, 2001; Hibbs et al., 1987; Tamir et al., 1996]. The NO released from endothelial cells is a crucial regulation of arterial conductance and plays an important role in the central tissue perfusion [d’Alessio, 2004; Ignarro et al., 1999]. NO can induce necrosis and apoptosis depending on the cell, signal, source, molecule, amount, presence or absence of co-
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reactants [Brune, 2003; Kitajimal et al., 1994; Murphy, 1999]. The various biological functions of NO are briefed in Scheme 1.4.

1.6 NO in Pharmacology

Eventhough NO has been involved in therapeutics for more than 100 years, its actual physiological mechanism of action had been little revealed since 1980's. It is now confirmed that the high or very low concentration of NO production leads to many diseases. The pharmacological use of NO can be achieved by stimulating NO or inhibiting NOS or giving NO directly. NO-donor drugs fall into the first category. The major classes of NO-donors are listed in the Table 1.1 [Wang et al., 2002]. Other NO donor drugs are NO donor/drug hybrids, enzyme-activated NO donors, NO releasing aspirins and NO-nonsteroidal antiinflammatory drugs. The clinical importance of NO is now revealed in the areas of cardio-respiratory [Barnes, 1993; Thiemermann et al., 1993], central nervous system (CNS) [Calver et al., 1993; Zaba et al., 1991], diabetes [Chan et al., 2001; Johnstone et al., 1993], gastrointestinal system [Rand, 1992; Sanders et al., 1992] and impotence [Burnett et al., 1992].
### Table 1.1: NO-donors and their pharmacological uses [Wang et al., 2002].

<table>
<thead>
<tr>
<th>Name</th>
<th>Example</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Nitrates</td>
<td>Gyceryl Trinitrate</td>
<td>To relieve angina pectoris, relaxation of vascular smooth vessels</td>
</tr>
<tr>
<td>Organic Nitrates</td>
<td>Tert-butyl nitrite</td>
<td>To relieve angina pectoris</td>
</tr>
<tr>
<td>Metal-NO complexes</td>
<td>Sodium nitroprusside</td>
<td>Vasodilatory activity</td>
</tr>
<tr>
<td>N-nitrosamines</td>
<td>N-nitrosoureas</td>
<td>Anti tumor activity, potent bacterial mutagens</td>
</tr>
<tr>
<td>N-Hydroxy-N-nitrosamine</td>
<td>Cupferron</td>
<td>Potent anti-hypertensive agent, inhibitor of platelet aggregation</td>
</tr>
<tr>
<td>N-Nitrosimines</td>
<td>1,3-disubstituted nitroimino benzimida zoles</td>
<td>Inhibit platelet aggregation, antithrombic and BP lowering abilities</td>
</tr>
<tr>
<td>Nitrosothiols</td>
<td>GSNO, Sugar-SNAPs, S-Nitrosopeptides</td>
<td>Antiplatelet agent, vasodilators, protection from oxidative stress, antitumor</td>
</tr>
<tr>
<td>C-Nitroso compounds</td>
<td>2-Methyl-2-nitroso propane</td>
<td>Inhibited aggregation of blood platelets</td>
</tr>
<tr>
<td>Diazetine dioxides</td>
<td>3,3,4,4-Tetra methyl-1,2-diazetine-1,2-dioxide</td>
<td>Strong vasodilator, anti aggregation</td>
</tr>
<tr>
<td>Furoxans and Benzofuroxans</td>
<td>Furazano benzo furoxan</td>
<td>Cytotoxicity, mutagenicity, BP lowering activity, anti leukemic and immuno suppression, vasodilators</td>
</tr>
<tr>
<td>Oxatriazole-5-imines</td>
<td>3-Cyclohexyl-1,2,3,4-oxatriazole-5-irine hydrochloride</td>
<td>Vasorelaxant, inhibit platelet aggregation</td>
</tr>
<tr>
<td>Sydnonimines</td>
<td>Molsidomine</td>
<td>Antihypertensive agent, anti anginal agent</td>
</tr>
<tr>
<td>Oximes</td>
<td>NOR-3</td>
<td>Vasorelaxant, anti platelet agent</td>
</tr>
<tr>
<td>Hydroxylamines</td>
<td>FerriMB</td>
<td>Vasodilator, inhibit insulin release and activate K⁺ channels</td>
</tr>
<tr>
<td>N-Hydroxy guanidine</td>
<td>N-Aryl-N-hydroxy guanidine</td>
<td>Anti hypertensive, anti tumor activity</td>
</tr>
<tr>
<td>Hydroxy urea</td>
<td>Hydroxyurea</td>
<td>Anti tumor agent, possible candidate for AIDS therapy</td>
</tr>
<tr>
<td>Diazoniumdiolates (NONOates)</td>
<td>DETA NONOate</td>
<td>Vasodilator, inhibit platelet aggregation</td>
</tr>
</tbody>
</table>
1.7 S-Nitrosothiols

The history of S-nitrosothiols (RSNOs) was dated from 1909. On that year RSNOs were first synthesised [Tasker et al., 1909]. In 1969, scientists found that during curing of meat with nitrite, RSNOs are generated by the interaction of nitrite with protein and non-protein sulfhydryl groups present in the meat and this helps to inhibit the growth of Clostridium and even prevent the lipid oxidation [Kanner et al., 1980; Minor et al., 1990]. After this observation, not much interest was given till 1980s when Ignarro et. al. postulated a role of RSNO in the activation of GC [Ignarro et al., 1980] and in 1990s endogenously occurring RSNOs were detected. RSNOs were detected in plasma at which 85% is of S-nitrosoalbumin (AbSNO) [Stamler et al., 1992]. S-nitrosoglutathioner (GSNOs) were also found in plasma, brain and alveolar fluids [Jia et al. 1996; Kluge et al., 1997]. It is now clear that the S-nitrosylation of hemoglobin (HbSNO) takes place on the cysteine residue located at position 93 on the βchain [Gow et al., 2001]. Recently S-nitrosocysteinyglycine is detected in the brain [Salt et al., 2000].

1.8 The Chemistry of RSNOs

RSNOs are thio esters of nitrite and are analogous with nitrite esters of alcohol. The primary and secondary RSNOs are red or pink in colour while tertiary RSNOs are green in colour. The UV-VIS spectra show three peaks
at 225-261 nm, 330-350 nm and 550-660 nm which corresponds to $\pi \rightarrow \pi^*$. $n_0 \rightarrow \pi^*$ and $n_N \rightarrow \pi^*$ with $\varepsilon \sim 10^3$, $10^2$ and $20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ respectively [Mason, 1969; Williams, 1996]. But the last two peaks are important to monitor RSNOs. The third band corresponds to the colour of the compound and the small $\varepsilon$ value for this is because of the forbidden transition. The IR spectrum shows stretching of N-O at 1479-1514 and N-S at 650-670 cm$^{-1}$. In $^1$H and $^{13}$C NMR a deshielding is observed for both $\alpha$-proton and $\alpha$-carbon resonances upon S-nitrosation of thiols [Wang et al., 1999]. Primary and secondary RSNOs adopt syn configuration while anti in tertiary. The bulky RSNOs are stable [Grossi et al., 2001].

1.9 Synthesis of RSNO

RSNOs can be synthesised by treating NO with thiols. But these reactions will takes place only if there is any electron acceptor like oxygen [Goldstein et al., 1996; Kharitonov et al., 1995].

$$NO + O_2 \rightarrow N_2O_3$$  \hspace{2cm} (1.9)

$$N_2O_3 + RSH \rightarrow RSNO + NO_3^- + H^+$$  \hspace{2cm} (1.5)

A second method is nitrosation reaction of RSH using sodium nitrite (NaNO$_2$) and HCl [Hart, 1985; Soulere et al., 2001].

$$NaNO_2 + HCl \rightarrow HNO_2 + HCl$$  \hspace{2cm} (1.10)
\[ RSH + HNO_2 \rightarrow RSNO + H_2O \]  

(1.11)

Another method is by reacting RSH with alkyl nitrites such as \( t \)-butyl nitrite. This is a pH dependent reaction. The reaction takes place via the formation of a thiolate anion \([\text{Patel et al., 1989; Roy et al., 1994}]\).

For the preparation of S-nitrosoproteins along with the above reactions, transnitrosation can also be utilized where the low molecular RSNO is treated with proteins \([\text{Broillet, 1999; Stamler, 1994}]\). Under \textit{in vivo} conditions, nNOS can yield GSNO \([\text{Schmidt, 1996}]\). Furthermore, it is now believed that transnitrosation is the suitable method for the formation of RSNO \textit{in vivo}.

1.10 Biological importance of RSNOs

RSNOs are believed to be the candidate for EDRF along with NO \([\text{Myers et al., 1990}]\). RSNOs were found to be potent vasodilators and an inhibitor of platelet aggregation \([\text{Ignarro, 1989}]\). They are responsible for vascular smooth muscle relaxation \([\text{Ignarro et al., 1981}]\), neuro transmission \([\text{Talman et al., 1993}]\), inhibition of cell growth and the regulation of autonomic processes \([\text{Barbier et al., 1994}]\) and blood flow \([\text{Stamler et al., 1997}]\). RSNOs were proved as effective cytotoxic and immuno suppressant \([\text{Merryman et al., 1993}]\) and they also relax airways \([\text{Gaston et al., 1994}]\). At very low concentrations, GSNO inhibits the growth of malarial parasite
[Rockett et al., 1991]. Oxygenated HbSNO circulates through the body and releases oxygen where there is a shortage of it. Subsequently, the NO is transformed to GSH forming GSNO, which leads to GC activation [Lipton, 2001]. GSNO in fusion is useful in coronary balloon angioplasty, coronary artery bypass and in thrombic embolic diseases [Langford et al., 1994; Salas et al., 1998]. S-Nitroso human serum albumin (AbSNO) protects skeletal muscles from ischemia/reperfusion injury [Hallstrom et al., 2002]. GSNOs are now used to treat preeclampsia in pregnant women [de Belder et al., 1995]. High concentration of RSNOs inhibits blood clot formation [Catani et al., 1998] and intracellular signalling through S-nitrosylation of transcription factor [dela Torre et al., 1998]. It also affects ion channel regulation [Bolotina et al., 1994], host defence [Persichini, 1998] and apoptosis [Tenneti et al., 1997].

RSNOs are now used as drugs. Some of the commercially available drugs are S-nitroso-N-acetyl penicillamine (APSNO), S-nitroso glutathione (GSNO) and S-nitroso-N-acetyl-D,L-penicilaminyl glycine methyl ester. The importance of RSNO drug is that it doesn’t induce tolerance while organic nitrate or inorganic nitrate develops tolerance [Richardson et al., 2002]. The use of RSNOs in photodynamic therapy is an ongoing research
area, which makes use of the cytotoxic nature of NO [Tannous et al., 2000].

1.11 Biochemistry of RSNOs

Homolytic and heterolytic cleavage determine the production of NO, NO$^-$ or NO$^+$. In therapeutic area, the understanding of the mechanism of NO release, the rate of release, oxidation state of NO liberated, the organic by-products released etc, are very important.

1.11.1 With metals

The interaction of Cu$^+$ is one among the most important reactions in this category. Cu$^+$ leads to the reductive cleavage of S-N bond. When Cu$^+$ is removed from the system by adding chelators, the decomposition of RSNOs are found to be halted. The reaction is strongly depended on the structure of RSNOs. The rate constant for this reaction is reported as diffusion controlled [Noble et al., 2000]. It is now clear that Cu$^+$ is actually formed by the reduction of Cu$^{2+}$ by thiolate ion [Williams, 1999; Dicks et al., 1996]. The reaction mechanism is as shown below

\[
Cu^{2+} + RS^- \rightarrow \frac{1}{2} RSSR + Cu^+
\]  \hspace{1cm} (1.12)

\[
Cu^+ + RSNO \rightarrow RS^- + NO + Cu^{2+}
\]  \hspace{1cm} (1.13)

In some cases the disulfide formed will complex with Cu$^{2+}$ and terminate the RSNO decomposition [Dicks et al., 1996]. The in vivo
importance of this reaction is because of the thiolate reduction of \( \text{Cu}^{2+} \). Copper containing proteins such as ceruloplasmin and unidentified metalloproteins are proved to decompose RSNOs [Al-Sa'doni et al., 1997; Dicks et al., 1997; Dicks et al., 1996]. Any agents that can reduce \( \text{Cu}^{2+} \) will be able to initiate RSNO decomposition [Williams, 1999].

The other metals of interest are mercury and silver ions. Both produce nitrite and metal thiolate [Swift et al., 1997; Williams, 1996]. They form S-bound intermediate complexes and the mechanism of the decay in the presence of \( \text{Hg}^{2+} \) is shown in reaction 1.14 and 1.15.

\[
\text{RSNO} + \text{Hg}^{2+} \rightarrow [\text{RS(Hg)NO}]^{2+} \quad (1.14)
\]

\[
[\text{RS(Hg)NO}]^{2+} + \text{H}_2\text{O} \rightarrow \text{RSHg}^+ + \text{HNO}_2 + \text{H}^+ \quad (1.15)
\]

Other metal ions, such as \( \text{Zn}^{2+}, \text{Ca}^{2+}, \text{Mg}^{2+}, \text{Ni}^{2+}, \text{Co}^{2+}, \text{Mn}^{2+} \) and \( \text{Cr}^{3+} \) were found to have no effect on RSNOs. \( \text{Fe}^{2+} \), on the other hand, shows some influence in the decomposition of RSNOs [McAninly et al., 1993; Sorenson et al., 2000; Vanin et al., 1997]. The metal induced decomposition of RSNOs can be effectively blocked by using metal chelators such as EDTA [Williams, 1999].

1.11.2 With nucleophiles

In the reaction of RSNOs with nitrogen nucleophiles like amines and azides, a nucleophilic substitution at nitroso nitrogen takes place. The
secondary amines yield the stable nitrosamine products [Munro et al., 1999].

\[ RSNO + Y^- \rightarrow RS^- + YNO \]  \hspace{1cm} (1.16)

1.11.3 With heme and non-heme models

When RSNO reacts with metalloporphyrins a trans addition of RS and NO groups across the metal center takes place [Lee et al., 2001; Yi et al., 1996]. Similar direct NO transfer from RSNO to non-heme iron complexes are also reported [Butler et al., 2001].

1.11.4 With hydrogen peroxide

Hydrogen peroxide decomposes RSNO by nucleophilic attack at nitrosonium nitrogen atom to produce thiol and peroxynitrite anion. At pH >13, peroxynitrite is observed while at physiological pH peroxynitrite anion decompose to nitrite or nitrate depending on the pH [Coupe et al., 1999].

\[ RSNO + HOO^- \rightarrow RS^* + ONOO^- + H^+ \]  \hspace{1cm} (1.17)

\[ ONOO^- + H^+ \rightleftharpoons ONOOH \]  \hspace{1cm} (1.18)

\[ ONOOH \rightarrow NO_2^- + H^+ \]  \hspace{1cm} (1.19)

\[ ONOO^- \rightarrow NO_2^- + \frac{1}{2}O_2 \]  \hspace{1cm} (1.20)

1.11.5 With Enzymes

GSNO-reductase reduces GSNO to disulfide and ammonia [Liu et al., 2001]. Glutathione peroxidase, xanthine oxidase and thioredoxin
decompose RSNOs in vitro [Hou et al., 1996; Trujillo et al., 1998]. CuZn-SOD decomposes GSNO to NO [Jourd'heuil et al., 1999].

1.11.6 With reducing agents

Compounds with reducing nature are found in vivo (fairly high in liver and kidney) and hence the reductive reactions with RSNOs are also important [Kashiba-Iwatsuki et al., 1997]. The major reactions in this category are the reaction with ascorbate and superoxide radical.

a) With ascorbate

Ascorbic acid is one of the important vitamins essential to our body, and hence its reaction with RSNO is of interest. Reduction by ascorbic acid proceeds according to an outer sphere electron transfer process [Smith et al., 2000]. It is now clear that the concentration of ascorbate is very important. This reaction is found to have two distinct pathways, at high concentration of ascorbate the Cu²⁺ dependent pathway takes place and the reaction is completely stopped in the presence of EDTA, whereas at low concentration of ascorbate, the Cu²⁺ independent pathway will follow. In the former case, the ascorbate acts as a reducing agent to produce Cu⁺ from Cu²⁺. The Cu⁺ converts RSNO to NO and thiolate ion and gets converted itself to Cu²⁺. The Cu²⁺ formed then leads to the final product, RSSR.
In the Cu$^{2+}$ independent pathway, ascorbate attacks the nitroso nitrogen atom of the RSNO and produce thiol and O-nitroso ascorbate. This O-nitroso ascorbate gets converted to dehydroascorbic acid (DHA) and NO via a free radical pathway [Holmes et al., 1998; Holmes et al., 2000; Kashiba-Iwatsuki et al., 1996; Scorza et al., 1997]. This pathway is dependent on pH. Here the dianion form of ascorbate is more reactive.

The general mechanism is as follows.

\[
GSNO + HA^- / A^{2-} \rightarrow GS^+ + \text{Dehydroascorbic acid} \quad (1.21)
\]

b) With thiols

The storage and transfer of NO \textit{in vivo} is now demonstrated by the transfer of NO from RSNO to RSH [Feldman et al., 1993]. The report shows that transfer readily takes place in aqueous solution. In biological systems the importance depends on the environment of the protein thiol and on the chemical nature of the RSNO. The reaction with thiol can be divided into two types; transnitrosation and S-thiolation.

i) Transnitrosation

Transnitrosation is important because it is understood as the way which helps thiols to transport NO \textit{in vivo} [Stamler et al., 1995]. The reaction

\[
Cu^{2+} + RS^- \rightarrow \frac{1}{2} RSSR + Cu^+ \quad (1.12)
\]

\[
Cu^+ + RSNO \rightarrow RS^- + NO + Cu^{2+} \quad (1.13)
\]
represents the transfer of NO$^+$ from the RSNO to the thiol. The reaction occurs via thiolate anion by nucleophilic attack at nitrosonium nitrogen. The reaction is found to be second order and the rate enhancement takes place with the introduction of electron withdrawing groups in RSNO [Barnett et al., 1995; Wang et al., 2001].

\[ RSNO + R'S^- \rightleftharpoons RS^- + R'SNO \]  
\[ \text{(1.22)} \]

The presence of Cu$^{2+}$ enhances the decomposition of RSNOs induced by thiols. The thiol converts Cu$^{2+}$ to Cu$^+$. In some cases the thiols act as a complexing agent for Cu$^{2+}$ and thus increase the stability of RSNO [Dicks et al., 1997; Gorren et al., 1996].

\[ Cu^{2+} + 2RS^- \rightleftharpoons \text{Dithiolate complex} \]  
\[ \text{(1.23)} \]

ii) S-Thiolation

In this reaction, thiolate anions attack the sulfur center of the RSNOs. Disulfide and NO$^-$ are formed via heterolytic cleavage of RSNO [Arnelle et al., 1995; Park, 1988]. Nitrite, ammonia, hydroxylamine and N$_2$O are formed as products from NO$^-$, depending on the concentration of oxygen and thiol [Hogg et al., 1996; Singh et al., 1996]. Exposure to nitrogen oxides lead to intracellular S-thiolation and this is regarded as an intracellular response to oxidative stress [Mohr et al., 1999; Pudgett et al., 1998].

\[ RSNO + R'S^- \rightarrow RSSR^- + NO^- \]  
\[ \text{(1.24)} \]
1.11.7 With free radicals

Free radical reactions are important because of their *in vivo* production. Hydroxyl radicals (\(\cdot{OH}\)) and superoxide radicals (\(O_2^{*-}\)) are the most important entities among the free radicals. The enzymes present inside the body, i.e., NADPH oxidase, leads to the formation of \(O_2^{*-}\) [Afanasiev et al., 1995]. Recent studies reveal that \(\cdot{OH}\) is produced during oxidative stress [Riley, 1994]. The reactions of \(O_2^{*-}\), produced from the enzyme xanthine oxidase, with RSNOs were reported. The reports have shown that the end products are nitrite, nitrate and disulfide. While, at high pH, peroxynitrite is one of the major products [Aleryani et al., 1998; Jourd'heuil et al., 1998].

\[2GSNO + O_2^{*-} + H_2O \rightarrow GSSG + NO_3^- + NO_2^- + 2H^+ \] \hspace{1cm} (1.25)

The rate of this reaction was found to be inconsistent in various reports [Afanasiev et al., 1995; Aleryani et al., 1998; Riley, 1994]. These may be due to the interference reactions of RSNOs with the enzymes. Radiation technique is one of the most suitable and reliable methods to study the properties of the intermediates and for determining the rate of free radical reactions. Recently Ford et al. calculated the rate of the reaction of RSNO with \(O_2^{*-}\) using the pulse radiolytic technique [Ford et al., 2002]. This avoids the possibility of interference of other competitive reactions. The
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fundamentals of free radical formation by radiation chemical processes have been discussed in Section 1.12.

1.11.8 Photolytic and thermal decomposition

The mechanism of photolytic and thermal decomposition of RSNO is believed to be via homolytic cleavage of S-N bond [Askew et al., 1995; Wood et al., 1996]. The homolytic bond dissociation energy is found to be between 20-32 kcal mol$^{-1}$ [Barberger et al., 2001; Lu et al., 2001]. RSNOs undergo thermal decomposition and form disulfide and NO [Bainbrigge et al., 1997]. UV light induces decomposition of RSNO and the products are disulfide and NO [Sexton et al., 1994]. This is an important reaction because of their possible use in photodynamic therapy [Shishido et al., 2000]. The fundamentals of its photochemistry are discussed in Section 1.13.

\[ RSNO \stackrel{hv/\Delta}{\longrightarrow} \frac{1}{2} RSSR + NO \]  

(1.26)

1.12 Aqueous Radiation Chemistry: Production of Free Radicals

Madame Currie was the first who suggested that primary effect of high-energy ionizing radiation on any substance is the formation of ions, which are the precursors of chemical changes. Charged particles like electrons, protons, deuterons, $\alpha$-rays etc and electromagnetic radiation of short wavelength namely $\gamma$-rays and X-rays come under ionizing radiation. Radiation Chemistry deals with chemical changes produced by the
interaction of high energy ionizing radiation of KeV or MeV range. One photon or particle can ionize more than one molecule. In the case of dilute solutions, it is the solvent that absorbs almost all of the energy and produce solvent excited states, ions and radicals, and they in turn react with the solute giving solute excited states, ions and radicals. The absorption of energy from light waves entirely depends on the atomic composition and not on molecular structure. The energy deposition takes place inhomogeneously in the medium so that the initial products are also distributed inhomogeneously till the process of diffusion takes place.

1.12.1 Radiation Sources

These can be mainly classified as radionuleide sources, particle accelerators and reactors. Sources can be chosen depending on the nature and size of the object to be irradiated. Chemical change during interaction depends both on the total quantity of radiation energy available and on the rate at which the energy is deposited. Linear Energy Transfer (LET), the energy actually deposited per unit distance along the track, increases in the order of γ-rays, high energy electrons < low-energy X-rays, β-particles < protons < deuterons < α-particles < heavy ions < fission fragments from nuclear reactions.
1.12.2 Ionization and excitation

When moving charged particle interact, particles get slow down with energy loss. This gives rise to both excited and ionized atoms and/or molecules in the path of the particle. This energy deposition path is known as *track*.

Electrons ejected during this process may themselves be sufficient to produce further ionization/excitation. If the energy of these secondary electrons is \(< 100\, \text{eV}\) their range in liquid or solid materials will be short. If they produce any secondary ionization, it will be close to the original ionization which gives rise to a small cluster of excited and ionized species called *spur*.

The energy transferred from the high-energy radiation to the interacting medium will decide the size of the spurs. If the energy transformation is between 100-500 \(\text{eV}\) then the secondary electrons can travel more distance before its energy is reduced to subexcitation level. Thus the reactive species are distributed in a larger volume called *bolb*. If the energy transferred is between 500-5000 \(\text{eV}\), the secondary electrons travel much greater distance and the path followed by these electrons are called a *short track*. For still much higher energy transfer, the secondary electron itself acts as a high energy particle and the path is called a
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*branched track* since it is branching off from the main track. When the spurs overlap; as in the case of densely ionizing radiation like $\alpha$-particles; a column of ions and excited species are formed. The tracks thus formed are called *columnar ionization*. Less ionizing radiation - low LET radiation - produce spurs at longer intervals along the track. After the formation, the reactive species diffuse in space with time. By around $10^{-7}$ s, a uniform distribution in the medium takes place and the species concentration is reduced considerably compared to that in the spurs. The products thus formed are termed as *primary products*. That is the primary products in any medium can be cations, anions, electrons, radicals and excited states.

### 1.12.3 Radiation Techniques

The radiation techniques can be divided into two *viz.* steady state radiolysis and pulse radiolysis. In steady state techniques, the irradiation is carried out with X-ray or $\gamma$-ray. The end products are then analysed with the help of spectrophotometers like UV-VIS or IR and also using high performance liquid chromatography, gas chromatography, liquid chromatography-mass spectrometry etc. While in pulse radiolysis method, particle accelerators such as linear accelerator (LINAC), van de graaf generator etc. are used to generate high-energy radiation. This technique is usually used for detecting the transient species formed during fast reactions and the detection part
involve optical absorption spectroscopy, electron spin resonance spectroscopy, Raman spectroscopy, Rayleigh scattering, conductance etc. This technique is also used for kinetic studies of fast reactions.

1.12.4 Radiolysis of Water

Water is the most important component in biological systems. The behaviour of water is somewhat different from other materials since the initial radiolytic products do not react readily with the solvent itself because of the high O-H bond strength. Water now provides a very convenient way of generating a variety of unstable species under well defined conditions. The time scale of various events in the radiolysis of water is shown in Scheme 1.5.

Recent experimental evidences using a subpicosecond laser pulse to generate excess electrons by photo-ionization have shown that electron is solvated in less than $10^{-12}$ s, which is much shorter than the dielectric relaxation time of water ($10^{-11}$ s) [Kozawa et al., 1999]. This means that the electron finds a preferred site in the medium that largely satisfies its solvation requirements. The spur expansion is complete by about $10^{-7}$ sec. When dilute aqueous solutions ($\leq 10$ mol m$^{-3}$) are irradiated, all the energy absorbed is deposited in water molecules and the observed chemical changes are brought about indirectly by the radical and molecular products
of water radiolysis. This means, the direct action due to energy deposited directly in the solute is not important in dilute solutions.

**Scheme 1.5:** Time events in the radiolysis of water

1.12.5 Primary Species and Their Properties

Radiolysis of water and dilute aqueous solutions leads to the formation of radical species such as hydrated electron ($e_{aq}^-$), hydroxyl radical ($^\cdot$OH) and
hydrogen radical (\( {^\bullet}H \)), and molecular products such as hydrogen gas and hydrogen peroxide as shown in equation 1.27.

\[
H_2O \rightarrow e^{-}\text{aq}, {^\bullet}H, {^\bullet}OH, H_2O_2, H_2, H_3O^+ \quad (1.27)
\]

These radical and molecular species are known as primary species. \( {^\bullet}OH \) is oxidizing in nature while \( e^{-}\text{aq} \) and \( {^\bullet}H \) are reducing in nature. \( e^{-}\text{aq} \) has a strong absorption band at 715 nm while \( {^\bullet}OH \) and \( {^\bullet}H \) have weak absorption bands in the UV region (Fig 1.2).

---

**Fig. 1.2:** Absorption spectra of \( {^\bullet}OH, e^{-}\text{aq}, {^\bullet}H \) and \( \text{HO}_2^\bullet \).
The properties of the major free radicals are given below.

**a. Hydroxyl radicals (•OH) and Oxide radical anion (O•−)**

In acidic solution, the standard reduction potential of •OH is 2.72 V while in neutral solution it is 1.89 V against NHE [Schwarz et al., 1984].

With organic compounds, •OH can undergo four types of reactions.

i) Electron transfer

\[ •OH + X^- \rightarrow X + OH^- \] (1.28)

ii) Hydrogen abstraction

\[ •OH + RH \rightarrow R + H_2O \] (1.29)

iii) Addition

\[ •OH + RH \rightarrow HROH \] (1.30)

iv) Displacement

\[ •OH + RX \rightarrow X + ROH \] (1.31)

It is difficult to follow the decay of •OH absorption and therefore, the rate constants for •OH reactions are generally determined by following the product formation or by using the competition method, where two solutes compete for •OH and one of the products can be measured.

In alkaline solutions, •OH is converted to its conjugate base O•−

\[ •OH + OH^- \Leftrightarrow H_2O + O^- \] (1.32)
O$^*$ can react rapidly with oxygen to give O$_3^*$

$$\text{O}^* + \text{O}_2 \rightarrow \text{O}_3^*$$  \hspace{1cm} (1.33)

$'$OH is unreactive towards oxygen. $'$OH behaves like an electrophile in its reactions with organic molecules where as O$^*$ is a nucleophile. The major properties of $'$OH are given in Table 1.2.

| Table 1.2: The properties of $'$OH [Dorfman et al., 1973] |
|----------------|-----------------|
| **Diffusion constant** | $2.3 \times 10^{-9}$ m$^2$ s$^{-1}$ |
| $\lambda_{\text{max}}$ | 230 nm |
| $\varepsilon_{230 \text{ nm}}$ | 530 dm$^3$ mol$^{-1}$ cm$^{-1}$ |
| $E^0 (\text{^*OH} + H^+ + e \rightarrow H_2O)$ | 2.7 V (in acidic solution) |
| $E^0 (\text{^*OH} + e \rightarrow \text{OH}^-)$ | 1.8 V (in acidic solution) |
| $pK_a (\text{^*OH} \iff \text{O}^* + H^+)$ | 11.9 ± 0.2 |

b. **Hydrated electron ($e^-_{\text{aq}}$)**

Electrons can be observed in molecules such as water, simple alcohols, ethers and amines, which have no low lying vacant orbitals. Organic molecules with low lying vacant orbitals react rapidly with $e^-_{\text{aq}}$, which act as a nucleophile. Its reactivity is generally enhanced by electron withdrawing substituents adjacent to the double bonds or attached to the aromatic rings. Although the precise details of the structure of $e^-_{\text{aq}}$ in liquid water are not yet settled, they may be visualized as a localized electron surrounded by oriented water molecules [Hart et al., 1970]. Using
Resonance Raman Spectroscopy, Tauber et al. proposed a 'solvated anion cluster' where an electron bonded to more than one water molecule. Here a large number of water molecules are directly hydrogen bonded to the electron [Tauber et al., 2003]. In another observation based on hybrid super molecule-continuum approach, Zhan et al. proposed the stable structure in which electron forms two strong electron-hydrogen bonds of the $\cdots\text{HO}$ type with H-bonded water cluster and two of the H bonds in the neutral water cluster are broken to accommodate these new bonds [Zhan et al., 2003]. $e^-_{\text{aq}}$ is a powerful reducing agent and its reactions are one electron transfer process.

$$e^-_{\text{aq}} + S^{n} \rightarrow S^{n-1}$$

(1.34)

where n is the charge of the solute S.

Because of its intense absorption band in the visible region it is simple to follow its reaction by using pulse radiolysis combined with kinetic spectrophotometry. The standard reduction potential of $e^-_{\text{aq}}$ is $-2.9$ V. The major properties of $e^-_{\text{aq}}$ are summarized in Table 1.3.

**Table 1.3: Properties of $e^-_{\text{aq}}$ [Dorfman et al., 1973]**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius of charge distribution</td>
<td>0.25-0.3 nm</td>
</tr>
<tr>
<td>Diffusion constant</td>
<td>$4.9 \times 10^{-5}$ cm$^3$ s$^{-1}$</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td>715 nm</td>
</tr>
<tr>
<td>$\varepsilon_{715 \text{nm}}$</td>
<td>$1.85 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$</td>
</tr>
<tr>
<td>Standard reduction potential</td>
<td>-2.9 V</td>
</tr>
</tbody>
</table>
c. Hydrogen Radical (\(\cdot\)H)

\(\cdot\)H is the major reducing radical in acidic media, but it is less important in neutral and alkaline solutions. It is a weak reducing agent compared to \(e^-_{aq}\). It is a weak acid with \(pK_a\) 9.6.

\[
\cdot\text{H} + H_2O \rightleftharpoons e^-_{aq} + H_3O^+ \tag{1.35}
\]

It has \(\lambda_{max}\) at about 200 nm. It can abstract hydrogen atom from organic molecules to give rise to \(H_2\). In strong acidic solutions it reacts with \(H^+\) to give \(H_2^{++}\).

\[
\cdot\text{H} + H^+ \rightarrow H_2^{++} \tag{1.36}
\]

d. Perhydroxyl radical (\(\text{HO}_2^-\))

It is not a significant primary radical with low LET radiation but it is an important secondary radical in oxygenated aqueous solutions. It can also be produced by the reaction of \(\cdot\text{OH}\) with \(H_2O_2\).

\[
e^-_{aq} + O_2 \rightarrow O_2^- \rightleftharpoons \text{HO}_2^+ (pH < 5) \tag{1.37}
\]

\[
\cdot\text{OH} + H_2O_2 \rightarrow H_2O + \text{HO}_2^- \tag{1.38}
\]

\[
\cdot\text{H} + O_2 \rightarrow \text{HO}_2^- \tag{1.39}
\]

The anion of \(\text{HO}_2^+\), superoxide ion (\(O_2^{--}\)) \(\text{HO}_2^+ \rightleftharpoons O_2^{--} + H^+ (pK_a = 4.8)\), has considerable importance in living systems. \(O_2^{--}\) mediated reactions in
cellular medium is one of the actively pursued research areas in life science. In human body, xanthine oxidase produces $O_2^{*-}$ during bacterial attack.

The standard reduction potentials of $HO_2^*$ is $-0.05 \text{ V}$ and of $O_2^{*-}$ is $-0.33 \text{ V}$. Both have characteristic absorption spectra with $\lambda_{\text{max}}$ at 225 and 245 nm, respectively, with $\varepsilon_{225 \text{ nm}} = 1400$ and $\varepsilon_{245 \text{ nm}} = 2300 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. In the absence of any reactive substrate, they undergo disproportionation.

$$HO_2^* + HO_2^* \rightarrow H_2O_2 + O_2 \quad (1.40)$$

$$HO_2^* + O_2^{*-} + H_2O \rightarrow H_2O_2 + O_2 + OH^- \quad (1.41)$$

e. Hydrogen peroxide ($H_2O_2$)

$H_2O_2$ is a weak acid with $pK_a 11.6$. It is one of the molecular products of the radiolysis of water.

$$H_2O_2 \rightarrow HO_2^- + H^+ \quad (1.42)$$

It can act as a weak oxidant or reductant.

1.12.6 Yields of primary radicals

The yields of primary radicals are usually represented based on $G$ values. $G$ value is defined as the number of molecular or radical products produced or destroyed by the absorption 100 eV of energy. The material balance
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equation for the yield of radiolytic products of water is as follows [Allen, 1954].

\[ G(-H_2O) = 2G(H_2) + G(H^+) + G(e^-_{aq}) = 2G(H_2O_2) + G(\cdot OH) \] (1.43)

It is possible to measure the yields of the radicals directly by using pulse radiolysis. But \(e^-_{aq}\) is only measured in this way because \(\cdot H\) and \(\cdot OH\) have weak absorption spectra in UV. Both of them can be measured by adding solutes that react to give observable products under pulse radiolytic conditions. The G values of primary products of water in neutral pH are given in Table 1.4.

Table 1.4: Yield of primary species formed in the radiolysis of water at neutral pH [Spinks et al., 1990]

<table>
<thead>
<tr>
<th>Species</th>
<th>G value / (\mu) mol J(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\cdot OH)</td>
<td>0.27</td>
</tr>
<tr>
<td>(e^-_{aq})</td>
<td>0.27</td>
</tr>
<tr>
<td>(H_3O^+)</td>
<td>0.27</td>
</tr>
<tr>
<td>(\cdot H)</td>
<td>0.06</td>
</tr>
<tr>
<td>(H_2)</td>
<td>0.045</td>
</tr>
<tr>
<td>(H_2O_2)</td>
<td>0.07</td>
</tr>
<tr>
<td>(HO_2^*)</td>
<td>0.0027</td>
</tr>
</tbody>
</table>
1.12.7 Secondary radicals

Secondary radicals are produced by the reaction of primary radicals with suitable radical scavengers and solutes under appropriate conditions. Example: \( \text{CO}_2^* \), \((\text{CH}_3)_2^*\text{COH}, \text{CH}_2\text{OH}, \text{Cl}_2^* \), \( \text{SO}_4^* \), \( \text{CH}_2\text{CHOHCH}_3 \) are few among them and their production in aqueous medium are presented in reaction (1.44-1.52).

\[
\begin{align*}
* \text{H} / *\text{OH} + \text{HCO}_3^- & \rightarrow \text{CO}_2^- + \text{H}_2 / \text{H}_2\text{O} \quad (1.44) \\
* \text{H} / *\text{OH} + (\text{CH}_3)_2\text{CHOH} & \rightarrow (\text{CH}_3)_2^*\text{OH} + \text{H}_2 / \text{H}_2\text{O} \quad (1.45) \\
* \text{H} / *\text{OH} + \text{CH}_3\text{OH} & \rightarrow *\text{CH}_2\text{OH} + \text{H}_2 / \text{H}_2\text{O} \quad (1.46) \\
* \text{H} / *\text{OH} + \text{SO}_4^{2-} & \rightarrow \text{SO}_4^- \quad (1.47) \\
\text{e}_{\text{aq}} / \text{H}^+ + \text{S}_2\text{O}_4^{2-} & \rightarrow \text{SO}_4^- + \text{SO}_4^{2-} \quad (1.48) \\
* \text{H} / *\text{OH} + \text{Cl}^- & \rightarrow \text{HOCl}^- \quad (1.49) \\
\text{HOCl}^- + \text{H}^+ & \rightarrow \text{H}_2\text{O} + \text{Cl}^+ \quad (1.50) \\
\text{Cl}^+ + \text{Cl}^- & \rightarrow \text{Cl}_2^- \quad (1.51) \\
\text{N}_3^- + *\text{OH} & \rightarrow \text{N}_3^- + \text{OH}^- \quad (1.52)
\end{align*}
\]

1.13 Photochemistry

In photochemistry, the reactions are initiated by electronically excited molecules. Photochemistry deals with chemical changes produced by
photons in the UV-VIS region. The energy of photon involved is only a few eV. Only a single molecule can be excited by one photon. When a dilute solution of compound A in water is photolysed it is A which absorbs the energy and produce excited states. The absorption of energy from light waves depends on the molecular structure of the compound and not on the atomic composition. The energy is deposited homogeneously in a plane perpendicular to the direction of incident light. So the intermediate products are also distributed homogeneously in the medium.

**Table 1.5: Properties of electromagnetic radiation**

<table>
<thead>
<tr>
<th>Type of Radiation</th>
<th>Frequency Range (Hz)</th>
<th>Wavelength Range</th>
<th>Type of Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-rays</td>
<td>$10^{20}$-$10^{24}$</td>
<td>$&lt;1$ pm</td>
<td>Nuclear</td>
</tr>
<tr>
<td>X-rays</td>
<td>$10^{17}$-$10^{20}$</td>
<td>1 nm-1 pm</td>
<td>inner electron</td>
</tr>
<tr>
<td>Ultraviolet</td>
<td>$10^{15}$-$10^{17}$</td>
<td>400 nm-1 nm</td>
<td>outer electron</td>
</tr>
<tr>
<td>Visible</td>
<td>$4-7.5 \times 10^{14}$</td>
<td>750 nm-400 nm</td>
<td>outer electron</td>
</tr>
<tr>
<td>Near-infrared</td>
<td>$1 \times 10^{14}$-$4 \times 10^{14}$</td>
<td>2.5 $\mu$m-750 nm</td>
<td>outer electron, molecular vibrations</td>
</tr>
<tr>
<td>Infrared</td>
<td>$10^{13}$-$10^{14}$</td>
<td>25 $\mu$m-2.5 $\mu$m</td>
<td>molecular vibrations</td>
</tr>
<tr>
<td>Microwaves</td>
<td>$3 \times 10^{11}$-$10^{13}$</td>
<td>1 mm-25 $\mu$m</td>
<td>molecular rotations, electron spin flips</td>
</tr>
<tr>
<td>Radio waves</td>
<td>$&lt;3 \times 10^{11}$</td>
<td>$&gt;1$ mm</td>
<td>Nuclear spin flips</td>
</tr>
</tbody>
</table>
1.13.1 Quantum Efficiency or Quantum Yield ($\Phi$)

The efficiency of a photochemical reaction is expressed in terms of quantum yield and is defined as the number of molecules decomposed or formed to the number of quanta absorbed. According to Stark-Einstein law, the sum of quantum yields for all primary processes including deactivation must be unity. If a secondary reaction takes place then the quantum yield is greater than unity while the quantum yield is less than unity for a chain reaction.

1.13.2 Electronic Energy States

The molecular electronic states can be classified into singlet states (S) or triplet states (T), in terms of spin multiplicities. $S_0$ denotes the ground state singlet state while higher singlet states are designed as $S_1$, $S_2$, $S_3$ etc. The triplet state energies are always lower than the corresponding singlet state of the molecule because of Hund's rule. The lower energy states are having higher multiplicities.

1.13.3 Interaction with Matter

When light interacts with molecules, the electric field of former will interact with the dipole moment of the latter. The oscillating electric field of the frequency of incident radiation becomes equal to molecular transition, a resonance occurs and the ground state molecule can absorb a
photon to go into the excited state, that is absorption process takes place. But if the molecule is already in the excited state, then the incident radiation can stimulate the molecule to emit a photon, which results an emission process and thus the molecule come back to the ground state.

1.13.4 Jablonski Diagram

The excited state will try to release its excess energy by some means to attain minimum energy conditions. The various pathways, photophysical and photochemical processes, by which an excited molecule dissipates its excess energy can be schematically represented using a pictorial diagram, the Jablonski diagram (Fig. 1.3)

![Jablonski Diagram](image)

**Fig. 1.3:** Jablonski Diagram (where ABS – Absorbance, S₀, S₁, S₂ – Singlet electronic energy levels, FL – Fluorescence, T₁, T₂ – Corresponding triplet states, IC – Nonradiative internal conversion, ISC – Intersystem crossing, PH – Phosphorescence)
Radiative and non-radiative processes come under photophysical process. Fluorescence, phosphorescence etc are radiative processes while internal conversion (IC) and inter system crossing (ISC) are non-radiative. Radiative and non-radiative processes in $S_1$ occur in $10^{-7}$ to $10^{-9}$ s while in $T_1$ these take place at $10^{-3}$ to $10^{-6}$ s. Most photo induced chemical reactions are known to occur in $T_1$ state of the molecule while some fast reactions are also possible in $S_1$ state of the molecules. The time scale of different photochemical processes is shown in Fig. 1.4.

**Fig. 1.4:** The time scale of different photochemical processes

The chemical behaviour of molecules depends on the weakly bound electrons. The reactions can be of photo fragmentation or photo dissociation and isomerization or rearrangement, former case becomes very
probable when the energy absorbed is equal to or more than the bond dissociation energy. When the molecule dissociates from an excited state it is called photolysis.

1.13.5 Types of Photochemical Reactions

The molecule in its excited state differs from the ground state with respect to both energy and electron wave function and hence there is a difference in chemistry too. Excited state of a molecule can be achieved by irradiating the molecule with light of appropriate wave length. The excited electronic state alters the reactivity of molecules. The main photochemical reactions include photo dissociation, isomerization, redox reaction, dimerization and cyclo addition (Fig. 1.5).

![Fig. 1.5: Fate of the electronically excited species](image_url)
1.14 Scope of the work

Nitric oxide (NO), produced by a variety of cell types such as endothelial cells, neutrophils, neurons and hepatocytes in biological systems, has gained lot of attention in recent years due to its possible role in various biological activities. The involvement of NO can be seen in several biological events including photoreceptor signalling [Horio et al., 1991], platelet deaggregation [Rodomski et al., 1987], and neuronal communication [Zhang et al., 1995]. Murine macrophages and other cells release NO which acts as a cytotoxic molecule for invading intracellular microorganisms and tumor cells [Barnes, 1995]. Most of the bioregulatory actions of NO have been attributed to the formation of S-nitrosothiol (RSNC) by its reaction with the thiol group of proteins [Aravindakumar et al., 1999] and hence this molecule has gained considerable attention in recent years. The formation and degradation of RSNO can be considered as the most suitable general way to store, to transport and to release NO [Stamler et al., 1995]. Among the various biological functions, it is reported that the cytotoxicity depends on the decomposition time of the RSNO. The compounds that release NO rapidly are less cytotoxic than the compounds that release NO slowly [Sexton et al., 1994]. Therefore, it is important to know the kinetics as well as the mechanistic aspect of the degradation of RSNO. The present study is focused on the investigation of the degradation of a variety of
RSNO obtained from low molecular weight thiols. The effects of visible light, UV light and ionising radiations in aerated and argon saturated conditions have been investigated.

1.1.5 Objectives

The major objectives are


2. Determination of the second order rate constant of the reaction of free radicals such as hydroxyl radical (\(^{*}\)OH) and hydrated electron (\(e^{-}_{aq}\)) with RSNOs at acidic and neutral pH using radiation chemical techniques.

3. Investigation of the end products of the reaction of \(^{*}\)OH and \(e^{-}_{aq}\) with RSNOs at acidic and neutral pH under aerated and argon saturated conditions.

4. Determination of the kinetics of the degradation of RSNOs induced by sunlight and UV light in aerated and argon saturated conditions.

5. Analysis of the photo degradation products of RSNOs at acidic and neutral pH in aerated and argon saturated conditions.

6. Elucidation of reaction mechanism of the formation of end products from 3 and 5.

7. Discussion on the biological significance of these reactions.