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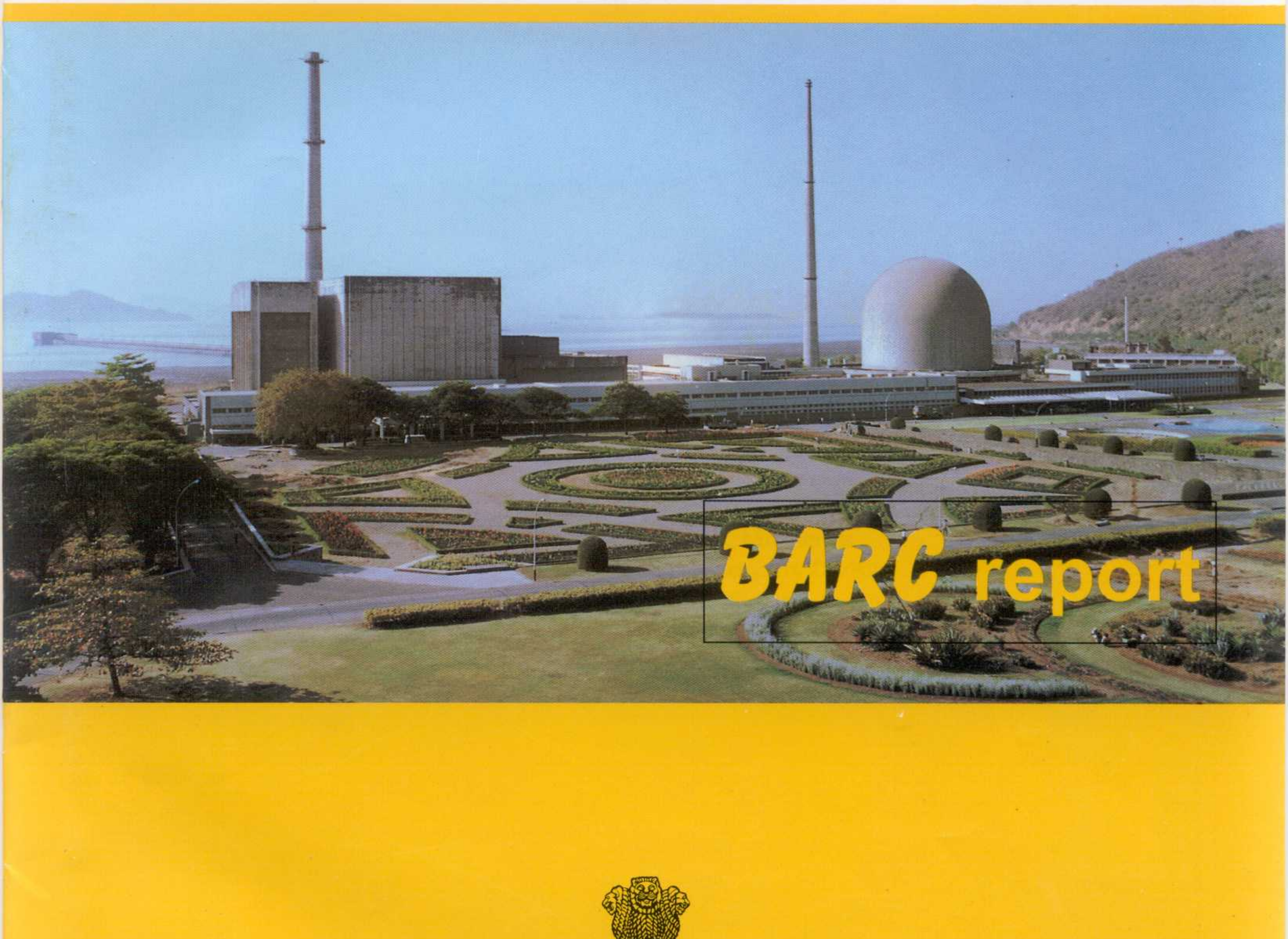

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एकक आक्सीजन कार्बनिक अणुओं के साथ फोटो सुग्राहित जनन, संसूचक एवं अभिक्रिया

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ए.वी. सप्रे, जे.पी. मित्तल एवं टी. मुखर्जी

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सारांश

एकक आण्विक आक्सीजन ($^1O_2$) आण्विक आक्सीजन की एक उत्प्रेरित स्थिति है जिसमें उसी π^* प्रति – आबंधन आर्बिटल में प्रतिसमांतर चक्र मौजूद है । एकक आक्सीजन उत्पादन एवं अभिक्रिया का अध्ययन संश्लिष्ट रसायनिकी, बहुलक रसायनिकी, प्रकाशगतिक चिकित्सा आदि जैसे क्षेत्रों में समृद्ध एवं विविध अवसर के रूप में उभरा है । एकक आक्सीजन के उत्पादन के लिए अनेक ज्ञात पद्धतियां हैं और उनके साथ–साथ इसके संसूचन हेतु विभिन्न तकनीक भी हैं । इनमें से फोटो–सुग्राहीकरण पद्धति सबसे लोकप्रिय है । इस लेख में प्रकाश की उपस्थिति में त्रिक आक्सीजन एवं फोटो–सुग्राहीकारकों से एकक आक्सीजन का फोटो–सुग्राहित उत्पादन, एवं 1270 nm पर अवरक्त संदीप्ति द्वारा इसका संसूचन प्रस्तुत है । इसके अतिरिक्त विविध प्रकार के फोटो–सुग्राहकों का प्रयोग करते हुए एकक आक्सीजन क्वान्टमी लब्धि एवं जीवनकाल पर विलायक के प्रभाव के कुछ परिणामों की चर्चा की गई है । एकक आक्सीजन के शमन दर स्थिरांकों को थायोयूरिया व्युत्पन्न, उसके अनुरूपों, हाइड्राक्सिल इंडोल एवं प्रतिऑक्सीकारक जैसे विभिन्न प्रकार के कार्बनिक अणुओं के साथ निर्धारित कर इसका परिणाम दिया गया है ।

Contents

Singlet oxygen: Photosensitized generation, detection and reaction with organic molecules

Abstract

Singlet molecular oxygen ($^1O_2$) is an excited state of molecular oxygen, having anti parallel spin in the same π^* antibonding orbital. The study of singlet oxygen production and reactivity has emerged as a rich and diverse area, with implication in diverse fields, such as synthetic chemistry, polymer chemistry, photodynamic therapy, etc. There are several known methods to produce singlet oxygen, and also various techniques employed to detect it. Out of these, photosensitization method is the most popular one. In this article, photosensitized production of singlet oxygen from triplet oxygen and photosensitizers in presence of light, and its detection by the infrared luminescence at 1270 nm have been presented. Further, some results using different types of photosensitizers, effect of solvent on singlet oxygen quantum yields and lifetime have been discussed. The quenching rate constants of singlet oxygen have been determined with different types of organic molecules such as derivatives of thiourea and its analogues, hydroxy indoles and antioxidants and the results have been presented.

1. Introduction

Reactions of singlet oxygen ($^1O_2$) are of great current interest due to their importance in many photo-oxidation reactions in chemistry and biology, including those used in photo-chemotherapy [1-6]. Oxygen was discovered by Josheph Priestley in the year 1775 [7]. In 1811, Avogadro reported that oxygen is a diatomic molecule. Faraday observed the paramagnetic nature of molecular oxygen in 1884 [8]. Although discovered in 1924, singlet molecular oxygen ($^1\Delta_g$) came into the focus of research only after 1963, when Khan and Kasha interpreted the chemiluminescence of the hypochlorite-peroxide reaction as caused by liberation of singlet oxygen [9]. In the last four decades, there are several reports on singlet oxygen chemistry and its reaction with organic compounds, which are important in medicine, biochemistry, food chemistry and environmental chemistry [8]. Singlet oxygen is a highly reactive, electrophilic and non-radical molecule. Early work with singlet oxygen showed that this active species could oxidize substrates that are unaffected by oxygen in its normal energy state. Due to high electrophilicity, it rapidly reacts with unsaturated carbon-carbon double bonds, and is capable of oxidizing phenols, sulphides, amines, etc. [10]. Consequently, organic chemists have found utility in singlet oxygen as a versatile synthetic reagent [11]. Singlet molecular oxygen is implicated in photosensitized processes, such as photodynamic inactivation of viruses and cells, cancer therapy, photo degradation of polymers, and as a potential therapeutic agent for the treatment of malignant tumors [12-17]. Many cellular organelles and biomolcules have high reactivity for singlet oxygen.

Electronic structure of molecular oxygen could be understood in a better way, considering molecular orbital theory. The lowest electronic state of oxygen is a triplet ground state ($^3\Sigma_g^-$), with two unpaired electrons distributed in the highest occupied orbital. Rearrangement of the electron spins, within these two degenerate orbitals, results in two possible excited states (table-1).

The $^1\Delta_g$ state has both the electrons paired in a single orbital, leaving the other π orbital vacant, which is 94 kJ mol^{-1} higher energy than the ground state [18]. The other singlet state ($^1\Sigma_g^+$) comes from spin pairing of the electrons in different π orbitals, and is 157 kJ mol^{-1} higher than the ground triplet oxygen ($^3\Sigma_g^-$). In both the forms of $^1O_2$, the spin restriction is removed so that the oxidizing ability is greatly increased. Hereafter, the word singlet oxygen refers to the $^1\Delta_g$ state only.

Table-1: *Electronic distribution in molecular oxygen*

States of the oxygen molecule	**Occupancy of highest orbital**	**Energy above ground state**
Second excited state	$^1\Sigma_g^+$ $(\uparrow)_\pi$ $(\downarrow)_\pi$	157 kJ / mol
First excited state	$^1\Delta_g$ $(\uparrow\downarrow)_\pi$ $(\ )_\pi$	94 kJ / mol
Ground state	$^3\Sigma_g^-$ $(\uparrow)_\pi$ $(\uparrow)_\pi$	

The electronic transitions $^1\Delta_g \leftarrow {}^3\Sigma_g^-$ and $^1\Sigma_g^+ \leftarrow {}^3\Sigma_g^-$, although highly forbidden, are readily observed by absorption and emission in the upper atmosphere with zero-zero transition at 1270 and 762 nm, respectively. The estimated radiative lifetimes of these two states in the gas phase are 45 min and 7-12 s, respectively [19]. In solution, the lifetimes of these states are reduced drastically, depending on the solvent polarity. $^1\Delta_g$ state has a relatively long lifetime (1 to 10 μs) in the absence of molecules with which it can undergo rapid reaction. On the other hand, $^1\Sigma_g^+$ state is short-lived, and usually undergoes rapid decay (with $k \sim 10^{11}$ s^{-1}), rather than chemical reaction [20].

The most versatile source of singlet oxygen generation is by electronic energy transfer from suitable photosensitizers to molecular oxygen. However it can also be generated by a number of other processes, such as irradiation with Nd:YAG lasers of Freon solutions subjected to high pressures of O_2, irradiation of aerated water at 600 nm via vibrationally excited water, absorption by O_2-organic molecule charge-transfer band, sensitized production by pulse radiolysis, microwave generation and chemical production [21-28]. Chemically it is generated by the reaction of hydrogen peroxide with hypochlorite (NaOCl)

The singlet oxygen can be detected by following its luminescence at 1270 nm, time-resolved thermal lensing, energy transfer, etc. [29-34]. In time-resolved thermal lensing method, absorption of a laser pulse increases the local temperature of liquids and gases, which changes the density and refractive index of the medium, which act as a diverging lens. EPR provides the measurement of unpaired electrons in the system and their surroundings. The ground state of oxygen has two unpaired electrons in two π* antibonding orbitals, and thus ground state molecular oxygen is EPR active. In the excited state of molecular oxygen, there is no unpaired electron, and therefore it is EPR inactive. Detection of singlet oxygen and the related kinetics are easily measured from the luminescence of singlet oxygen at 1270 nm [35,36].

1.1 Photosensitized singlet oxygen generation

Photo-excitation of a sensitizer in the presence of oxygen often leads to the production of singlet oxygen by a bimolecular energy transfer process involving excited sensitizer and ground state triplet oxygen. The energy transfer occurs during collision of the excited state of the sensitizer with triplet oxygen. Photosensitized generation is a simple and controllable method for production of singlet oxygen. It requires only oxygen, light of an appropriate wavelength, and a photosensitizer capable of absorbing and using that energy to excite oxygen to its singlet state. Sensitizer excitation is generally achieved via a one photon transition (hν) between the ground state (S_0) and a singlet excited state, which relaxes to the lowest vibrational level of lowest excited state of the sensitizer S_1. Intersystem crossing of the S_1 of sensitizer generates the triplet state (T_1). The lifetime of the T_1 state is longer (μs) than that of the S_1 state (ns), allowing it to react with molecular oxygen.

$$P(S_0) \xrightarrow{h\nu} P(S_1) \xrightarrow{k_{ISC}} P(T_1) \tag{1}$$

$$P(T_1) + {}^3O_2 \xrightarrow{k_{en}} P(S_0) + {}^1O_2 \tag{2}$$

Here P refers to the photosensitizer and S_0, S_1 and T_1 are the corresponding spin states of the photosensitizer. k_{ISC} and k_{en} are the intersystem crossing and energy transfer rate constant, respectively.

1.2 Commonly used sensitizers for generation of singlet oxygen

Several types of photosensitizers have been used for the generation of singlet oxygen. Molecule used as photosensitizer should exhibit the following properties: (a) high absorption coefficient in the spectral region of the excitation wavelength; (b) a well-defined triplet state with appropriate energy, $E_T \geq 95$ kJmol^{-1}, to allow for efficient energy transfer to ground state oxygen; (c) high quantum yield of the triplet state, $\Phi_T \geq 0.4$; (d) long triplet state lifetime, $\tau_T > 1$ μs, since the efficiency of the photosensitizer is dependent on the photophysical properties of its lowest excited triplet state; and (e) high photo-stability. Porphyrins, organic dyes, aromatic hydrocarbons, transition metal complexes, semiconductor nano-particles, etc., are found to be efficient sensitizers of singlet oxygen. Some of the well-known photosensitisers reported in the literature are given in table-2

Table-2: *List of common photosensitizers and their singlet oxygen yields [30]*

Sensitizer	Solvent	Singlet oxygen yield
Hematoporphyrin	Ethanol	0.57
Cercosporin	Benzene	0.81
Fullerene C_{60}	Benzene	1
Fullerene C_{70}	Benzene	1
Methyl violet	Ethanol	0.24
Haematoporphyrin (HP)	Methanol	0.74
Rose Bengal	Water	0.75
Methylene blue	Ethanol	0.52
Eosin blue	Water	0.52
$Ru(bpy)_3^{2+}$	Methanol	0.73
$Ru(phen)_3^{2+}$	Methanol	0.54

1.3 Other methods of generation of singlet oxygen

Chemically singlet oxygen can be generated in large concentrations (> 10%) using reaction of hydrogen peroxide with hypochlorite. Chlorine gas is bubbled through alkaline hydrogen peroxide to generate singlet oxygen

$$H_2O_2 + 2NaOH + Cl_2 \longrightarrow 2NaCl + 2H_2O + {}^1O_2 \qquad (3)$$

Efficiency of generation is ~ 80%.

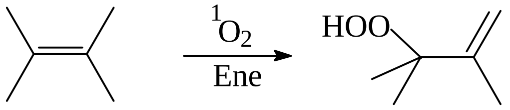
Singlet oxygen is also generated in the microwave discharge excitation of oxygen gas under certain conditions.

1.4 Singlet oxygen as a reagent for organic synthesis

The reactions of singlet oxygen with organic molecules can be categorized as follows

a. Ene and cycloadditions reactions

Due to electrophilic nature, singlet oxygen readily reacts with carbon-carbon double bond and with dienes it reaction under goes cycloaddition. The ene reaction generates allylic hydroperoxides, which can be converted to synthetically valuable allylic alcohols. The cycloaddition leads to formation of endoperoxide, which can be converted to oxygenated product through a variety of synthetic product.



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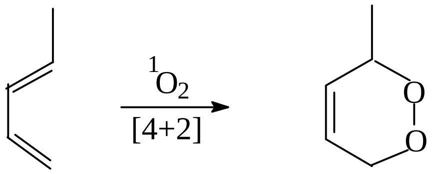

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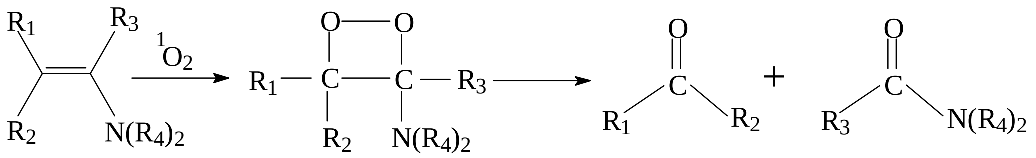
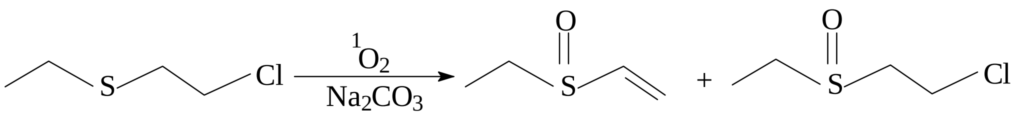
Reaction of singlet oxygen with π-electron rich an olefin forms dioxetane as an unstable intermediate, which readily decomposes to carbonyl groups.



(6)

b. Heteroatom oxidation

By virtue of electrophilic nature, singlet oxygen readily reacts with heteroatom having electron lone pair. Sulphur, selenium, phosphorous and nitrogen compounds are known to react with singlet oxygens, leading to formation of covalent adduct involving transfer of one or two oxygen atom to the heteroatom center.



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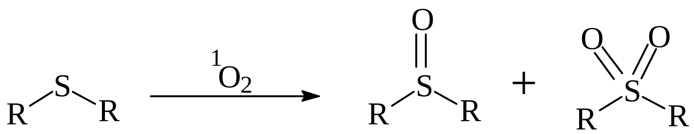

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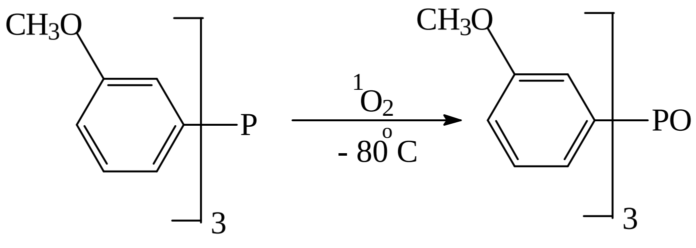

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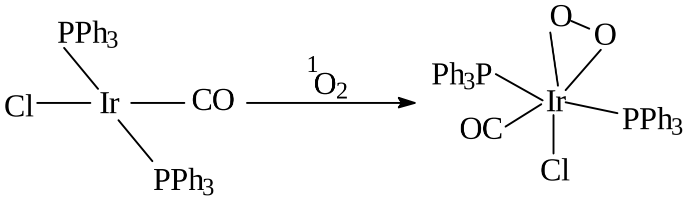
c. Oxidation of organometallic complexes

The chemical reactivity of singlet oxygen with organometallic complexes can be either ligand centered or metal centered. Vaska's complex is an example of metal-centred reaction of singlet oxygen to produce metal –dioxygen bond. In this reaction, Ir (I) is oxidized to Ir(III) peroxo complexes.



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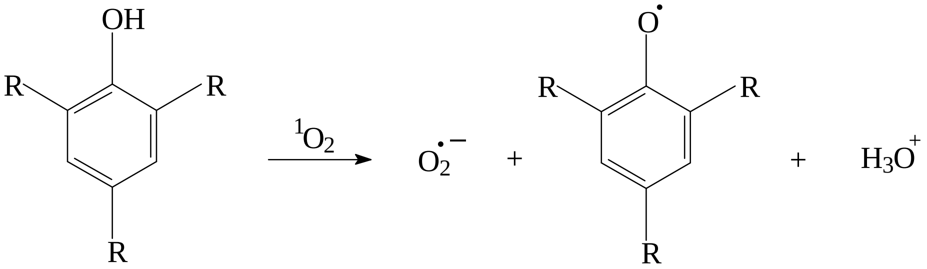
d. Electron transfer

Singlet oxygen reactions may proceed by electron transfer from the electron rich compound to the electrophilic singlet oxygen. Phenols can react with singlet oxygen via electron transfer. Photo oxidation of polystyrene by singlet oxygen has also been reported to proceed by formation of superoxide in addition to radical mechanism [37].



(11)

1.5 Singlet oxygen decay pathways

In the absence of a physical quencher or a chemical acceptor, singlet oxygen is deactivated in solution by collisions with the solvent molecules (main decay pathway, equation 12) and as well as by luminescence (equation 13). The radiative transition of singlet oxygen at 1270 nm is very weak, as the process is forbidden by spin, symmetry and parity consideration.

$$^1O_2 \xrightarrow{k_d} {}^3O_2 \tag{12}$$

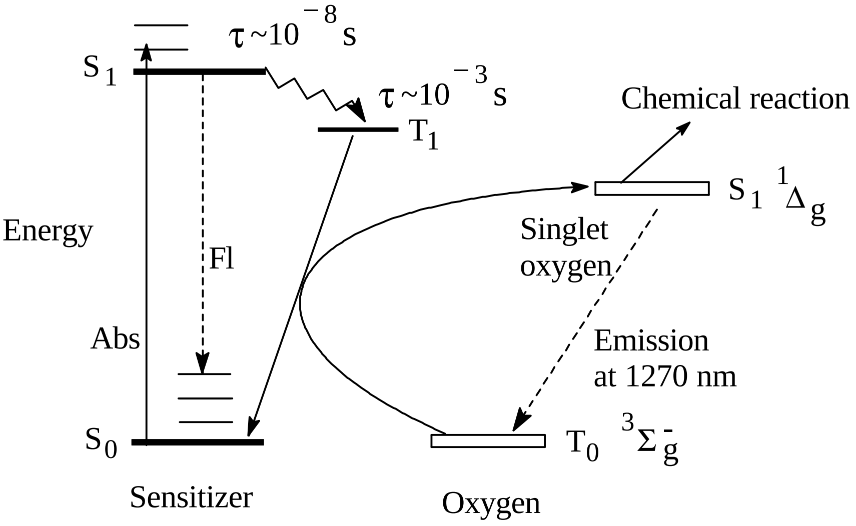
$$^1O_2 \xrightarrow{k_e} {}^3O_2 + h\nu' \text{ (1270 nm)} \tag{13}$$

Here, k_d and k_e are the rate constants of deactivation of singlet oxygen by the solvent molecules and rate constant for radiative transition for singlet oxygen luminescence, respectively.

Singlet oxygen shows "dimeric" emissions, which is known as the dimol emission at ~635 nm. The process is described as “energy pooling” and is similar to triplet-triplet annihilation process

$$^1O_2 + {}^1O_2 \longrightarrow {}^3O_2 + {}^3O_2 + h\nu(635\text{nm}) \qquad (14)$$

All these physical processes are summarized in the scheme-I given below.



Scheme-1

Reaction of singlet oxygen with iodine is exploited for the working of first electronic transition chemical laser COIL (chemical oxygen iodine laser) which operates at 1315 nm [38]. The reactions are

$$2O_2(^1\Delta_g) + I_2 \longrightarrow 2O_2(^3\Sigma_g) + 2I(^2P_{3/2}) \qquad (15)$$

$$O_2(^1\Delta_g) + I(^2P_{3/2}) \longrightarrow O_2(^3\Sigma_g) + I(^2P_{1/2}) \qquad (16)$$

$$I(^2P_{1/2}) \longrightarrow + I(^2P_{3/2}) \quad + \quad 1315 \text{ nm } \textit{laser transition} \qquad (17)$$

CW powers of few kilowatts have already been realized using COIL. This laser finds uses in space and communication type of applications.

Since the IR emission from singlet oxygen is a forbidden process, the emission yield is very low, and very difficult to detect. In the last few years, as a result of the development of suitable and sensitive detectors, it has been possible to detect this weak signal. The use of high intensity laser pulses and high-gain, wide-band amplifiers provides a method to detect time-resolved IR luminescence.

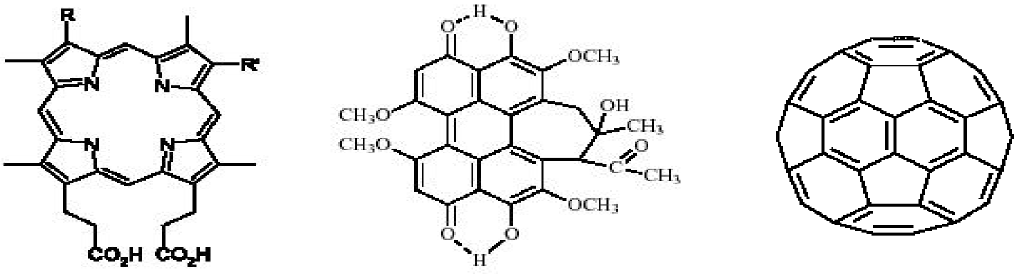
2. Experimental methods

Under the X plan project, a new facility, based on luminescence lifetimes was set up for generation, detection and reactions of singlet oxygen. For this, a transient luminescence spectrometer (Edinburgh Instruments Ltd., U.K, TL 900) has been installed. The spectrometer is based on production of singlet oxygen by photosensitization method and simultaneous detection by infrared emission at 1270 nm. Block diagram of the luminescence spectrometer and its different components are shown in figure-1. The instrument consists of an excitation laser, monochromator, germanium detector coupled to an oscilloscope and computer. For the excitation, Nd:YAG laser (Minilite Continuum USA, flashlamp pumped, Q-switched) was employed. It can be operated at the fundamental (1064 nm, 50 mJ), or its second harmonic (532 nm, 25 mJ), third harmonic (355 nm, 8 mJ) and fourth harmonic (266 nm, 4 mJ) frequencies. The pulse width of the laser is 3-5 ns, with beam size of < 3 mm and repetition rate of 10 Hz. The sample compartment consists of a cell holder in which 1 cm x 1 cm suprasil square cuvette can be placed. The sample holder can be cooled to any desired temperature between –10° to + 90° C, by circulating a thermostated liquid (± 0.5° C), using a variable temperature bath (Julabo Germany; model F12-ED).

The fluorescence emission from the sample is focused on to the entrance slit of a grating monochromator (focal length F/3.5, holographic grating with linear dispersion of 8 nm/mm), which can be used in 800 – 1700 nm region. Low luminescence signals can be detected by replacing the monochromator with suitable filters. The exit slit of the monochromator is fixed to a liquid nitrogen-cooled EI-P germanium detector with optimum spectral response in 800 – 1700 nm region. The detector has an active area of 25 mm^2, is powered by a highly filtered and stable power supply. The output signal from the germanium detector is fed to a 100 MHz digital storage oscilloscope (Tektronix TDS 3012B, 1.25 GS/s) to allow single-shot monitoring and data averaging. The scattered radiation from the fundamental laser pulse (1064 nm) is filtered with the help of a silicon filter placed before the emission monochromator. The transient signal from the oscilloscope can be loaded into a computer using F900 software available with the instrument. Time-resolved luminescence signal of singlet oxygen was monitored at 1270 nm. The software allows data presentation, computation and numerical data reconvolution. The curve fitting procedure for exponential fit and lifetime measurements is based on the Marquard-Levenberg algorithm. This yields the lifetime of singlet oxygen, which is directly related to the total rate of deactivation both physical and chemical quenching. This method is much more reliable than the indirect trapping method or the detection of steady-state IR luminescence of singlet oxygen. Some of the studies carried out, using the above equipment, are discussed below.

3. Results and Discussion

In the present study, hematoporphyrin, hypocrellin-A and fullerenes are used as sensitizers in different sets of experiments. The structures of the sensitizer are shown below. All these sensitizers are used in photodynamic therapy for treatment of cancer.



R = R’ = CH(OH)Me
Haematoporphyrin

Hypocrellin-A

Fullerene (C_{60})

***Scheme-2:** Different types of photosensitizers*

The role of haematoporphyrin in photodynamic therapy is well understood, and it is considered as the first generation of photosensitizers. There are several reports where the photophysical properties of haematoporphyrin have been discussed [39-43]. Hypocrellin-A is a perylene quinone derivative bearing two hydroxyl groups, which form strong hydrogen bonds with the carbonyl groups. Direct excitation of hypocrellin-A produces high yield of triplets, which on reaction with oxygen produce singlet oxygen [44].

Fullerenes are more efficient photosensitizers than organic dyes because of very high triplet quantum yield ($\Phi_T \sim 1$) [45-49]. This is due to very effective intersystem crossing, which is driven with efficient spin-orbit coupling [45]. The energy of the triplet state of C_{60} is 157.5 kJ mol^{-1}, which is very favourable for transfer of the energy to the ground state oxygen ($^3O_2$), giving singlet molecular oxygen ($^1O_2$), with very high quantum yield (~1) [49]. Excitation of C_{60} in benzene (7.0×10^{-4} mol dm^{-3}) at 532 nm showed very good emission due to singlet oxygen (figure-2) with a lifetime 29 μs. The relative yield and lifetime of singlet oxygen remained independent of temperature from 10 to 60 ^{o}C. However, its yield increased with C_{60} concentration up to 4.0×10^{-4} mol dm^{-3}, and with further increase in concentration, the relative yield decreased (inset of figure-2). The decrease in the yield of the singlet oxygen may be due to the decrease in the triplet yield of C_{60} because of the aggregation of C_{60}, which in turn may reduce intersystem crossing rate [50].

3.1 Quantum yield of singlet oxygen in different solvents

To understand the effect of solvents on the yield of singlet oxygen, the quantum yields were determined by direct analysis of IR luminescence at 1270 nm in different solvents. The measurements were done with two independent solutions, one containing the molecule under investigation and the other a reference R, for which singlet oxygen quantum yield is known. The concentrations were adjusted such that the two solutions showed identical absorbances at the excitation wavelength [51,52]. Under these conditions, for identical conditions of laser energy, the ratio of the luminescence intensity is related to the ratio of the quantum yields of singlet oxygen production by the two sensitizers according to equation 18.

$$\frac{\Phi_\Delta}{\Phi_\Delta^R} = \frac{S_e}{S_e^R} \times \frac{(n)^2}{(n^R)^2} \tag{18}$$

where Φ_Δ and Φ_Δ^R are quantum yields of the singlet oxygen from the molecule under investigation and the reference R, respectively. S_e and S_e^R are the measured luminescence signal (in mV) at 1270 nm for the molecule under investigation and the reference R, respectively. Refractive index of the solvent is denoted by n.

Using TL900, quantum yield of singlet oxygen was measured in dichloroethane, chloroform and acetonitrile. Hypocrellin-A in benzene was used as the reference sensitizer to determine the yield of the singlet oxygen in other solvents. The quantum yields of singlet oxygen in different solvents estimated by this are given in table-3. The ability to produce singlet oxygen depends largely on the production of a preferably long-lived triplet state. Excitation of hypocrellin-A gives the triplet state in very high yield. The value of singlet oxygen yield is a function rate of intersystem crossing of the sensitizer, which, in turn, depends on the structure of the sensitizer. Depending on the electronic interaction of the carbonyl moiety of hypocrellin-A with the arene groups and the substituents attached to them, the lowest singlet and triplet states of aromatic ketones may exhibit the characteristics of n,π* or π,π* electronic configuration. The energetic ordering within these states also depends on the solvent. These states are destabilized to different extent with the variation in solvent polarity. In general, the triplet states are polar than the singlet states, as these are far apart from the nucleus. With increasing solvent polarity, the energy gap between the triplet state and the singlet state increases, and thereby reduces intersystem crossing. This, in turn, reduces the yield of singlet oxygen produced by energy transfer from the triplet state of hypocrellin A.

Table -3: *Quantum yield of singlet oxygen in different organic solvents*

Solvent	**Quantum yield of $^1O_2$**
Benzene	0.74
Dichloroethane	0.37
Chloroform	0.35
Acetonitrile	0.18

3.2 Effect of temperature on the lifetime of singlet oxygen

Lifetime of singlet oxygen was studied at different temperatures, maintained by circulating water from a thermostated water bath to the sample chamber in a loop. Since water was used as the bath fluid, the range of temperature was restricted between 10 to 50 °C. Under such conditions lifetime of singlet oxygen was measured in benzene, using C_{60} and haematoporphyrin as sensitizers and listed in table-4.

Table-4: *Effect of temperature on the lifetime of singlet oxygen*

Temperature/°C	t /µs A	t /µs B	t /µs C
10	45	31	31
20	46	--	--
30	44	30	30
40	44	30	31
50	45	30	30

A : Sensitizer hematoporphyrin in acetonitrile
B : Sensitizer hematoporphyrin in benzene
C : Sensitizer C-60 in benzene

There variation in the lifetime of singlet oxygen in the specified temperature range was negligible. The lack of an observed temperature effect on the lifetime and quenching constant of singlet oxygen suggests that bulk properties of the solvent are not involved in the rate-determining step of the energy transfer [53]. Similar results were reported by several authors [53-56].

3.3 Solvent effects on the lifetime of singlet oxygen

The lifetime of singlet oxygen in a collision-free environment (gaseous) is of the order of 45 mins. However, in solution, the lifetime ranges from one to several hundred microseconds, depending on the solvent. Since oxygen does not have as many vibrational degrees of freedom as a polyatomic solvent molecule, the medium may not serve to induce transitions, but may provide a sink for the electronic excitation energy of oxygen. Electronic transitions in molecular oxygen are dependent on the perturbations offered by the solvent [53]. An important aspect of studying the generation of singlet oxygen is the choice of solvent. The following simple rules anticipate the changes of lifetime of singlet oxygen as a function of solvent [56,58-60]. (a) The longest lifetimes are observed in perhalogenetaed solvents. (b) The singlet oxygen lifetime decreases on increasing the number of hydrogen atoms in the solvent molecule. (c) The shortest lifetime are observed in solvents having O-H groups, particularly water. (d) The presence of heavy atom reduces the lifetime. (e) Solvent deuteration invariably increases singlet oxygen lifetime.

Table-5: *Life time of singlet oxygen in some common solvents [56]*

Solvent	t /µs	Solvent	t /µs
H_2O	4.5	CH_2Cl_2	94
D_2O	58	$C_2H_4Cl_2$	66
CH_3OH	23	CH_3OCH_3	50
CD_3OD	224	CD_3OCD_3	860
C_2H_5OH	10	*C_6H_6	31
C_2D_5OD	230	C_6D_6	700
*CH_3CN	61	C_6F_6	3900
CD_3CN	440	C_6H_{12}	23
*CH_3Cl	266	CS_2	200
CD_3Cl	640	Freon 11	1000

* Lifetime obtained from the present study

The rate of decay of singlet oxygen is related to the polarity of the solvent. This trend was observed by Young et.al., when the lifetimes of singlet oxygen in alcohol and mixed alcohol solvents were compared [57]. The decrease in lifetime in protic solvents indicates that hydrogen bonding may play an important role in the deactivation of singlet oxygen. This interaction is weakened considerably by replacement of the hydrogen with deuterium, and further, by replacement of the deuterium with fluorine [56]. Therefore, deuterated solvents provide longer lifetimes of singlet oxygen. Table-5 lists the singlet oxygen lifetimes in different protonated and deuterated solvents.

3.4 Determination of the rate constants of quenching by the singlet oxygen

In the absence of a quencher, singlet oxygen decays by emission of light and as well as by some non-radiative processes discussed above. The rate constant for all these processes can be summed up by the inverse of emission lifetime. In the presence of a quencher, the deactivation rate increases due to additional parallel pathways, like physical and chemical quenching. As a result, the emission lifetime decreases. With increasing concentration of the quencher, the emission lifetime decreases further. This change in lifetime as a function of the quencher concentration can be represented by Stern–Volmer equation (equation 19) [61,62],

$$\tau^{-1} = \tau_0^{-1} + k_q[\mathrm{Quencher}] \qquad (19)$$

where τ and τ_0 are lifetime of singlet oxygen in the presence and absence of a quencher, respectively, and k_q is the quenching rate constant. Thus the plot of reciprocal lifetime of singlet oxygen with concentration of a quencher will be a straight line with the slope giving the rate constant for the quenching of singlet oxygen.

Thiourea and its counter parts (group 16 of the periodic table) were used as quenchers. Thiourea is commonly used in several industries such as fertilizers, dyes, rubber etc. It is one of the important industrial wastes that need to be removed from affluent water. Since reactions of organic molecules with singlet oxygen can be employed for waste-water treatment, we studied the quenching reactions of thiourea with singlet oxygen. [63]. Figure-3a shows the absorption-time plot of singlet oxygen generated on photo-excitation of haematoporphyrin (~1.2×10^{-4} mol dm^{-3}, absorbance at 532 nm ~0.5) in acetonitrile. On the addition of thiourea (1.0×10^{-3} mol dm^{-3}), the decay of singlet oxygen becomes faster (figure-3b). The pseudo-first order rate constant increases linearly with thiourea concentration (inset of figure-3). The slope of this linear plot gives the bimolecular rate constant value of 1.54 x 10^7 dm^3 mol^{-1} s^{-1} (table-6). This high rate constant indicates that thiourea can be removed by reaction with singlet oxygen. Alternatively can also be employed as an efficient quencher of singlet oxygen, where its generation and reactions are unwanted as in dye lasers.

Table-6: *Bimolecular rate constants (k_q) for the deactvation of singlet oxygen with urea derivatives*

Compound	**k_q / dm^3 mol^{-1} s^{-1}**
Urea	---
Thiourea	1.54 x 10^7
Diethyl thiourea	1.73 x 10^7
Tetramethyl thiourea	1.30 x 10^7
Phenyl thiourea	5.43 x 10^7
Thioacetamide	6.63 x 10^6
Selenourea	1.90 x 10^8

Similar studies have been carried out on a number of thiourea derivatives, and their bimolecular rate constant values obtained for the quenching of singlet oxygen are listed in table-6. It can be seen that substitution of thiourea does not have significant effect on the singlet oxygen quenching rate constant. Singlet oxygen quenching reactions were also studied with thiourea and its analogues, from the same groupurea and selenourea. While urea did not show any reactivity with singlet oxygen, there is a significant increase in the rate constant for quenching the singlet oxygen for selenourea, which is a analogue of thiourea. The increase of the quenching rate constant for singlet oxygen could be attributed to the decrease of ionization potential from urea to thiourea to selenourea which vary as 7.32 eV, 6.12 eV, 5.11 eV, respectively [64]. From this, it has been concluded that the substitution does not change the value of bimolecular rate constant, whereas replacement of sulfur with selenium increases it significantly.

3.5 Reactions of singlet oxygen with antioxidants and other biomolecules

Singlet oxygen reacts with a wide range of biological molecules, including DNA, RNA, proteins, lipids and sterols [20]. It is known to react with lipids, forming lipid hydroperoxide, which cause oxidative events that leads to the cell death. As discussed earlier, singlet oxygen interacts with potential targets by two major pathways, namely, physical and chemical. The former results in energy transfer and de-excitation of the singlet state, but causes no chemical change in the acceptor. The latter results in chemical modification of the target [20]. In human physiological condition, antioxidant defense against reactive oxygen species requires modulation of cellular enzyme, which scavenges the initial radical produced to prevent the oxidative damage brought by reactive oxygen species. Antioxidants also have to be efficient singlet oxygen quenchers. Rate constants of singlet oxygen with well known phenolic antioxidants namely: curcumin, resveratrol, ferulic acid, caffeic acid, o-hydroxy cinnamic acid and some indole derivatives, have been determined. The singlet oxygen, produced on photo-excitation of haematoporphyrin (~1.2×10^{-4} mol dm^{-3}, Absorbance at 532 nm ~0.5) in acetonitrile, was used to measure the reactivity of these antioxidants. The decay of singlet oxygen became faster in the presence of these antioxidants and the quenching rate constants determined are shown in table-7. All of the phenolic antioxidants show moderate quenching ability with singlet oxygen and the quenching rate constant were in the order of~10^6-10^5 dm^3 mol^{-1} s^{-1}.

The singlet oxygen quenching rate constants for two indolic derivatives, tryptophol and 5-hydroxy tryptophol, were determined to be 3.2×10^7 and 9.3×10^7 dm^3 mol^{-1} s^{-1}, respectively. These values of the rate constants for indole derivatives show close agreement with melatonin and other related indoles studied by Matuszak et.al. [65]. From these studies, it appears that indoles show high reaction rate constants with singlet oxygen. As curcumin and resveratrol are known to be better antioxidants than ferulic acid, caffeic acid and o-hydroxy cinnamic acid, it is expected that they show higher quenching rate constant for singlet oxygen. This is reflected in the observed singlet oxygen quenching rate constant in table-7.

Table-7: Bimolecular rate constants (k_q) for the reaction of singlet oxygen with antioxidants

Compound	**kq / dm^3 mol^{-1} s^{-1}**
Curcumin	1.30×10^6
Resveratrol	1.14×10^6
Ferulic acid	4.00×10^5
Caffeic acid	8.12×10^5
o-Hydroxy cinnamic acid	1.42×10^5
Tryptophol	3.20×10^7
5-Hydroxy tryptophol	9.30×10^7
5-Hydroxy indole	9.55×10^6

The rate constants are also dependent on the one-electron reduction potential of the radical. Protection by any typical antioxidant is closely related to its ability to chemically deactivate the reactive oxygen species that are toxic. Although all these antioxidant molecules seem to react with free radicals or singlet oxygen, at different rates, their contribution to total antioxidant capacity should be proportional to their respective physiological concentrations. Thus, from the rate constant, it can be inferred that high concentration ($\sim 10^{-3}$ mol dm^{-3}) of antioxidant is required to scavenge singlet oxygen efficiently inside the cells. However, none of these antioxidants react with singlet oxygen as efficiently as β-carotene, which is considered to be the most powerful quencher for singlet oxygen. It was observed that one molecule of β-carotene can quench 250 to 1000 molecules of singlet oxygen with a rate constant of 1.3×10^{10} dm^3 mol^{-1} s^{-1} [4]. Most of the carotenoids have singlet oxygen quenching rate in the order of $\sim 10^9$ dm^3 mol^{-1} s^{-1} [8].

Quenching studies on singlet oxygen were carried out in different types of solvents, viz., acetonitrile, dichloroethane, and chloroform. Hypocrellin A was used as a sensitizer, which was excited at 532 nm, and curcumin as quencher for singlet oxygen. The quenching rate constants for curcumin were 1.2×10^6, 4.6×10^5 and 1.6×10^5 dm^3 mol^{-1} s^{-1} in these solvents, respectively. Increase of the quenching rate constant in polar aprotic solvent is explained in terms of the charge-transfer exciplex stabilization of dipolar interactions. Stabilization increases if solvents have a large capacity as a hydrogen bond acceptor [62]. Systematic study with more number of solvents would give deeper insight into this aspect.

4. Summary

The major findings of the present article can be summarized as follows

1) Singlet oxygen could be efficiently produced by photosensitization method. It was detected by infrared luminescence at 1270 nm using liquid nitrogen cooled germanium detector.

2) Porphyrins, perylene quinone dyes, fullerenes act as very good sensitizers for singlet oxygen generation.

3) Fullerenes are the best sensitizers due to very high triplet quantum yield (close to unity).

4) The yield of singlet oxygen produced from C_{60} depends on the concentration of C_{60} used. Singlet oxygen yield increases with increase in C_{60} concentration up to $4.0x10^{-4}$ mol dm^{-3} and then decreases with further increase in C_{60} concentration. This was attributed to the formation of aggregates at higher concentration.

5) The yield of singlet oxygen depends on the nature of the solvent and the yield of triplet state of the sensitizer in that solvent.

6) The lifetime of singlet oxygen varies with the solvent. In deuterated solvent the lifetime increases by a considerable amount.

7) Temperature has negligible effect on the decay of singlet oxygen studied in solvents like benzene and acetonitrile.

8) The quenching rate constant increased from urea to thiourea to selenourea, whereas substitution in the thiourea analogues had negligible effect on the quenching rate constant.

9) Well known antioxidants exhibit high rate constant with singlet oxygen, but to protect cellular damage from singlet oxygen, the required concentration of these antioxidant should be high in the order 10^{-3} mol dm^{-3}.

10) The quenching rate constant for singlet oxygen with curcumin, was found to increase in an aprotic polar solvent, probably because the exciplex formed by the reaction of singlet oxygen can get stabilized by dipolar interactions.

11) Due to the high selectivity towards organic molecules, singlet oxygen can be used for removal of sulfides from mining effluent, phenol and its derivatives from wastewater of paper and dye industries, and destruction of viruses and bacteria in drinking water.

5. Future Programmes

The main objective for studying photosensitized singlet oxygen reaction is to identify new photosensitizers with better efficacy for photodynamic therapy. An improved understanding of mechanisms of photosensitizer quenching, photobleaching, and localization in the tissues is required for this purpose. Investigation of new photosensitizers is also crucial for further development in this field.

Singlet oxygen, being a constituent of ROS in biological system, it is necessary to scavenge it to reduce its damaging effects. Antioxidants can be used to scavenge the harmful effect of singlet oxygen. The quenching rate constant of these antioxidants with singlet oxygen will provide information on the concentrations that are required for selectively protecting crucial biomolecules.

Semiconductors, with wide band gaps in the visible region, have been used for light emitting diodes and photovoltaic devices. The efficiencies of these devices are largely dependent on the excited state of these semiconductors. The excited states are often sensitive to oxygen, so a better understanding of the reactivity of oxygen with the semiconductor material will help to improve the efficiency.

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7. Figure legends

Figure-1: Schematic diagram of transient luminescent spectrometer

Figure-2: Emission-time profile of singlet oxygen at 1270 nm produced on photo-excitation of C_{60} (7.0x10^{-4} mol dm^{-3}) in benzene. Inset shows the variation of relative intensity of singlet oxygen produced on photo-excitation of C_{60} in benzene as a function of C_{60} concentration.

Figure-3: Figure-3: Absorption-time profile of singlet oxygen at 1270 nm, produced on photo-excitaion of haematoporphyrin (~1.2x10^{-4} mol dm^{-3}) in acetonitrile (a) in absence and (b) presence of thiourea (1.0x10^{-3} mol dm^{-3}). Inset shows variation of pseudo-first order rate constant (k_{obs}) as a function of thiourea concentration.

8. References

1. Lang K., Mosinger J., Wagnerová D. M.; *Coord. Chem. Rev.* 2004, **248**, 321.
2. Gorman A. A.; *Adv. Photochem.* 1992, **17**, 217.
3. Dougherty T. J.; *Adv. Photochem.* 1992, **17**, 275.
4. Foote C. S.; *“Free Radicals in Biology”* W. A. Pryor (Ed.) Academic Press, New York, 1976, **Vol.2**, page 85.
5. Ali H., vanLier J. E.; *Chem. Rev.* 1999, **99**, 2379.
6. Boyle R. B., Dolphin D.; *Photochem. Photobiol.* 1996, **64**, 469.
7. Priestley J.; *“Experiments and observation on different kinds of air”*, London, (Experiments, Alembic Club Reprints No. 7, Edinburgh), 1775.
8. Min D. B., Boff J. M.; *Comprehensive Reviews in Food Science and Food Safety* 2002, **1**, 58.
9. Khan A. U., Kasha M.; *J. Chem. Phys.* 1963, **39**, 2105.
10. Foote C. S., Clennan E. L.; *“Active Oxygen in Chemistry”* Foote C. S., Valentin J. S., Greemburg A., Liebman J. F. (Eds.), Chapman and Hall, London, 1995, page 105.
11. Clennan E. L.; *Tetrahedron* 2000, **56**, 9151.
12. Schaap A. P.; *“Singlet Molecular Oxygen”*, Dowden, Hutchinson and Ross, Strondsburg, PA, 1976.
13. Frimer A. A.; *“Singlet Oxygen”*, CRC Press, Boca Ration, FL, **Vol. I-IV**, 1985.
14. Mathews-Roth M. M.; *“The Science of Photomedicine”*, Regan J. D., Parris J. A. (Eds.), Plenum Press, New York, 1978.
15. Krasnovsky A. A.; *“Light in Biology and Medicine”*, Douglas R. H., Moan J., Ronto G. (Eds.), Plenum Press, New York, 1991, **Vol.2**, page 431.
16. Ranby B., Rabek J. F.; *“Singlet Oxygen Reactions with Organic Molecules and Polymers”*, Wiley, New York, 1978.
17. Dougherty T. J.; *Adv. Exp. Med. Biol.* 1983, **160**, 3.
18. Herzberg G.; *“Molecular spectra and molecular structure I: Spectra of diatomic molecules”*, 2nd Ed. VonNostrand, New York, 1950.
19. Arnold S. J., Kubo M., Ogryzlo E. A.; *Adv. Chem. Ser.* 1968, **77**, 133.
20. Davies M. J.; *Biochem. Biophys. Res. Commun.* 2003, **305**, 761.
21. Wilkinson F., Brummer J. G.; *J. Phys. Chem. Ref. Data* 1981, **10**, 809.
22. Wilkinson F., Helman W. P., Ross A. B.; *J. Phys. Chem. Ref. Data* 1993, **22**, 113.
23. Matheson I. B. C., Lee J.; *Photochem. Photobiol.* 1979, **29**, 879.
24. Singh A., Koroll G. W., Antonsen S. A.; *J. Photochem.* 1984, **25**, 99.
25. Birks J. B.; *“Photophysics of Aromatic Molecules”*, Wiley Interscience, New York, 1970.
26. Gorman A. A., Lovering G., Rodgers M. A. J.; *J. Am. Chem. Soc.* 1978, **100**, 4527.
27. Carlsson D. J., Suprunchuk T., Wiles D. M.; *Can. J. Chem.* 1974, **52**, 3728.
28. Foote C. S., *Acc. Chem. Res.* 1968, **1**, 104.
29. Ogilby P. R., Kristiansen M., Martire D. O., Scurlock R. D., Taylor V. L., Clough R. L.; *Adv. Chem. Ser.* 1996, **249**, 113.
30. Redmond R. W., Gamlin J. N.; *Photochem. Photobiol.* 1999, **70**, 391.
31. Hurst J. R., McDonald J. D., Schuster G. B.; *J. Am. Chem. Soc.* 1982, **104**, 2065.
32. Gorman A. A., Hamblett I., Land E. J.; *J. Am. Chem. Soc.* 1989, **111**, 1876.

33. Rossbroich G., Garcia N. A., Braslavsky S. E.; *J. Photochem.* 1985, **31**, 37.
34. Farmilo A., Wilkinson F.; *Photochem. Photobiol.* 1973, **18**, 447.
35. Bilski P., Chignell C. F.; *J. Biochem. Biophys. Methods.* 1996, **33**, 73.
36. Cosa G., Scaiano J. C.; *Photochem. Photobiol.* 2004, **80**, 159.
37. DeRosa M. C., Crutchley R. J.; *Coord. Chem. Rev.* 2002, **233-234**, 351.
38. Basis R., Churassy S. in Gas flow and Chemical lasers, Ed. S. Roseawacka, Springer Verlag, Berlin, 1986, p. 142.
39. Keene J. P., Kessel D., Land E. J., Redmond R. W., Truscott T. G.; *Photochem. Photobiol.* 1986, **43**, 117.
40. Bonnett R., McGarvey D. J., Harriman A., Land E. J., Truscott T. G., Winfield U. J.; *Photochem. Photobiol.* 1988, **48**, 271.
41. Bonnett R., Lambert C., Land E. J., Scourides P. A., Sinclair R. S., Truscott T. G.; *Photochem. Photobiol.* 1983, **38**, 1.
42. Reddi E., Jori G., Rodgers M. A. J., Spikes J. D.; *Photochem. Photobiol.* 1983, **38**, 639.
43. Tanielian C., Schweitzer C., Mechin R., Wolff C.; *Free Radic. Biol. Med.* 2001, **30**, 208.
44. Weng M., Zhang M-H., Shen T.; *J. Chem. Soc. Perkin Trans. 2* 1997, 2393.
45. Guldi M. D., Prato M.; *Acc. Chem. Res.* 2000, **33**, 695.
46. Arbogast J. W., Foote C. S.; *J. Am. Chem. Soc.* 1991, **113**, 8886.
47. Hamano T., Okuda K., Mashino T., Hirobe M., Arakane K., Ryu A., Masiko S., Nagano T.; *J. Chem. Soc. Chem. Commun.* 1997, 21.
48. Vileno B., Sienkiewicz A., Lekka M., Kulik A. J., Forró L.; *Carbon* 2004, **42**, 1195.
49. Arbogast J. W., Darmanyan A. P., Foote C. S., Rubin Y., Diederich F. N., Alvarez M. M., Anz S. J., Whetten R. L.; *J. Phys. Chem.* 1991, **95**, 11.
50. Nath S., Pal H., Sapre A. V., Mittal J. P.; *J. Photoscience* 2003, **10**, 105.
51. Aminian-Saghafi T., Nasini G., Caronna T., Braun A. M., Oliveros E.; *Helv. Chim. Acta.* 1992, **75**, 531.
52. Oliveros E., Suardi-Murasecco P., Aminian-Saghafi T., Braun A. M., Hans-Jürgen H.; *Helv. Chim. Acta.* 1991, **74**, 79.
53. Ogilby P. R., Foote C. S.; *J. Am. Chem. Soc.* 1983, **105**, 3423.
54. Bisby R. H., Morgan C. G., Hamblett I., Gorman A. A.; *J. Phys. Chem. A* 1999, **103**, 7454.
55. Long C. A., Kearns D. R.; *J. Am. Chem. Soc.* 1975, **97**, 2018.
56. Hurst J. R., Schuster G. B.; *J. Am. Chem. Soc.* 1983, **105**, 5756.
57. Young R. H., Brewer D., Keller R. A.; *J. Am. Chem. Soc.* 1973, **95**, 375.
58. Schmidt R.; *J. Am. Chem. Soc.* 1989, **111**, 6983.
59. Rodgers M. A. J.; *J. Am. Chem. Soc.* 1983, **105**, 6201.
60. Catalán J., Díaz C., Barrio L.; *Chem. Phys.* 2004, **300**, 33.
61. Cristina V. U., Lemp E., Zanocoo A. L.; *J. Chil. Chem. Soc.* 2003, **48(4)**, 717.
62. Lemp E., Cristina V., Zanocoo A. L.; *J. Photochem. Photobiol. A* 2004, **168**, 91.
63. Wang W-f., Schuchmann M. N., Schuchmann H. P., Knolle W., von Sonnatg J., von Sonnatg c.; *J. Am. Chem. Soc.* 1999, **121**, 238.
64. Mishra B., Maity D. K., Priyadarsini K. I., Mohan H., Mittal J. P.; *J. Phys. Chem. A* 2004, **108**, 1552.
65. Matuszak Z., Bilska M. A., Reszka, K. J., Chignell C. F., Bilski P.; *Photochem. Photobiol.* 2003, **78**, 449.

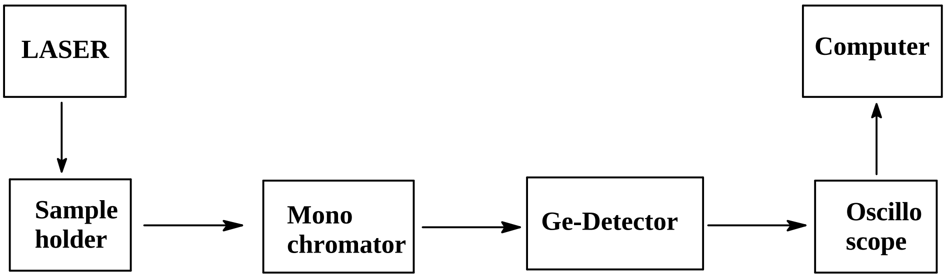

Figure-1

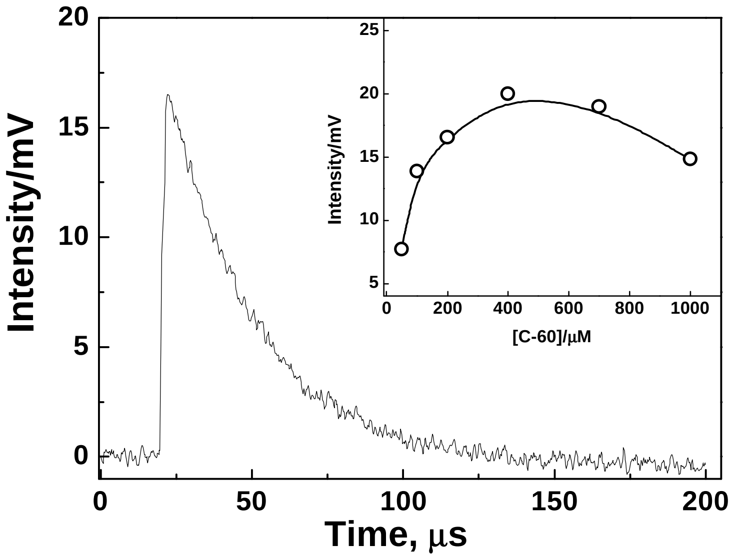

Figure-2

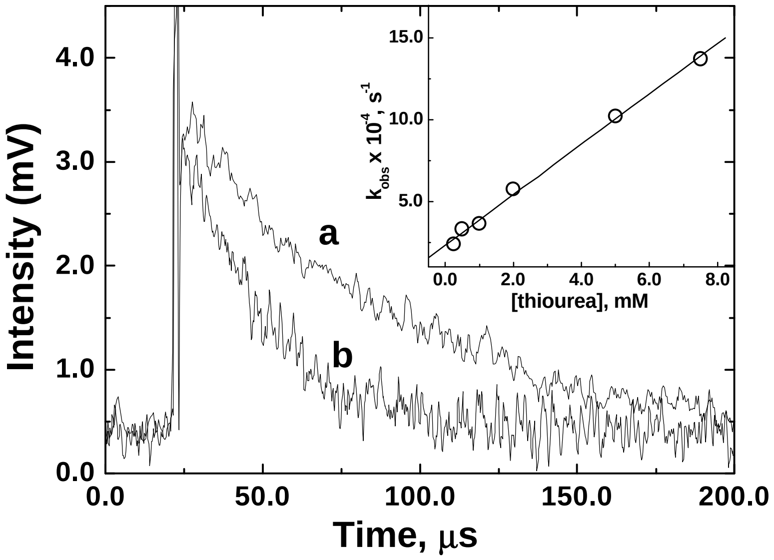

Figure-3