Numerical modelling of photodynamic therapy

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BACHELORS OF TECHNOLOGY

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By

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CERTIFICATE

This is to certify that the thesis entitled *"Numerical modelling of photodynamic therapy"* by Mr Vishnu B Kumar submitted to the National Institute of Technology, Rourkela for the Degree of Bachelors of Technology in Biomedical Engineering, is a record of bona fide research work, carried out by him in the Department of Biotechnology and Medical Engineering under my supervision.

I believe that the thesis fulfills part of the requirements for the award of Bachelors of Technology. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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Vishnu Baby Kumar

ABSTRACT:

Photodynamic Therapy (PDT) is a new and minimally invasive cancer treatment modality based on the photo-activation of a light-sensitive drug '**Photosensitiser**' (PS). PS can be administered in various forms (e.g. as injection or ointment) and accumulates selectively in the target tumour tissue. This activated drug in turn generates singlet oxygen ($^1O_2$) in the malignant tissue and induces tissue necrosis or apoptosis. In this thesis, we have implemented a simulation model utilising the primary processes that the photosensitiser undergoes in the presence of irradiating light and molecular oxygen ($^3O_2$). We have used spectroscopic data of the commercially available and clinically significant photosensitiser 'Photofrin®' (porfimer sodium, Axcan Pharma, Montreal, Canada, a heterogeneous mixture of porphyrins). This model encourages predictive statements to be made regarding the efficiency of photodynamic modalities at various initial conditions, including PS concentration and tissue oxygen concentration.

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CHAPTER 1: INTRODUCTION

(1.1) MOTIVE

(a) CANCER:

The focus of my thesis lies on a novel clinical treatment modality called the '**Photodynamic Therapy'** which is based primarily on the interaction of light with human tissue and certain cancer-specific agents which can be exploited and utilised in our struggle against cancer.

The W.H.O has described **Cancer**[1] as the generic term given to a large group of diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms[1]. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as **metastasis**[1]. Metastases are the major cause of death from cancer.

Cancer spares no one – the young or old, the rich or poor, men, women or children; it is one of the leading causes of death in the world, particularly in developing countries and accounted for 7.6 million deaths (around 13% of all deaths) in 2008[2]. Deaths from cancer worldwide are projected to continue to rise to over 11 million by the year 2030[1]. Many of these deaths can be avoided. Over 30% of all cancers can be prevented[1]. Others can be detected early, treated and cured.

The utilisation of lasers in medicine[11] has been overwhelming due to the possibility of varying its biological impact via the selection of wavelength, output power and, illumination time and mode. Conventionally, the thermal interaction of light with tissues is being exploited as a replacement

for the "cold knife"[11]. But this thesis considers a non-thermal regimen of utilising light within medicine referred to as the Photodynamic Therapy (PDT).

In PDT, the tissue is sensitised to light by applying photosensitive agents called Photosensitisers (PS) administered topically or systemically. The therapeutic effect of PDT is attributed to the Reactive Oxygen Species (ROS)[17,22-25] generated by the reaction of the excited photosensitiser molecules with oxygen following the non-ionising electromagnetic irradiation. In summary, a photochemical reaction is induced where the activated photosensitisers generate tissue damage through oxygen free radicals.

(b) ADVANTAGES OF PDT OVER CONVENTIONAL MODALITIES:[15]

Due to the selectivity of drug uptake by target cancer tissues and the control of light delivery, PDT has the potential of inducing effective cytotoxicity in the malignant tissue and limited damage to the surrounding healthy tissues. PDT has superior properties compared with conventional cancer therapies such as chemotherapy[3] and radiotherapy[3], these are;

(1) it is selective[15]

(2) it is non-invasive[15], and

(3) has fewer side effects[15]

At the cellular and tissue levels, the free radicals can cause cellular or vascular damages and have immunological effects as mentioned above[15]. Over the past 30 years, PDT has been successfully applied for the treatment of a variety of types of cancers. Another commonly used term for PDT is "**Photo Chemotherapy**" (PCT).[7,11] Although this designation encompasses a

wider definition, it is often preferred, more so since it emphasises on the actual mechanism involved i.e. the photochemistry behind PDT.

(1.2) HISTORICAL BRIEFING ON THE PHOTODYNAMIC THERAPY:

Light as a therapeutic agent[9] has been in use for more than three thousand years. The Ancient Egyptian, Chinese and Indians civilisations used the combined action of natural plant extracts like chlorophylls with sunlight in attempts to cure disorders such as vitiligo, rickets, psoriasis, skin cancer and psychosis[9].

In 1898, the medical student Oscar Raab[11] discovered by chance that the toxicity of the dye acridine to paramecia (alga) was dependent on the ambient light when performing the experiments, rather than to the concentration of the drug. This pioneering work was performed in the laboratories of Professor Herman von Tappeiner[11] of Munich. Prof. Tappeiner continued the work and with Jodlbauer[11] reported that the presence of oxygen was an indispensable factor. With this, he first coined the term 'Photodynamic Therapy'[11] to describe the phenomenon of oxygen dependent photosensitisation.

The discovery of PDT then paved way for the first medical application where Tappeiner together with dermatologist Jesoniek[11] went on to perform PDT on humans, reported in 1903, with eosin as a photosensitiser.

Thus, the application of photodynamic reactions to medicine gathered momentum but it was not until 1950s when the ability of a few tetrapyrrolic compounds called Porphyrins, to selectively accumulate in certain tissues i.e. in malignant tissues, was reported. Hausmann[11] in Vienna performed the first studies on the biological effects of hematoporphyrin. In 1911, he reported on the combined effect of hematoporphyrin and light on a paramecium and red blood cells and

described skin reactions in mice exposed to light after hematoporphyrin administration. He, in particular described photosensitivity changes and some phototoxicity with intense light. The first report on the human photosensitisation by porphyrins was in 1913 by the German Friedrich Meyer-Betz[11]. Determined to find whether the same effects could be induced in humans as well as mice, he injected himself with 200 mg of hematoporphyrin and subsequently noticed prolonged pain and swelling in light-exposed areas.

In a landmark paper, the concept that the combination of tumour localising and phototoxic properties of porphyrins could be exploited to produce an effective treatment for cancer was first proposed in 1972 in 'The Lancet' by Diamond et al.[15,26] from San Francisco. These authors validated the hypothesis that hematoporphyrin may serve as a selective photosensitising agent to destroy tumour cells exposed to light. They further concluded that PDT offered a new approach to the treatment modality of brain tumours and other neoplasms resistant to other existing forms of therapy. Refer Figure 1 for a historical overview of the photodynamic therapy.

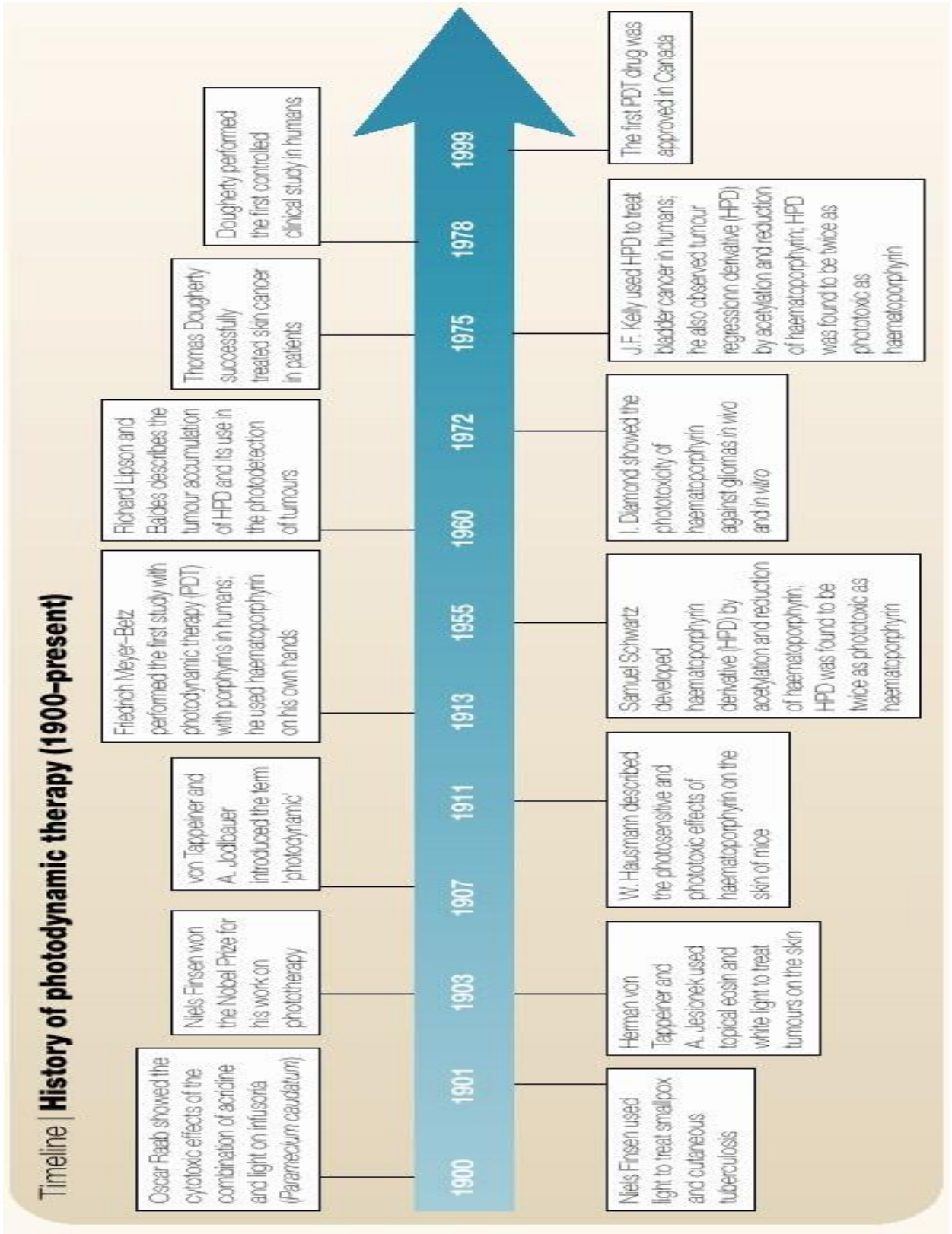

Figure 1: Historical outlook of the photodynamic therapy, 1900 to present[5]

(1.3) PHOTODYNAMIC THERAPY: THE PROCESS

The process of **Photodynamic Therapy** begins with the photo excitation of the photosensitiser (PS) drug that has been administered to the target tissue. Following this, is a cascade of processes that occur after a molecule of PS absorbs a photon having the appropriate energy ($E=h\nu$). The photochemical processes that take place during this irradiation process lead to the production of singlet oxygen that ultimately does the cancer cell damage.

In the first step, the photosensitiser (PS) in its fundamental/ground singlet state (S_0) absorbs light and is excited into its excited singlet state (S_1). This process is reversible since the S_1 state can revert back to the S_0 state by a fluorescence emission[8]. Then, the excited state is converted by the **Inter-System Crossing**[17,19] (ISC) process to the triplet state (T) of the photosensitiser. This triplet state can:

(1) Either transfer its energy to the in-vivo dissolved molecular oxygen, which exits in the fundamental triplet state ($^3O_2$), generating singlet oxygen ($^1O_2$), or,

(2) Revert to the fundamental S_0 state via a phosphorescence[8] process. According to the ***Frank-Condon Principle***[15], the time for an electronic transition (on the order of 10^{-15} s) is much shorter than that for a nuclear rearrangement. (on the order of 10^{-13} to 10^{-14} s) And so, this electronic transition occurs without causing any significant displacement of the nuclei.

In the second step, we have the excited PS in its triplet form (T) which reacts with the molecular triplet oxygen ($^3O_2$) present in tissue to give singlet oxygen ($^1O_2$). Singlet oxygen is a highly reactive species of oxygen radicals having the innate ability to react spontaneously with cellular targets causing their apoptosis. The generated singlet oxygen may also undergo a

phosphorescence-mediated[8] relaxation process back to its fundamental state. Ultimately, the generated singlet oxygen ($^1O_2$) also has the potential to react with the photosensitiser (PS) by a process called **Photobleaching**[19], which hampers the overall singlet oxygen generation efficiency.

The above reading suggests that in the course of Photodynamic Therapy, healthy cells are not necessarily spared, therefore it is imperative for the photosensitiser to accumulate selectively in the tumoural tissue to produce cancer cell necrosis (used interchangeably with 'apoptosis') and to abate healthy tissue damage. As mentioned above, besides the reaction with cells, singlet oxygen can also react with the photosensitiser drug. This undesirable process photobleaching often leads to the irreversible inactivation of the photosensitiser hamepring the overall effectiveness of the photodynamic process. The efficiency of singlet oxygen generation depends on several factors, such as the molecular structure of the photosensitiser or its concentration and the in-vivo concentration of oxygen in the tissue of interest.

In this thesis, we are interested in a **Type II Photodynamic process**[20] which is the case when the excited PS reacts with molecular oxygen in tissue transforming it from its harmless triplet state to its highly potent singlet state; while a Type I Photodynamic process[20] is where the same excited PS transfers its energy to a neighbouring atom or molecule thereby forming a free radical species.

Summarising the reactions in step-wise fashion:[8,17]

- I - Irradiating target tissue with light post-application of the PS drug
- II - PS transforms from ground singlet S_0 state to excited singlet S_1state
- III - PS in S_1 state converts to the triplet T state via ISC

- IV - PS in S_1 state reverts to S_0 state via fluorescence
- V - PS in T state transfers energy to molecular oxygen, $^3O_2$ state to $^1O_2$ state
- VI - PS in T state reverts to S_0 state via phosphorescence
- VII - Oxygen in singlet $^1O_2$ state reverts to triplet $^3O_2$ state via phosphorescence
- VIII - Oxygen in singlet $^1O_2$ state reacts with PS via photobleaching

THREE KEY INGREDIENTS OF PHOTODYNAMIC THERAPY:

(a) PHOTOSENSITISERS:[11,18]

The term photosensitiser encompasses a family of substances which are activated by light causing a biological effect. Historically, much of the work on photosensitisers revolved around the experiments on porphyrins. The name porphyrin comes from a Greek word for purple.[4] One of the best-known porphyrins is heme[4], the pigment found in human red blood cells; heme is a cofactor of the oxygen-carrier protein hemoglobin. Porphyrins are heterocyclic macrocycles (i.e. cyclic macromolecules) composed of four modified pyrrole subunits interconnected at their α carbon atoms via methine bridges (=CH-). They are aromatic. The simplest porphyrin is porphine[4] (Figure 2), and in fact substituted porphines are called porphyrins.

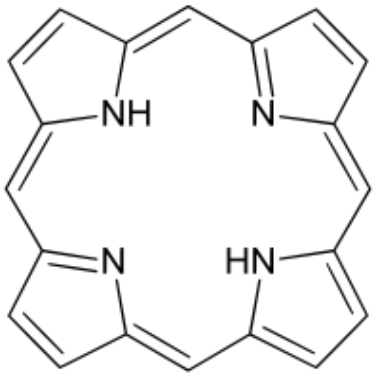

Figure 2: The structure of Porphine, the parent Porphyrin[4]

In solutions, photosensitising compounds can have either negligible solubility in water or alcohol or maybe freely soluble in water and/or alcohol at the physiological pH (~7.365 of blood)

As majority of the photosensitisers are large and possess a relatively complex molecular structure, they are usually administered by intravenous (IV) injection. An exception to this is d-aminolevulinic acid (ALA),[11] which is a precursor of the photosensitising compound Protoporphyrin IX (PpIX),[11] wherein the target tumour tissues can themselves produce the PpIX compound for sensitisation following the administration of the precursor ALA. Protoporphyrin IX is, presently, one of the most utilised photosensitisers in clinical trials.

Physico-Chemical properties of the photosensitiser are:[11,15]

(1) Chemical purity of the PS to avoid mutagenic effects.

(2) Capability to localise[11] specifically in malignant tissue. The fundamental requirement for optimum response to PDT is that a sufficient amount of the drug be localised in the target tissue. Initially, photosensitisers are taken up by most normal and hyperproliferating cells alike, but are retained longer in the tumour cells. Increased blood vessel permeability as well as poor lymphatic drainage in the malignant tissues contribute to the longer retention of the drug.

(3) Short time interval between the administration of the drug (either systemic or topical) and its maximum accumulation in the host target tissue.

(4) Rapid clearance[18] from normal tissues. The fast clearance rate should ideally apply to all normal tissues, inclusive of the liver, kidney and spleen as well. But at the same time, the clearance from the tumour tissues should be slower.

(5) Activation at wavelength with ideal tissue penetration. The marked feature of photosensitising agents is light absorption in a wavelength range where the ambient biological tissue is relatively transparent. The wavelength for optimal tissue penetration and that for maximal absorption should exhibit null disparity.

(6) High quantum yield[11] efficacies for the generation of the reactive singlet oxygen species i.e. a high yield of ROS per photon absorbed.

(7) Lack of dark toxicity[15] is another desirable feature of an ideal photosensitiser. A photosensitiser should exhibit a rather narrow absorption band, with little absorption at other wavelengths within the spectrum. This is to minimise the side effect of skin photosensitivity.[18]

All these are very important for the overall efficiency of photosensitisation and thus the PDT.

Other notable families of photosensitisers apart from Porphyrins are Chlorins, Purpurins, Pheophorbides, Napthalocyanines, and Porphycenes etc.[18]

DRAWBACKS OF PORPHYRIN-BASED PHOTOSENSITISERS:

As stated above, most of the initial work on photosensitisation was performed utilising porphyrins and their relatives. However, scientists and researchers are in the quest for newer substances which best meet the requisites of the ideal photosensitiser. This is because Porphyrin-based PS drugs suffer serious setbacks as a consequence of which, many different molecular structures are currently being investigated. But the whole field is in itself at its infancy.

Some of the deterrents in using porphyrin-based photosensitisers are:

(1) Significant tissue penetration[18] is achieved by light at 630 to 635 nm in the Infra-Red (IR) range, which however corresponds to the weakest absorption of most porphyrin drugs. So there is a trade-off between tissue penetration and degree of light absorption.

(2) Cutaneous accumulation of porphyrin-based photosensitising drugs (i.e. both normal and tumour tissues absorb the drug at the same time) and their slow clearance from the skin lead to long-lasting skin photosensitivity,[9] requiring the affected patient to avoid exposure to light from 4 to 6 weeks following a photosensitisation course.[18]

(b) LIGHT SOURCES:[11,18]

Irradiation with light induces several processes when photons pass through the tissue; these are **Absorption, Scattering, Transmittance** and **Fluorescence**,[11] all of which have been medically exploited. Photodynamic therapy as a treatment modality is governed by the accessibility of the target area to light application and facilitates the use of any light source with the appropriate wavelength spectrum. In the earlier days, photosensitisation was carried out with the help of conventional gas discharge lamps.

Common examples of light sources :

Metal halogen lamp, which emits 600 to 800 nm radiation at high power density, short-arc xenon lamp, tunable over a bandwidth between 400 and 1200 nm and the broad light beam emanating from incoherent lamps is useful for the treatment of large lesions.

The introduction of lasers coupled with optical fibres heralded a new era in photosensitisation and expanded its applicability in medicine, promoting the endoscopic delivery of light to nearly

any site in the human body. In contrast to traditional incandescent lamps, lasers are equipped to provide us with the exact selection of wavelengths and the precise targeted application of light.

FEATURES THAT MAKE LASERS UNIQUE:

(1) Monochromatic[11] i.e. containing one or a narrow range of wavelengths. This permits the light to match precisely an absorption band of the sensitiser in the region where its penetration in tissue is also at its largest and thus avoid thermally damaging the tissue with light that is not active in the photochemical reaction.

(2) Coherent[11] where the light waves emanating from a laser source are all in phase which makes lasers unique with respect to traditional lamps and permit the spot-on application of laser beam onto the target tissue.

(3) Concentrated beam[11] i.e. possessing a minimal angle of divergence facilitating easy focussing of lasers into optical fibres and thus favouring long distance near-lossless transmission. As a consequence was born various endoscopic techniques in which the laser-coupled optical fibres are transported into the body cavities or through the lumen of needles into tissue for interstitial illumination. Thus the laser has been considered the ideal choice as light source for PDT.

When it comes to dye lasers,[11] the apparent advantage is the possibility of being able to change the dye, and thereby also manipulate the emission wavelength within a certain region, making it possible to use the same laser in conjunction with various photosensitisers.

Common examples of clinically used lasers:

The gold vapour laser (GVL)[18] and the copper vapour laser-pumped dye laser (CVDL)[18] (both being types of pulsed lasers), neodymium (Nd:YAG)[11] laser (being a tunable solid-state laser) and gallium-arsenide (GaAlAs)[18] (being a semi-conductor laser) are particularly useful for PDT.

The conventionally used lasers like the GVC and CVDL are disadvantageous[18] in that these lasers are bulky, expensive, relatively immobile, and require frequent maintenance checks.[18] It has been acknowledged generally that regarding the source of light, much smaller and cheaper sources are to be investigated before PDT can become a future treatment modality for common use.

This is where the development of semiconductor diode lasers like the Nd:YAG and GaAlAs lasers gains momentum as a novel approach to alleviate the aforesaid disadvantages. Besides having a convenient size, diode lasers are also reliable, economical and easy to use.

A SMALL NOTE ON LAMPS AND LEDs:[11,18]

LAMPS:[11]

Filtered lamps constitute other possible light sources in PDT, which have already proved to be very useful. The advantages presented are a low price and a smaller form factor. In principle, lamps can only be used for superficial illumination. When filtered, the wavelength band achieved is quite broad unlike the narrow lasers. The emitted light outside the absorption band of the sensitiser was earlier presumed to have no other purpose than inducing hyperthermia[11] of surrounding tissues. Later this hyperthermia was revealed to give an increased tumouricidal

effect. The Infra-Red (IR) band can be used for adding hyperthermia, but if undesired, can also be easily removed with the help of a simple water filter.

LEDs:[11]

Light emitting diodes (LEDs) are also non-coherent light sources that can be employed for PDT. However given the infancy of the Photodynamic therapy and current technological progress, these alternate source have not yet been widely used. They emit in wavelength bands that are much broader than those from a laser and look promising in the future as they are predominantly simpler, cheaper and portable.

A SMALL NOTE ON LIGHT DOSIMETRY:[11,]

The degree of penetration of light through tissue is dependent not just on the characteristics of the target tissue but also on the wavelength of the light. Moreover, the free passage of light is hindered by optical scattering within the tissue, the absorption by endogenous (in-vivo) chromophores, and the absorption of light by the sensitising drug itself.

Using the present laser and low-powered LED technologies, the light needed to activate most photosensitisers cannot penetrate through more than one third of an inch (1 cm) of human tissue. Thus laser application of PDT is mostly confined to the treatment of tumours on or under the skin, or on the lining of some internal organs and consequently less effective in treatment of large tumours and metastasis.

(c) OXYGEN:[11,18]

Investigations have purported the idea that the efficiency of the photosensitisation step of PDT is a direct consequence of the yield of $^1O_2$ in the tumour environment and the yield of $^1O_2$ in turn depends on the in-vivo concentration of oxygen in the tissue. Hypoxic cells are very tolerant to photosensitisation and the photodynamic reaction mechanism may itself consume oxygen at a rate conducive to induce a state of temporary hypoxia and stifle further photosensitisation effects. On the contrary, it has been reported, that hyperbaric oxygen can improve the effect of photosensitisation.

Singlet oxygen $^1O_2$ is a member of the general class of reactive species called **Reactive Oxygen Species** (ROS)[9] that also comprises the free radicals: hydroxyl (OH^-) and superoxide (O_2^{2-}), together with other reactive molecules such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HClO). All these radical species are capable of oxidising biomolecules and induce cell death by apoptosis and/or necrosis mechanisms. We note that $^1O_2$ free radicals are generated by photosensitisation of exogenous and/or endogenous photosensitisers and do not interconvert to other ROS whereas many ROS are the products of physiological processes and an inter-conversion between them is feasible.

The hallmark feature of $^1O_2$ in the context of PDT is its lifetime.[9] The term lifetime of a sample refers to the time elapsed for a decline in its concentration by 1/e, or to ~37 % of the original concentration. Thus these $^1O_2$ free radicals can exist in their excited states hardly for a fraction of time before reverting to their ground states by the loss of energy via a phosphorescence process as observed in PDT.

(1.4) FUTURE DIRECTIONS:[9,11]

Now a century after Oscar Raab's ground-breaking work, the clinical propensity of PDT is finally being realised. PDT has been successfully employed in the treatment of many tumours, including skin cancer, oral cavity cancer, bronchial cancer, esophageal cancer, bladder cancer, head and neck tumours in addition to non-malignant diseases. The mechanism of action is continuously being defined along with the multitude of theoretical advantages over other cancer therapies.

Presently, clinical trials on PDT emphasise its role in both the curative treatment of early tumours and the palliative[11] (symptomatic) control of advanced cancer. However, PDT is not without its problems and before it becomes adopted as a clinical modality these have to be addressed. For instance, at the molecular level, the mechanisms for drug action, particularly the initial photochemical reactions leading to the generation of the ROS, have not been properly understood.[11] Moreover, the efficiency of currently used PDT drugs is limited by the light penetration depth in tissue, since the absorption of these drugs lies in the visible light wavelength range (400 to 780 nm)[11], where the attenuation of light in tissues is strong. So, despite there being a number of photosensitisers, right now, only a handful is approved for clinical use[15]. These are Photofrin[15], Levulan[15] (ALA) and Foscan[15]. The first health license was granted in Canada for Photofrin in 1993 for use in bladder cancer.[11] Photofrin is now licensed in many countries and reigns supreme as the sensitiser with the most indications. Further trials with other sensitisers will no doubt lead to their licensing for clinical use, and this will propagate the vital role that PDT will have as the treatment of the future for both malignant and benign diseases.

CHAPTER 2: MATHEMATICAL MODELLING

(2.1) OVERVIEW: THE MODELLING APPROACH [7,8]

Our modelling framework is aimed at a clearer understanding of the fundamental processes involved to help expand the range of PDT and improve its clinical efficacy. The schematic of the energy level diagram[7] (Figure3) illustrates various pathways of the PDT process and their corresponding rate constants.

We have devised a numerical approach to quantitatively model a Type II photodynamic therapy (PDT) process in the time domain and thereby corroborate our results with the observations made by Hu et.al[7]

In the course of modelling, we are considering the photochemical processes invoked by the irradiation of an aqueous solution of the photosensitiser drug with light; the resulting mathematical model is strongly based on the kinetic rate equations of the individual reactions. The relevant photoinduced processes have been represented in the Jablonski diagram[8,17] (Figure4) The PDT process starts with the absorption of photons by the PS in S_0 state, having an adsorption cross-section, σ_{psa}. Refer to Table 2 for a list of the various coefficients and parameters used.

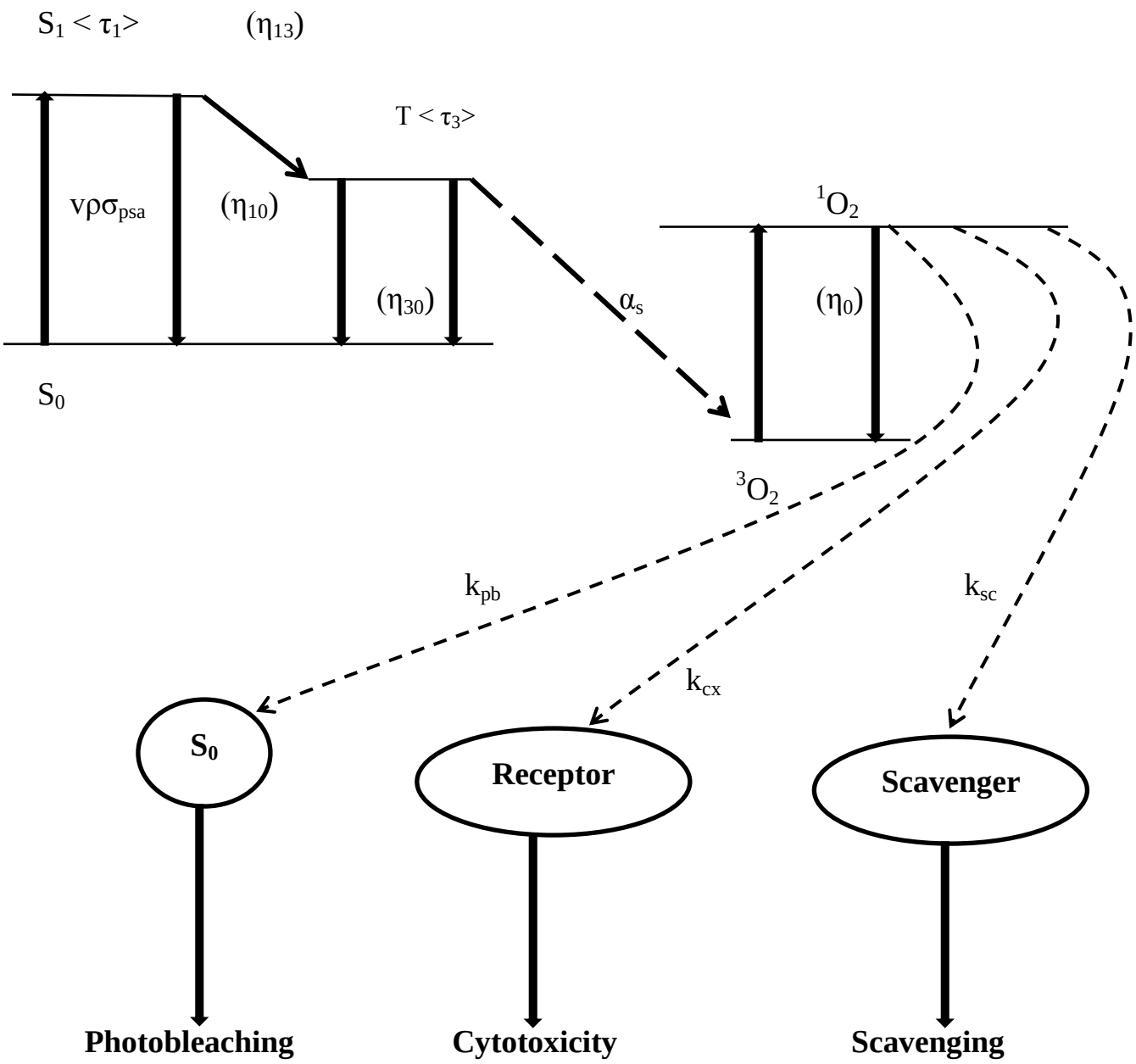

Figure 3: Schematic Representation of the PDT Process[7]

(Refer to Table 1 for a description of the symbols used)

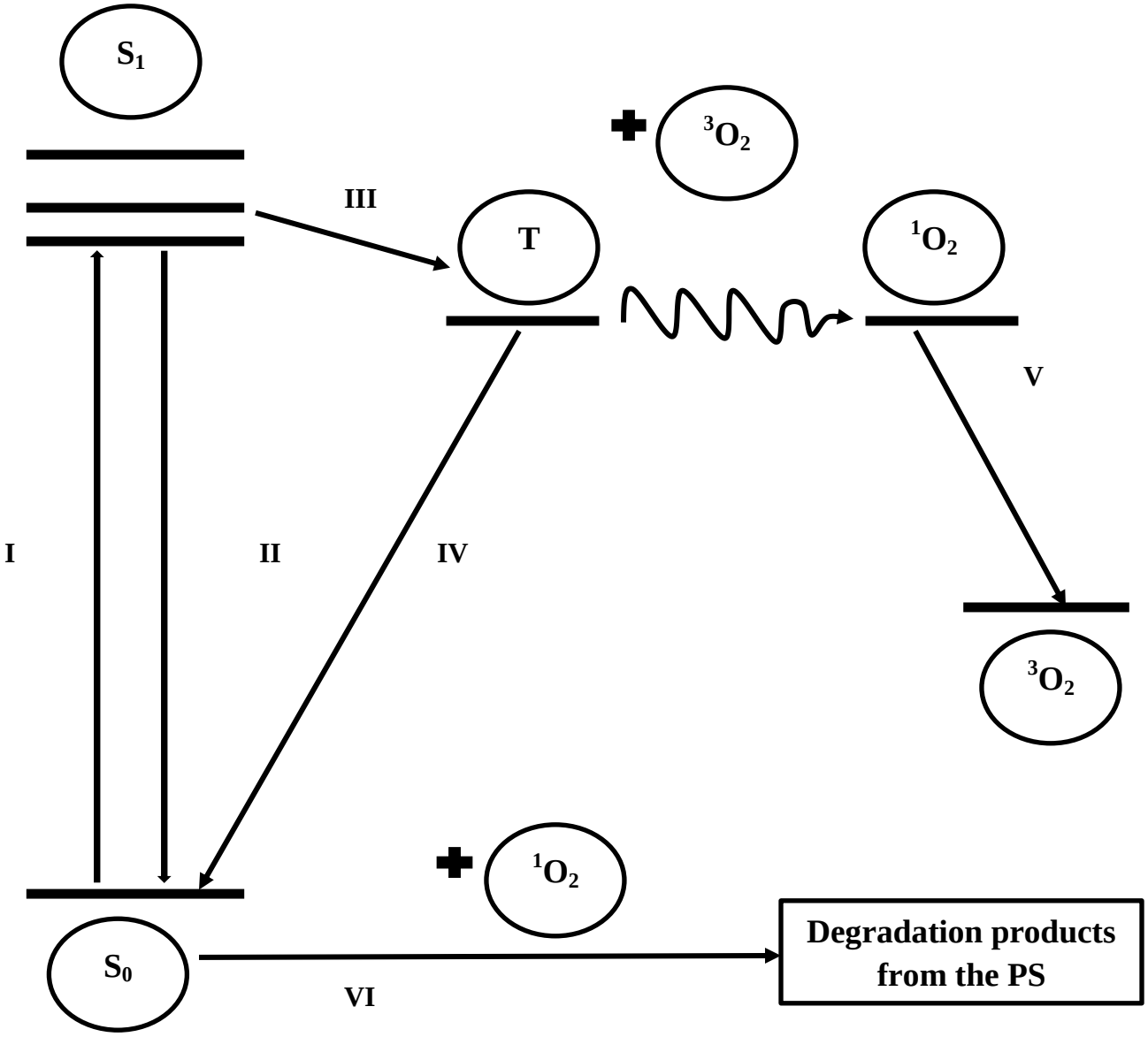

Figure 4: Simplified Jablonski diagram for PS-mediated singlet oxygen photogeneration[8]

Key:

I: Absorption **II: Fluorescence** **III: Inter System Crossing**

IV: Phosphoresence of PS **V: Phosphorescence of $^1O_2$** **VI: Photobleaching**

The following are the six first-order differential rate equations[7] that we come across in a Type II PDT process;

Rate equations

$$\frac{d[S_0]}{dt} = -v\rho\sigma_{psa}[S_0] - k_{pb}[{}^1O_2][S_0] + \frac{\vartheta_{10}}{\tau_1}[S_1] + \frac{\vartheta_{30}}{\tau_3}[T] + \frac{\alpha_S}{\tau_3}[T][{}^3O_2] \longrightarrow \quad \text{(i)}$$

$$\frac{d[S_1]}{dt} = -\frac{1}{\tau_1}[S_1] + v\rho\sigma_{psa}[S_0] \longrightarrow \quad \text{(ii)}$$

$$\frac{d[T]}{dt} = \frac{\vartheta_{13}}{\tau_1}[S_1] - \frac{\vartheta_{30}}{\tau_3}[T] - \frac{\alpha_S}{\tau_3}[T][{}^3O_2] \longrightarrow \quad \text{(iii)}$$

$$\frac{d[{}^3O_2]}{dt} = \frac{\vartheta_0}{\tau_0}[{}^1O_2] - \frac{\alpha_S}{\tau_3}[T][{}^3O_2] + P \longrightarrow \quad \text{(iv)}$$

$$\frac{d[{}^1O_2]}{dt} = -k_{pb}[{}^1O_2][S_0] - \frac{\vartheta_0}{\tau_0}[{}^1O_2] + \frac{\alpha_3}{\tau_3}[T][{}^3O_2] - k_{pb}[{}^1O_2][S_0] - k_{cx}[{}^1O_2][R] - k_{sc}[{}^1O_2][C_i] \longrightarrow \quad \text{(v)}$$

$$\frac{d[R]}{dt} = -k_{cx}[{}^1O_2][R] + U \longrightarrow \quad \text{(vi)}$$

For a summary of the coefficients and parameters used in these equations and their corresponding values, refer to Table 2.

The set of rate equations from (i) to (vi) have been solved using an implicit iterative method with the coding done in FORTRAN.

Table 1: Symbols used and their definitions[7]

Symbol	Definition	Symbol	Definition
τ_1	Relaxation time of S_1 to S_0	ρ	Photon density
τ_3	Relaxation time of T to S_0	σ_{psa}	Absorption cross-section of S_0 molecules
τ_0	Relaxation time of $^1O_2$ to $^3O_2$	$[S_0]_i$	PS drug concentration in cells and tissues 48h after injection
η_{10}	Quantum yield of S_1 to S_0 step	$[S_1]_i$	Initial concentration of $[S_1]$
η_{13}	Quantum yield of S_1 to T step	$[T]_i$	Initial concentration of [T]
η_{30}	Quantum yield of T to S_0	$[^3O_2]$	Initial concentration of $[^3O_2]$
η_0	Quantum yield of $^1O_2$ to $^3O_2$	$[^1O_2]$	Initial concentration of $[^1O_2]$
α_s	Efficiency factor for energy transfer from T to $^3O_2$	$[R]_i$	Initial concentration of intracellular molecular receptor for $^1O_2$
k_{pb}	Bimolecular photobleaching rate	$[C]_i$	Scavenger concentration
k_{cx}	Bimolecular cytotoxicity rate	P	Rate of O_2 diffusion and perfusion
k_{sc}	Bimolecular scavenging rate	U	Cell damage repair rate
V	Light speed in tissue = c/n = c/1.38		

We have resorted to using an implicit versus an explicit solution method for this time-dependent problem due to the numerical stability of an implicit method, which being more complex to program and requiring more computational effort in each solution step permits large time-step sizes. In this thesis, for e.g. we have considered time points ranging from 10^{-9}s to 10^{3}s.

Thus the time dependences of the photosensitiser (PS), oxygen (triplet and singlet states) and intracellular unoxidised receptor (R) concentrations were obtained and tandem decreases in the concentrations of the ground-state photosensitiser and receptor were observed.

(2.2) THE IMPLICIT METHOD:[6]

Consider the differential equation,

$$\frac{d[\boldsymbol{X}(t)]}{dt} = f(\boldsymbol{X}(t))$$

The implicit Euler method is represented by;

$$\boldsymbol{X}_{new} = \boldsymbol{X}_0 + hf(\boldsymbol{X}_{new}) \longrightarrow \text{(vii)}$$

That is, we are going to evaluate $\boldsymbol{f}$ at the current time (which is unknown), rather than previous time (which is known). Then the equation says "if you were at $\boldsymbol{X}_{new}$, and took a step - $hf(\boldsymbol{X}_{new})$, you would end up at $\boldsymbol{X}_0$." This means if our differential equation represents a system that is reversible in time, this step makes sense. It is about finding a point $\boldsymbol{X}_{new}$ such that if we reverse time, we would end up at $\boldsymbol{X}_0$. Unfortunately, we cannot in general solve for $\boldsymbol{X}_{new}$ directly instead we follow an iterative procedure.

We define $\Delta \boldsymbol{X}$ by $\Delta \boldsymbol{X} = \boldsymbol{X}_{new} - \boldsymbol{X}_0$.Using this, we rewrite equation (vii) as

$$\Delta \boldsymbol{X} = h(f(\boldsymbol{X}_0) + f`(\boldsymbol{X}_0)\Delta \boldsymbol{X})) \longrightarrow \text{(viii)}$$

In many types of problems, the matrix represented by f' will be sparse making solving process easier. For time-dependant problems involving stiff ODEs, the implicit method is the preferred choice.

Implicit schemes are converged using iterations. These iterations usually involve solving a set of linear system(s) of equations that are either discrete forms of the original linear equations or linearised forms if the original equations are non-linear.

This scheme is always numerically stable but usually more numerically intensive than the explicit method as it requires solving a system of numerical equations on each time step.

CHAPTER 3: RESULTS AND DISCUSSIONS

Literature on PDT research was surveyed to select values of coefficients and parameters (refer Table 2 for the range of values used) and the set of rate equations in the time domain were solved. From that, we plotted graphs showing the time dependence of concentrations of photosensitiser, oxygen and unoxidised receptor and got to understand the dependence of photobleaching and cytotoxicity on drug dose and photon density.

We plotted two graphs- Figure 5 , Figure 6 using the exact set of values taken by *Hu et al.* and successfully validated our graphs with those obtained by them, thus ensuring that our approach was heading in the right direction. Next we plotted a set of graphs by varying only the photosensitiser concentration in its ground state [S_0]- Figure 9, Figure 10 , and excited state [S_1]- Figure 7, Figure 8; keeping all other parameters fixed. Detailed discussions have been entailed after the graphs.

ASSUMPTIONS:[7]

(1) Differences in the optical parameters between tumour and normal tissues attributable to the physiological variations such as the extravasculature in the tumour and differential PS uptake.

(2) Molecular parameters of PDT processes vary in different cells and their environments and, therefore, uncertainty does exist for selecting the parameter values.

(3) Photobleaching or destruction of the PS can occur via two pathways : one by photochemical reaction of S_0 with $^1O_2$, and the other independent of oxygen . We limit our model on photobleaching to the former and described it by a reaction rate k_{pb} in equation (v).

(4) Multiple types of intracellular receptors exist that react with $^1O_2$ at different rates, hence k_{cx} and [R] should be regarded as the averaged values over different species of receptors involved in the PDT process.

(5) Refractive index n=1.38 for both tumour and normal tissue regions. The ranges of the tissue optical parameters were selected based on the values determined at light wavelength λ~630 nm. A total of $2.12x10^8$ photons in a diverging beam of light have been tracked within the tissue phantom. Used various time steps between 0 ns and t_{max}=3000 s to achieve desired modelling.

(6) Parameters set as $[^3O_2]_i=5.06x10^{17}$ (cm^{-3}), $[S_0]_i=5.00x10^{13}$ (cm^{-3}), $\rho=1.00x10^5$ (cm^{-3}), these concentrations were normalised by their initial values. (Figure 5)

(7) $[S_1]$, [T] and $[^1O_2]$ were plotted using the normalised values of their corresponding maximum values $[S_1]_m$, $[T]_m$ and $[^1O_2]_m$ respectively. (Figure 6)

Table 2: Values of coefficients and parameters in equations (i)-(vi)[7]

No:	Symbol	Definition	Values	Notes
1.	τ_1	Relaxation time of S_1 to S_0	10 ns	-
2.	τ_3	Relaxation time of T to S_0	30 or 300 μs	-
3.	τ_0	Relaxation time of $^1O_2$ to $^3O_2$	30 or 300 μs	-
4.	η_{10}	Quantum yield of S_1 to S_0 step	0.2	-
5.	η_{13}	Quantum yield of S_1 to T step	0.8	-
6.	η_{30}	Quantum yield of T to S_0	0.3	-
7.	η_0	Quantum yield of $^1O_2$ to $^3O_2$	0.3	-
8.	α_s	Efficiency factor for energy	$1x10^{-17}$ cm^3	-

		transfer from T to $^3O_2$		
9.	k_{pb}	Bimolecular photobleaching rate	$2x10^{-10}$ $cm^3.s^{-1}$	kpb/kcx[R]~80 M^{-1}
10.	k_{cx}	Bimolecular cytotoxicity rate	$2x10^{-9}$ $cm^3.s^{-1}$	-
11.	k_{sc}	Bimolecular scavenging rate	$1x10^{-9}$ $cm^3.s^{-1}$	-
12.	V	Light speed in tissue = c/n = c/1.38	$2.17x10^{10}$ $cm.s^{-1}$	-
13.	P	Photon density	$5x10^4$ to $5x10^7$ cm^{-3}	-
14.	σ_{psa}	Absorption cross-section of S_0 molecules	$5x10^{-13}$ cm^2	-
15.	$[S_0]_i$	PS drug concentration in cells and tissues 48h after injection	$2x10^{10}$ to $2x10^{14}$ cm^{-3}	-
16.	$[S_1]_i$	Initial concentration of $[S_1]$	0	-
17.	$[T]_i$	Initial concentration of [T]	0	-
18.	$[^3O_2]$	Initial concentration of $[^3O_2]$	$4.98x10^{17}$ or $5.06x10^{17}$ cm^{-3}	-
19.	$[^1O_2]$	Initial concentration of $[^1O_2]$	0	-
20.	$[R]_i$	Initial concentration of intracellular molecular receptor for $^1O_2$	$5x10^{17}$ cm^{-3}	-
21.	$[C]_i$	Scavenger concentration	$1x10^3$ cm^{-3}	-
22.	P	Rate of O_2 diffusion and perfusion	$1x10^{12}$ to $1x10^{13}$ $cm^3.s^{-1}$	-
23.	U	Cell damage repair rate	$2.6x10^{12}$ $cm^3.s^{-1}$	-

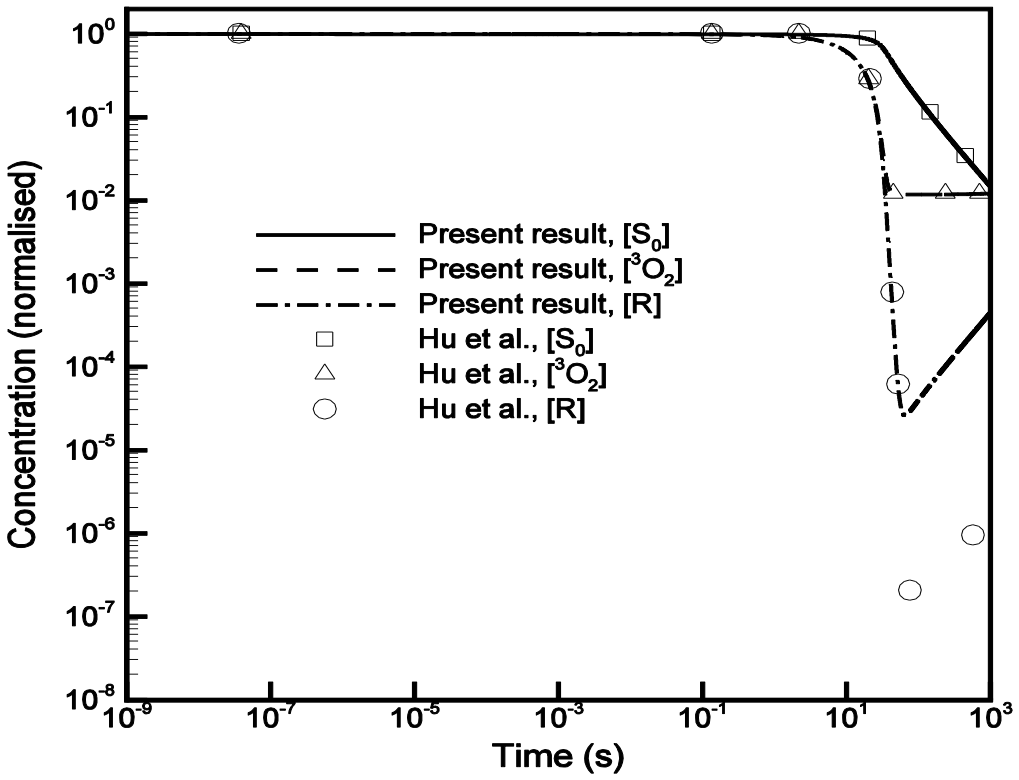

Figure 5: [S_0], [$^3O_2$], [R] versus time (validation graph 1)

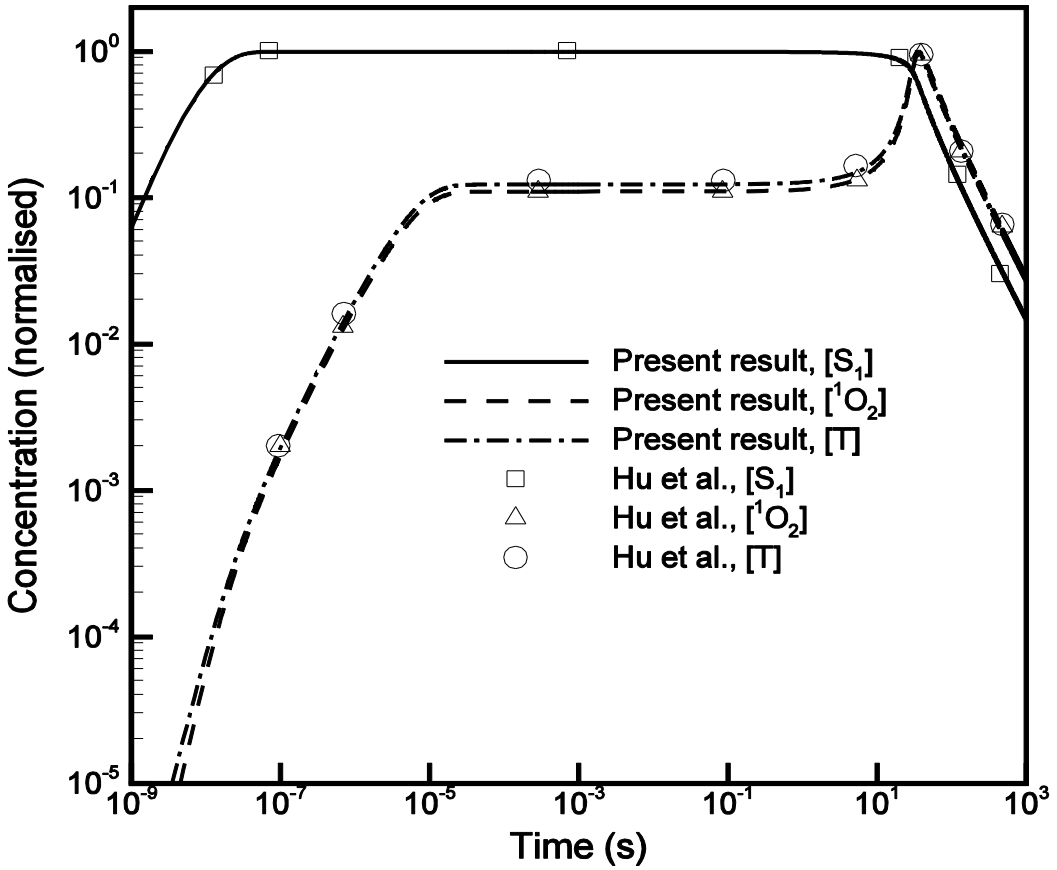

Figure 6: [S_1], [$^1O_2$], [T] versus time (validation graph 2)

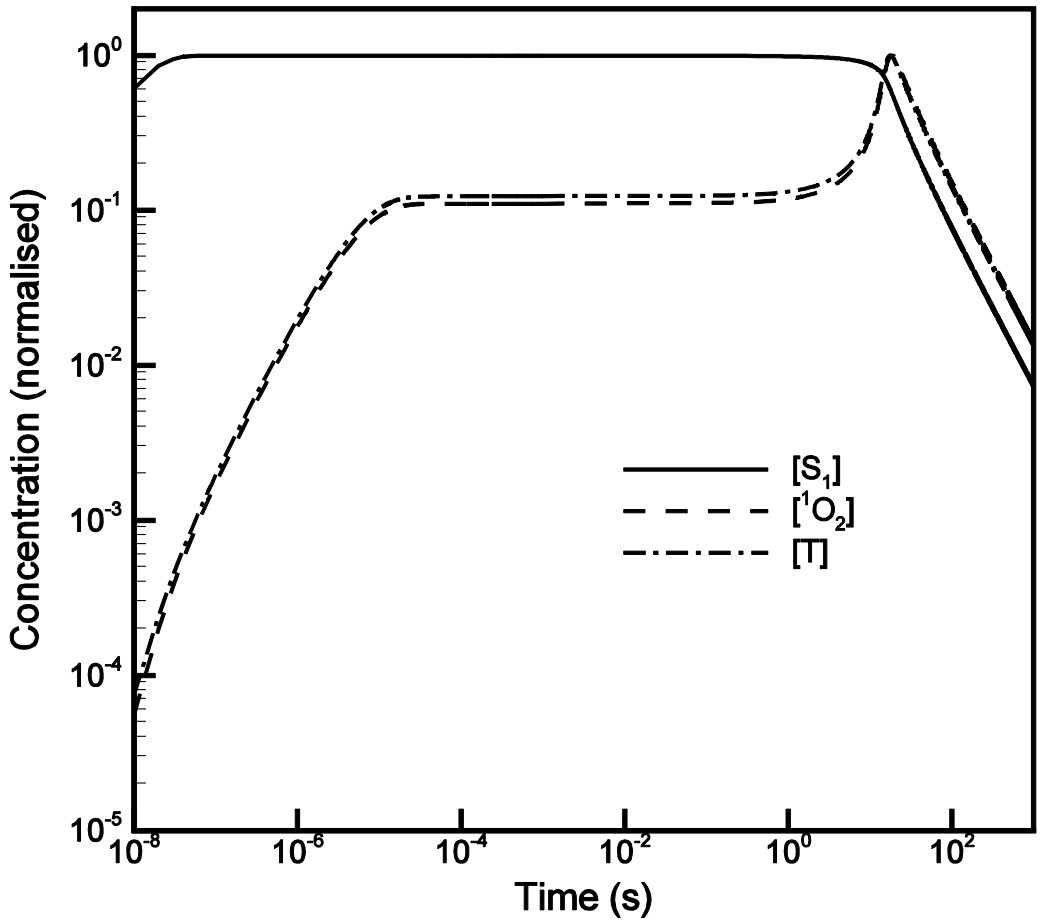

Figure 7: $[S_1]$, $[^1O_2]$, [T] versus time at $[S_1] \sim 10^{13}$ (cm^{-3})

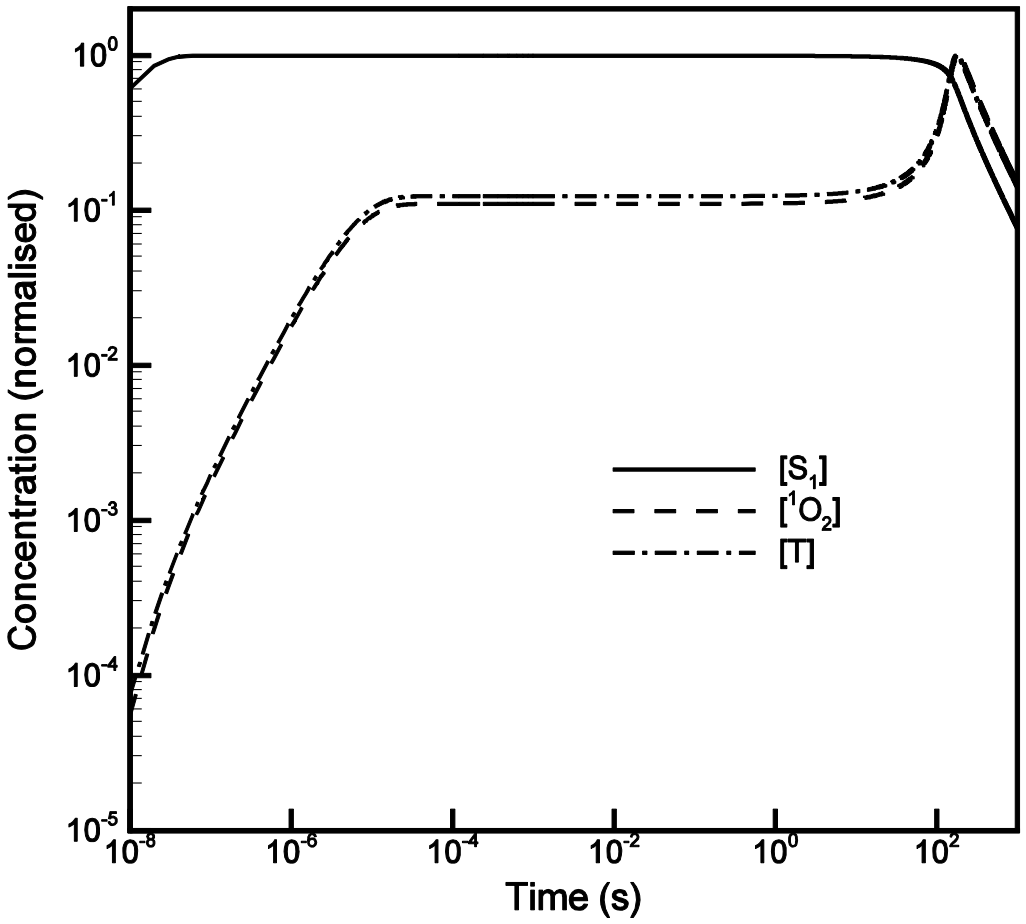

Figure 8: $[S_1]$, $[^1O_2]$, [T] versus time at $[S_1] \sim 10^{14}$ (cm^{-3})

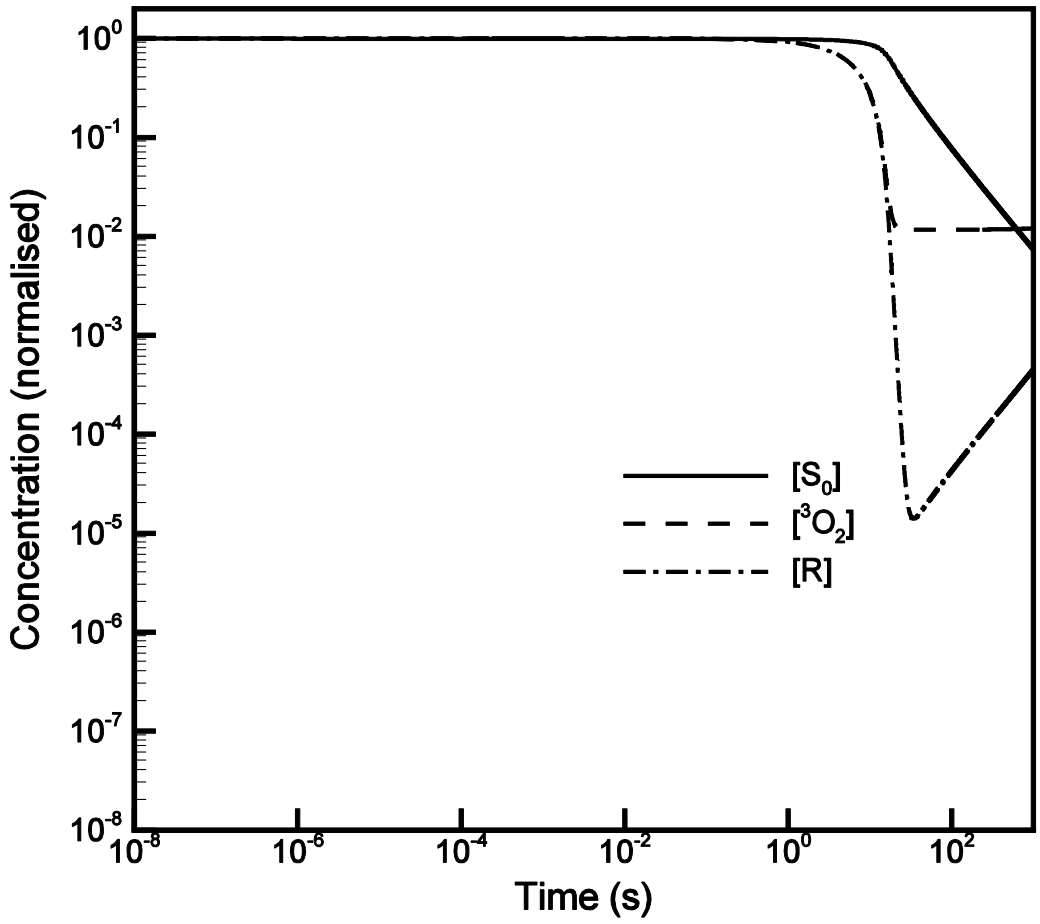

Figure 9: $[S_0]$, $[^3O_2]$, [R] versus time at $[S_0] \sim 10^{14}$ (cm^{-3})

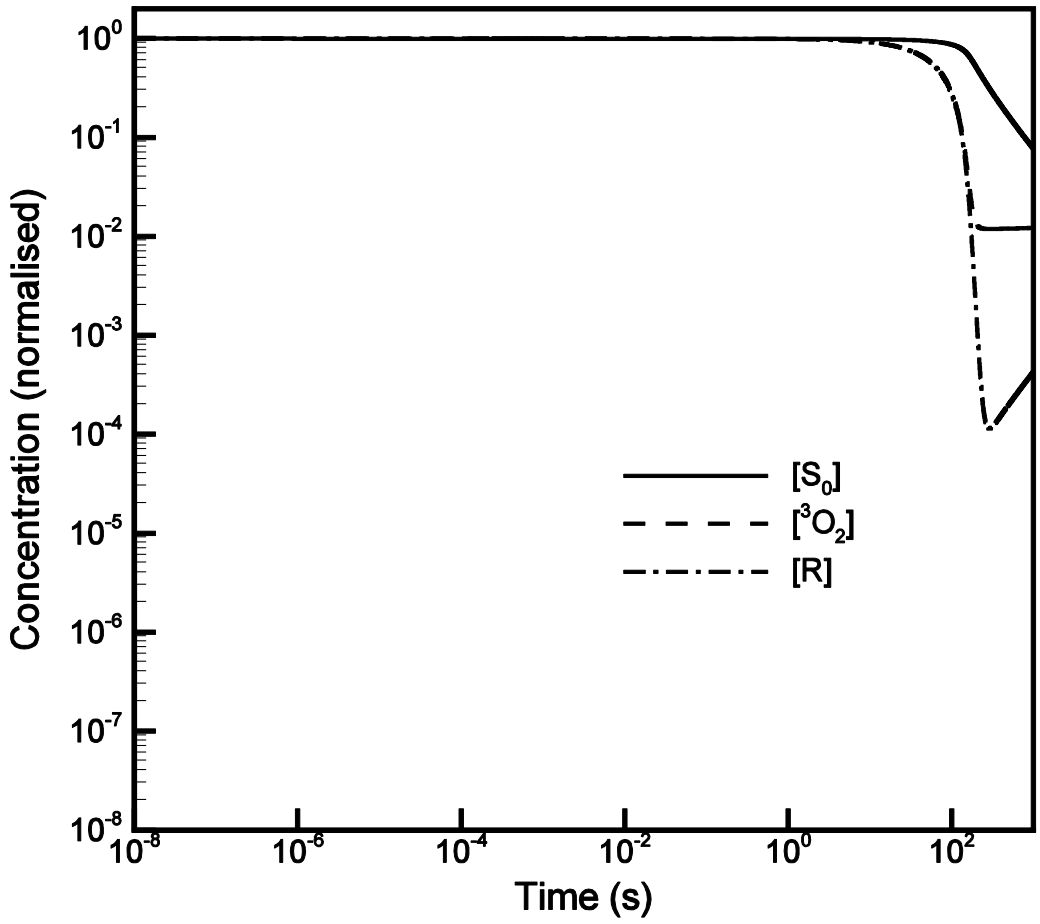

Figure 10: $[S_0]$, $[^3O_2]$, [R] versus time at $[S_0] \sim 10^{13}$ (cm^{-3})

These concentrations versus time graphs demonstrate two types of trends:

(1) those for the ground-state molecules of PS, oxygen and unoxidised receptors are slow-varying in response to the activation light, whereas

(2) those for the excited molecules exhibit transient responses depending on the specific choice of relaxation times.

The graphs show a decline in S_0 concentration which is due to the conversion of PS into excited S_1 and T states with time. Once a certain threshold limit is crossed, marked at time t=10s, S_0 concentration falls due to the process of photobleaching where the singlet O_2 degrades the PS. At the same time, it may rise due to fluorescence of excited S_1 back to S_0 or phosphorescence of excited T to S_0, hence the fluctuations in S_0 concentration.

The decline in $^3O_2$ concentration is due to in-vivo molecular oxygen ($^3O_2$) being consumed and converted to ROS, here we note that there is a threshold oxygen concentration below which there is no cytotoxicity and PDT process in fact creates a pseudo-hypoxic condition which slows down PDT temporarily. Considering vascularisation of tissues, there is also a possibility of blood-perfusion as well as gaseous diffusion of the oxygen which brings down its concentration though to a small extent. However phosphorescence of singlet oxygen to molecular oxygen can raise its concentration.

The concentration of the unoxidised receptors R shows a fall after a limit since till that point, the cell's inherent repair mechanism U was able to nullify the cytotoxic effects of the ROS. Singlet oxygen has yield which is proportional to both the PS drug and molecular oxygen concentrations and when they exceed tolerance limits, the repair machinery fails and cytotoxic damage begins by oxidisation of various intracellular receptors at the PS binding sites.

CONCLUSIONS:

One of the major hindrances in getting useful results from these simulations is the challenge of performing them such that the biological accuracy of the mathematical model is not compromised in the name of simplicity before we may perform the simulation. The ODEs found in these models are often nonlinear and stiff. The consequence of the stiffness is a trade-off between stability over accuracy.

Results indicate that appropriate combinations of PS drug and light doses may be explored to achieve cytotoxicity in a tumour without significant photobleaching. Also we observed collateral tissue damage in addition to the killing of tumour cells at higher drug doses.

To summarise, we have demonstrated the feasibility of modelling a Type II Photofrin-mediated PDT process by solving a set of rate equations to obtain the time dependence of the concentrations of PS, oxygen and intracellular unoxidised receptors. Each species followed differential decay rates. A threshold of oxygen was identified in our model under which no cytotoxicity occurred. With this approach, we elucidated the variations in drug dose at different concentrations of oxygen, which can be used to quantitatively investigate the photobleaching and cytotoxicity effect.

Future research will undoubtedly be directed towards the development of better photosensitisers with increased tumour selectivity and fewer side effects, in particular the systemic toxicity and duration of photosensitivity. Simultaneously we need to focus on more efficient light delivery systems and better comprehension of the optical properties of tissues. And when all these issues have been resolved, photodynamic therapy will fully realise its potential as a major cancer treatment modality.

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