Numerical simulation of singlet oxygen generation by a porphyrin-based photosensitizer

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Abstract: Photodynamic therapy is a new, minimally invasive method of treating cancer. It is based on the light activation of a photosensitizer, which can be administered in several forms (e.g. as an injection or an ointment) and accumulates selectively in the tumour. The activated photosensitizer in turn generates singlet oxygen in the malignant tissue, the action of which leads to tumor necrosis or apoptosis. In the present paper, we have developed and implemented a simulation model by using the main processes that the photosensitizer undergoes in the presence of irradiating light and molecular oxygen. We have carried out the simulation for the commercially available photosensitizer porfimer sodium (commercial name Photofrin), which is also currently used in clinical practice, regarding its ability to generate singlet oxygen. The model allows predictive statements to be made regarding the efficiency of photodynamic treatments at various initial conditions, including photosensitizer concentration and tissue oxygen concentration.

Key-Words: simulation, singlet oxygen, photosensitization, porphyrin, photodynamic therapy

1 Introduction

Photodynamic therapy (PDT, also known as photochemotherapy), is a minimally invasive method based on applying a photosensitiser compound onto the tumour and then irradiating with a light source [1-2]. The photosensitiser transfers its energy to the oxygen found in tumoral tissue, generating singlet oxygen, which can oxidize tumour cells and also induce cell death (apoptosis) [3]. Some common classes of compounds used as photosensitizers in PDT are porphyrins, metalloporphyrins, purpurins, phtalocyanines, chlorin and porphycenes, with porphyrins being the most widespread in clinical settings. Porphyrins are naturally occuring tetrapyrrolic compounds found, for example, in hemoglobin, to chlorophyll, myoglobin and cytochromes [4].

The photochemical processes that take place during the irradiation process lead to the production of singlet oxygen. In a first step, the photosensitizer (PS) in its fundamental singlet state (S_0) absorbs light and is excited into its excited singlet state (S_1). This process is reversible, and the inverse process is the fluorescence back to the fundamental state. In the second step, the transformation of the excited of the PS takes place, and the triplet form of the PS results. This triplet form then reacts with the molecular oxygen present in tissue to give singlet oxigen ($^1O_2$) [5]. Singlet oxygen then reacts very fast with cellular targets and causes their apoptosis. Since also healthy cells can be affected, it is important for the photosensitizer to accumulate selectively in tumoral tissue to produce cancer cell necrosis and to minimize healthy tissue damage. Besides the reaction with cells, singlet oxygen can also react with the photosensitizer. This process, called photobleaching [6] leads to the irreversible inactivation of the photosensitizer, reducing the overall efectiveness of the photodynamic process, and has to be minimized. The efficiency of singlet oxygen generation depends on several factors, such as the molecular structure of the photosensitizer or its concentration [7].

In order to be able to optimally administer the photosensitizer drug in a given oncological case, one has to know which is the amount of singlet oxygen produced by the photosensitizer as a function of various treatment parameters (drug concentration, type of drug used, irradiation intensity, oxygen concentration). For the achievement of this goal, the modeling and simulation of the singlet oxygen generation process comes at hand, and allows for the optimization of the therapeutic effect (by increasing the amount of singlet oxygen produced and minimizing unwanted side reactions-such as photobleaching). The computational model of the singlet oxygen generation process also allows the testing of various therapeutic situations (varying dosages, different photosensitisers) in order to establish the best treatment approach, without the high costs associated with the carrying out of time-intensive experiments.

The present paper focuses on the simulation of the generation of singlet oxygen from solutions of the drug Photofrin (porfimer sodium), which is widely used in clinical settings for the treatment of several cancers, such as esophageal cancer, non-small cell lung cancer or skin cancer [8-10].

Photofrin is not a single chemical entity, it is a mixture of oligomers formed by ether and ester linkages of up to eight porphyrin units [11]. It can be depicted using the structural formula in fig. 1.

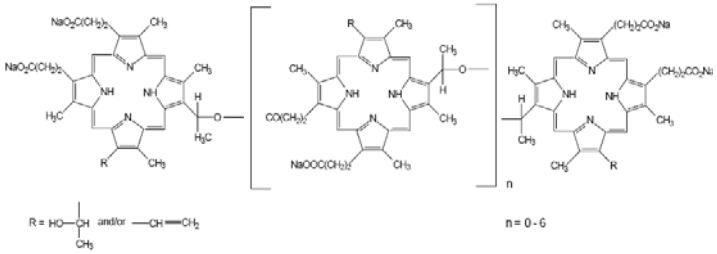

Fig. 1: Representative structural formula for components of Photofrin

Photofrin has cytotoxic and antitumor actions that are light-dependent [12-13]. Photodynamic therapy with Photofrin consists of two main stages: the first is the intravenous administration of the Photofrin solution, followed by an irradiation step with a 630 nm wavelength laser [14-15]. Especially when the illumination is carried out in internal organs (such as in endobronchial cancer), laser diffusers are used. The PDT session is minimally invasive, due to the fact, that Photofrin is selectively retained in tumour cells, and laser light is specifically delivered to the tumoral site [16-17]. Commonly used incident laser light doses are in ther range of 150-300 J/cm of diffuser length [18].

The simulation of the photosensitization and singlet oxygen generation process has been carried out by using the Comsol 3.3 simulation software, an implementation of the Matlab PDE (Partial differential equation) toolbox, based on the finite element mehod (FEM) [19-22]. This software allowed to solve the material and energy balances associated with the main reaction processes involved in singlet oxygen generation. In the present paper, we have carried out the simulation of the singlet oxygen generation process in a Photofrin-containing solution, when exposed to light. A time-dependent model has been developed for the determination of the concentrations of the active species.

2 Methods

2.1. Modeling approach

By considering the photochemical processes involved in the irradiation of an aqueous solution of Photofrin (porfimer sodium salt) with light, a simulation model has been developed, based on the kinetic equations of the individual reactions.

The relevant photoinduced processes during singlet oxygen generation can be represented in the Jablonski diagram (fig. 2).

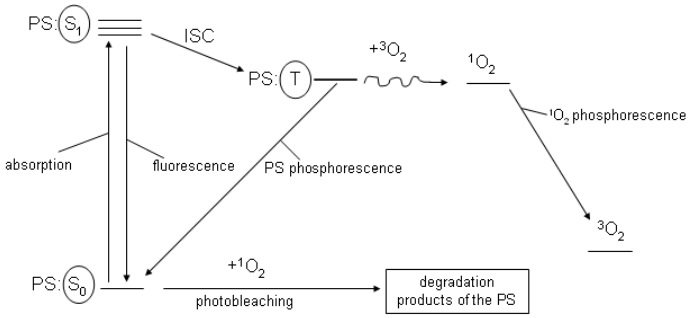

Fig. 2. Jablonski diagram for Photofrin-mediated singlet oxygen photogeneration

The process starts with the absorption of light by the photosensitizer (PS), which passes from the fundamental singlet state (S_0) to the excited singlet state (S_1). This process is reversible, the S_1 state can revert back to the fundamental state by fluorescence emission. Then, the excited state is converted by the intersystem crossing (ISC) process to the triplet state (T) of the photosensitizer. This triplet state can either transfer its energy to the dissolved molecular oxygen (fundamental state, $^3O_2$), generating singlet oxygen ($^1O_2$), or, return to the fundamental state via the phosphorescencence process. The generated singlet oxygen can also undergo a phosphorescence relaxation process back to the fundamental state. Finally, the generated singlet oxygen can also react with the photosensitizer, a process called photobleaching, which reduces the overall singlet oxygen generation eficiency.

The above-presented reactions, together with their rate constants, are presented in equations 1-6.

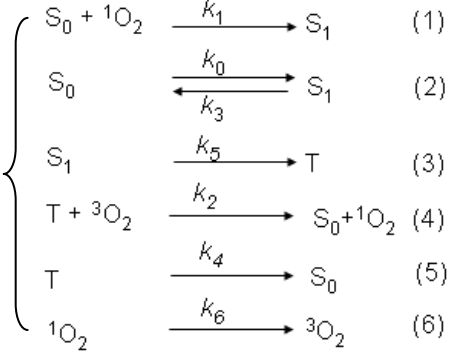

Fig. 3: Relevant reactions involved in photosensitization process with Photofrin (Porfimer sodium salt)

The corresponding kinetic equations are:

$$\begin{cases} \text{For reaction (1): } rate = -k_1 \cdot c(^1O_2) \cdot c(S_0) & (7) \\ \text{For reaction (2): } rate = -k_0 \cdot c(S_0) + k_3 \cdot c(S_1) & (8) \\ \text{For reaction (3): } rate = -k_5 \cdot c(S_1) & (9) \\ \text{For reaction (4): } rate = -k_2 \cdot c(^3O_2) \cdot c(T) & (10) \\ \text{For reaction (5): } rate = -k_4 \cdot c(T) & (11) \\ \text{For reaction (6): } rate = -k_6 \cdot c(^1O_2) & (12) \end{cases}$$

In order to compute the amounts of generated singlet oxygen and the concentrations of the rest of active species, we have to consider that the photogeneration process of $^1O_2$ is a time-dependent one. Thus, we have to calculate the mass balances for the species involved in more than one reaction. The mass balances for the fundamental (S_0) and excited singlet (S_1) state of the PS, the PS triplet state (T), $^1O_2$ and $^3O_2$, respectively, can be written as follows:

$$\frac{d[S_0]}{dt} = -k_1 \cdot c(^1O_2) \cdot c(S_0) - k_0 \cdot c(S_0) + k_2 \cdot c(T) \cdot c(^3O_2) + k_3 \cdot c(S_1) + k_4 c(T) \quad (13)$$

$$\frac{d[S_1]}{dt} = -k_3 \cdot c(S_1) - k_5 \cdot c(S_1) + k_0 \cdot c(S_0) \quad (14)$$

$$\frac{d[T]}{dt} = -k_2 \cdot c(T) \cdot c(^3O_2) - k_4 \cdot c(T) + k_5 \cdot c(S_1) \quad (15)$$

$$\frac{d[^3O_2]}{dt} = -k_2 \cdot c(T) \cdot c(^3O_2) - k_6 \cdot c(^1O_2) \quad (16)$$

$$\frac{d[^1O_2]}{dt} = -k_1 \cdot c(S_0) \cdot c(^1O_2) - k_6 \cdot c(^1O_2) + k_2 \cdot c(T) \cdot c(^3O_2) \quad (17)$$

In order to solve the above system of differential equations, a numeric simulation software is needed. Comsol 3.3. software has been used for this purpose.

The reaction rate constants have been obtained from literature and are presented in table 1.

Table 1: Reaction rate constants for Photofrin

Constant	Corresponding to	Value	Unit	Ref.
k_0	Absorption	1.6×10^5	1/s	[23]
k_1	Photo-bleaching	2×10^{-10}	cm^3/ s	[23]
k_2	Photosensitiza-tion	0.3×10^{-13}	cm^3/ s	[23]
k_3	Fluorescence	2×10^7	1/s	[23]
k_4	PS phosphorescence	1×10^7	1/s	[23]
k_5	ISC process	8×10^7	1/s	[23]
k_6	$^1O_2$ phosphorescence	1×10^6	1/s	[23]

2.2. Software implementation

The simulation model has been implemented by using the softvare Comsol version 3.3.

Process modeling mode has been used, in order to be able to study the influence of various operating conditions on the singlet oxygen generation process. The reactions from equations 1-6 have been implemented and the reaction rate expressions have been introduced (fig. 4).

Simulations have been carried out for different initial Photofrin concentrations (low-dose amount and high-dose amount of photosensitizer), and for different initial dissolved oxygen (DO) concentrations.

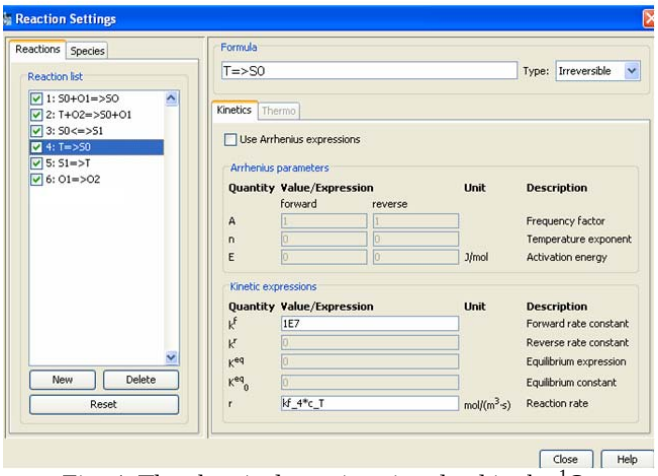

Fig. 4. The chemical reactions involved in the $^1O_2$ photogeneration, modeled in Comsol software

After specifying the reaction rate constants and the initial conditions for the simulation, the resulting model has been solved by using a time-dependent solver, based on the Backward-Euler algorithm (fig. 5). Step size has been allowed to be set automatically. Results have been plotted on a double-logarithmic scale.

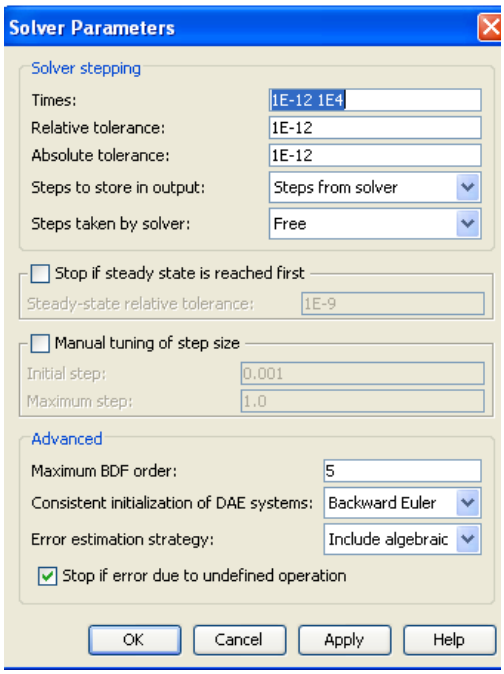

Fig. 6. Choosing the parameters of the time-dependent solver

3 Results and Discussion

We have carried out the simulations for two initial concentrations of the photosensitizer Photofrin in solution: 0.2 mg/L (low concentration) and 2 mg/L (high concentration) and two initial concentrations of dissolved oxygen (DO): 4.5 mg/L and 1.12 mg/L.

The results for the variation of the photosensitizer concentration are shown in figures 7 and

8. These results have been both obtained at a dissolved oxygen value of 2.65 mg/L.

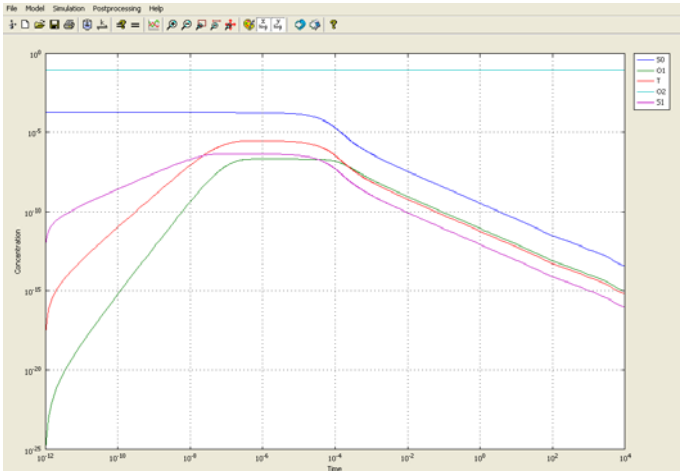

Fig. 7. Time-dependent concentration (mM) profiles of the involved species for an initial PS concentration of 0.2 mg/L (low- concentration) (time is in seconds)

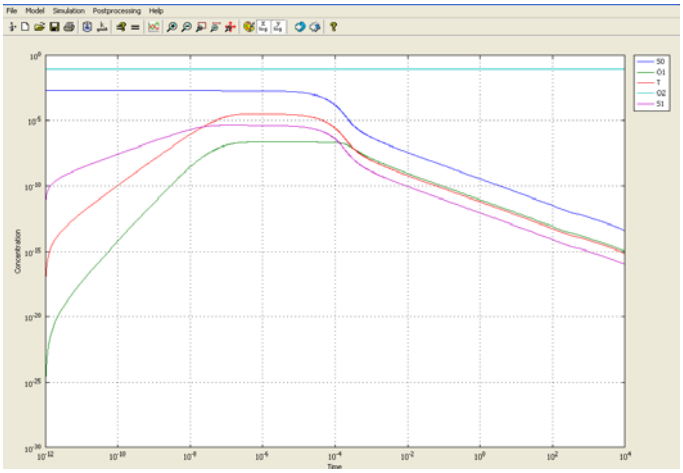

Fig. 8. Time-dependent concentration (mM) profiles of the involved species for an initial PS concentration of 2 mg/L (high concentration) (time is in seconds)

The results indicate, that the increased photosensitizer concentration determines also an increase in the number of excited singlet state PS molecules. It can be noted that at higher initial Photofrin concentrations, the triplet state is generated in a higher amount than in the low-dose PS case. Thus, the efficiency of the ISC (intersystem crossing) process increases at higher photosensitizer concentrations. Also, it can be seen that the amount of singlet oxygen generated is higher in the case of higher initial concentration of the photosensitizer.

The results of the variation of the dissolved oxygen concentration are presented in fig. 9 and fig. 10. It is to note that both cases have been computed at the photosensitizer concentration of 2 mg/L.

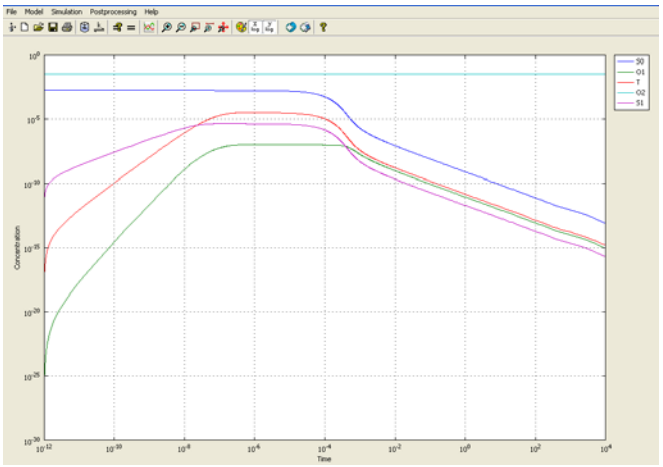

Fig. 9. Time-dependent concentration (mM) profiles of the involved species for low dissolved oxygen concentration: 1.12 mg/L (time is in seconds)

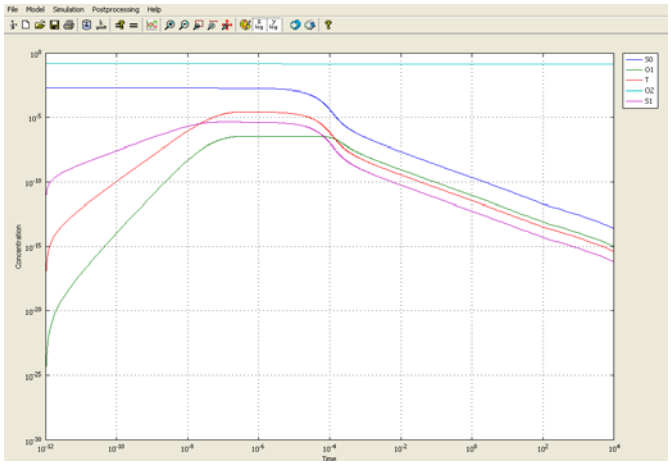

Fig. 10. Time-dependent concentration (mM) profiles of the involved species for high dissolved oxygen concentration: 4.5 mg/L (time is in seconds)

The results for the case of different initial concentrations of dissolved oxygen indicate that an increase of the DO value increases the amount of the generated singlet oxygen. Thus the increase in dissolved oxygen concentration positively affects the photosensitization process.

It can be observed, though, that the concentrations of the other species do not vary in such an extent as of singlet oxygen. For example, the concentration profile of the triplet state photosensitizer is almost the same for both initial disssolved oxygen concentrations (4.5 mg/L and 1.12 mg/L). Thus, we can observe that the variations in initial oxygen content do not affect the intersystem conversion process; this process is not sensitive to the parameter $c_i(^3O_2)$.

Thus, we can conclude that in order to increase singlet oxygen generation, it is indicated to increase the amount of administered photosensitizer and the oxygenation.

4 Conclusion

We have developed a time-dependent model for the numerical simulation of the singlet oxygen production from aqueous solutions of Photofrin (porfimer sodium salt), a porphyrin-based photosensitizing drug used in the photodynamic therapy of cancer. The model allows predictive statements to be made regarding the efficiency of photodynamic treatments at various initial conditions, including photosensitizer concentration and tissue oxygen concentration. The obtained computational results can also serve for the comparison with experimental studies in the field of photogenerated singlet oxygen formation in aqueous solutions of porphyrins [24].

The observations made in this paper are of interest for the future design of injectable photosensitizer drug preparations, where the PS concentration has to be adjusted in such a way that singlet oxygen generation is maximized, while reducing unwanted side-reactions such as photobleaching.

Further computational studies will focus on the simulation of singlet oxygen generation processes *in vitro.* Already, experimental studies have been carried out on cell lines for estimating the generated amount of singlet oxygen from photobleaching kinetics [25], and these experimental data can serve as starting points for the development of a simulation model for *in vitro* $^1O_2$ generation.

It is also planned to extend the singlet oxygen generation simulation to other porphyrins which can act as photosensitizers in PDT, such as meso-substituted porphyrins.

References:

[1] L. Danaila, M.L. Pascu, A. Popescu, M. Pascu, R. M. Ion, Spectrophotometric characterization of useful dyes in laser photodynamic therapy of cancer, Proc. SPIE, 712, 2000, pp. 4068.

[2] K. Berg, P. Selbo, A. Weyergang et al., Porphyrin-related photosensitizers for cancer imaging and therapeutic applications, J. Microsc., 218 (2), 2005, pp. 133-147.

[3] S. Agirtas, R. M. Ion, O. Bekaroglu, Spectral study of the supramolecular assemblies porphyrins–phthalocyanines, Mat. Sci. Eng.: C, 7(2), 2000, pp. 105-110.

[4] S. Varga, S. Patachia, R. Ion, M. Rinja, Targeted and directional photodynamic therapy with hydrogel controlled relase carriers like an attractive contribution to mainstream oncologic treatment, Proceedings of the 31st Congress of the American Romanian Academy of Arts and Sciences, Ed. by Presses Internationales Polytechniques, Quebec, ISBN 978-2-553-01412-3, pp. 573-576, 2007

[5] X.-Y. He et al., Photodynamic therapy with photofrin II induces programmed cell death in carcinoma cell lines, Photochemistry and Photobiology, 59(4) (2008) pp. 468 – 473

[6] I. Georgakoudi, The Mechanism of Photofrin Photobleaching and Its Consequences for Photodynamic Dosimetry, Photochemistry and Photobiology, 65(1) (2008) pp. 135 - 144

[7] F. Jiang et al., Photodynamic therapy using Photofrin in combination with buthionine sulfoximine (BSO) to treat 9L gliosarcoma in rat brain, Lasers in surgery and medicine, 23(3) (1998) pp. 161-166

[8] K. Furuse et al, A prospective phase II study on photodynamic therapy with photofrin II for centrally located early-stage lung cancer, Journal of clinical oncology 11(10) (1993) pp. 1852-1857

[9] H. Ya-Ju et al., Subcellular localization of Photofrin® determines the death phenotype of human epidermoid carcinoma A431 cells triggered by photodynamic therapy: When plasma membranes are the main targets, Journal of cellular physiology, 194(3) (2003) pp. 363-375

[10] M. Dellinger, Apoptosis or Necrosis Following Photofrin® Photosensitization: Influence of the Incubation Protocol, Photochemistry and Photobiology 64(1) (2008) pp. 182 - 187

[11] T. Dougherty, Studies on the structure of porphyrins contained in photofrin II, Photochemistry and Photobiology, 46 (5) (2008) pp. 569 – 573

[12] Thomas J. Dougherty, An Update on Photodynamic Therapy Applications, Journal of Clinical Laser Medicine & Surgery. 20(1) (2002) pp. 3-7.

[13] I. van Geel et al., Mechanisms for optimising photodynamic therapy : second-generation photosensitisers in combination with mitomycin C, British journal of cancer 72(2) (1995) pp. 344-350

[14] B. W. Henderson et al., Photofrin Photodynamic Therapy Can Significantly Deplete or Preserve Oxygenation in Human Basal Cell Carcinomas during Treatment, Depending on Fluence Rate, Cancer Research 60 (2000) pp. 525-529

[15] M. Dellinger, Apoptosis or Necrosis Following Photofrin Photosensitization: Influence of the Incubation Protocol, Photochemistry and Photobiology 64(1) (2008) pp. 182 – 187

[16] D. Eton, Inhibition of intimal hyperplasia by photodynamic therapy using photofrin, Journal of surgical research, 53(6) (1992) pp. 558-562

[17] B. C. Wilson, Subcellular Localization of Photofrin and Aminolevulinic Acid and Photodynamic Cross-Resistance in Vitro in Radiation-Induced Fibrosarcoma Cells Sensitive or Resistant to Photofrin-Mediated Photodynamic Therapy, Photochemistry and Photobiology, 65(1) (2008) pp. 166-176

[18] T. Dougherty et al., Cutaneous phototoxic occurences in patients receiving Photofrin, Lasers in surgery and medicine, 10(5) (1990) pp. 485-488
[19] S. Gilbert, G. Fix, An Analysis of the Finite Element Method, Prentice-Hall, Englewood Cliffs, 1973
[20] K. Bathe, Finite element procedures, Prentice Hall, New Jersey, 1996
[21] C. Johnson, Numerical solution of partial differential equations by finite elements methods, Cambridge Univ. Press, 1987
[22] G.R. Liu, S.S. Quek, The finite element method, Butterworth-Heinemann, Oxford, 2003
[23] X.H. Hu et al., Modeling of a Type II Photofrin-mediated Photodynamic Therapy Process in a Heterogeneous Tissue Phantom, Photochemistry and Photobiology, 81 (2005) pp. 1460-1468.
[24] S. Yu. Egorov, A. A. Krasnovskii Jr., D. B. Papkovskii, G. V. Ponomarev, A. P. Savitskii, Photoinduced singlet oxygen formation in aqueous solutions of covalent porphyrin-antibody conjugates, Bulletin of Experimental Biology and Medicine 109(4) (1990) pp. 454-456
[25] J. Dysart et al., Characterization of Photofrin photobleaching for singlet oxygen dose estimation during photodynamic therapy of MLL cells in vitro, Phys. Med. Biol. 50 (2005) pp. 2597-2616