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Practical identifiability of photophysical parameters in photodynamic therapy

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Abstract—Photodynamic therapy (PDT) is an alternative treatment for cancer that involves the administration of a photosensitizing agent, which will be activated by light at a specific wavelength. In order to compare different photosensitisers for their cytotoxic activity, photophysical parameters provide good indicators. These parameters are generally estimated one by one from *in vitro* dedicated experiments, but they cannot always predict the *in vivo* cytotoxic action. So far, the estimation of photophysical parameters from *in vivo* data sets has never been regarded as a system identification problem. This paper deals with the practical identifiability of photophysical parameters. Practical identifiability deals with the uniqueness of the model parameters estimates, given the experimental data. The practical identifiability approach and its application to the photoreaction model of PDT are developed in this paper. It is shown that that the photophysical parameters involved in the kinetic model of photoreactions are identifiable in a practical framework with only one measurement - the intracellular photosensitizer concentration, and a wide square pulse as irradiation signal.¹

I. INTRODUCTION

Photodynamic therapy (PDT) [1], [2], [9] is a therapy for displastic tissues such as cancers. This therapy involves selective uptake and retention of a photosensitive drug (photosensitizer, PS) in a tumor, followed by irradiation with light at an appropriate wavelength. The activation of the photosensitizer is thought to produce singlet oxygen at high doses and thereby to initiate apoptotic and necrotic death of tumour.

For the PDT application, many different photosensitisers have been developed. It is important to compare these photosensitisers for their cytotoxic activity. It is generally accepted that the most important mechanism of cell killing is *via* the production of singlet oxygen. Currently, these photosensitisers are compared upon some photophysical properties: (1) the absorption coefficient, σ_S of the PS in the spectral region of the excitation light; (2) the quantum yield of the triplet state, Φ_T ; (3) the triplet state lifetime τ_T and (4) the quantum yield of singlet oxygen production, Φ_Δ . Most of the time, these parameters are determined from dedicated *in vitro* experiments, and unfortunately are rarely determined from *in vivo* conditions. So far, few studies

have reported some developments in the measurement of the singlet oxygen concentration through the detection of its luminescence intensity at 1272nm [3], [4]. Moreover, these developments are still difficult to reproduce in practice, due in particular to the interlesion and interpatient variations. This paper proposes an alternative approach.

A model of the photoreaction phase is introduced, based on the kinetics equations describing the type-II reactions specific to PDT. The singlet oxygen yield explicitly depends on photophysical parameters of these photoreactions. In other terms, these photophysical parameters provide good indicators to compare the cytotoxic activities of PS. So far, the estimation of photophysical parameters from *in vivo* data sets has never been regarded as a system identification problem [11]. The objective of this paper is thus to assess the practical identifiability of photophysical parameters in PDT by using an approach recently reported in [5]. The latter approach requires the dynamic model to be expressed as a block diagram implemented into the simulation environment *Simulink*® for the symbolic computation of the sensitivity functions.

II. MODELING OF PHOTOREACTIONS

This part is dedicated to the modelling of the main reactions involved in the mechanism of singlet oxygen production. A summary of the notations used in the sequel is given in Tab. I, and a list of all the reactions considered in this modelling study is given in Tab. II. Considering the reactions presented in Tab. II and adopting the notations in Tab. I, the photoreaction phase can be described by a nonlinear state-space model, defined as follows

$$\begin{cases} \dot{\mathbf{x}} = f(\mathbf{x}, \mathbf{u}, t, \Theta) \\ y = \gamma \cdot [S_0] \\ \mathbf{x}(0) = \mathbf{x}_0 \end{cases} \quad (1)$$

where \mathbf{x} , \mathbf{u} and y denote the state vector, the input vector and the output variable (fluorescence intensity) respectively, with $\mathbf{x} = ([S_0], [S_1], [T_1], [^3O_2], [^1O_2], [M])^T$ and $\mathbf{u} = (V_A, U_P, U_{O_2})$. \mathbf{x}_0 denotes the initial value of the state variable and t is the time variable. $f(\cdot)$ contains the state equations and γ is the gain of the measurement system (spectrofluorimeter). All the photophysical parameters are gathered in Θ . The

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TABLE I
NOTATIONS

Symbol	Definition	Units
V_A	Rate of photon absorption	$M \cdot s^{-1}$
U_P	Uptake rate of photosensitising molecules	$M \cdot s^{-1}$
U_{O_2}	Uptake rate of oxygen molecules	$M \cdot s^{-1}$
Φ_i	Irradiance of the incident light	$mW \cdot cm^{-2}$
$[S_0]$	Photosensitizer ground state	M
$[S_1]$	Photosensitizer singlet excited state	M
$[T_1]$	Photosensitizer triplet excited state	M
3O_2	Triplet ground - state oxygen	M
1O_2	Singlet excited - state oxygen	M
$[M]$	Cellular targets	M

TABLE II
SUMMARY OF PHOTOCHEMICAL REACTIONS

Photochemical Reaction	Rate Constant	Units
1. $S_0 + h\nu_A \rightarrow S_1$	V_A	$M \cdot s^{-1}$
2. $S_1 \rightarrow S_0 + h\nu_F$	k_F	s^{-1}
3. $S_1 \rightarrow S_0$	k_{IC}	s^{-1}
4. $S_1 \rightarrow T_1$	k_{ISC}	s^{-1}
5. $T_1 \rightarrow S_0 + h\nu_P$	k_P	s^{-1}
6. $T_1 \rightarrow S_0$	k_{TS}	s^{-1}
7. $T_1 + {}^3O_2 \rightarrow S_0 + {}^1O_2$	k_T	$M^{-1} \cdot s^{-1}$
8. ${}^1O_2 + S_0 \rightarrow {}^3O_2 + S(O)$	k_{pb}	$M^{-1} \cdot s^{-1}$
9. ${}^1O_2 \rightarrow {}^3O_2 + h\nu_L$	k_r	s^{-1}
10. ${}^1O_2 \rightarrow {}^3O_2$	k_{nr}	s^{-1}
11. ${}^1O_2 + M \rightarrow {}^3O_2 + M(O)$	k_{ox}	$M^{-1} \cdot s^{-1}$

state equations corresponding to the photoreactions defined in Tab. II are

$$\begin{cases} \frac{d[S_0]}{dt} = U_P(t) + (k_F + k_{IC})[S_1] - k_{pb}[{}^3O_2][S_0] \\ \quad + (k_P + k_{TS})[T_1] + k_T[T_1][{}^3O_2] - V_A(t) \\ \frac{d[S_1]}{dt} = V_A(t) - (k_F + k_{IC} + k_{ISC})[S_1] \\ \frac{d[T_1]}{dt} = k_{ISC}[S_1] - (k_P + k_{TS})[T_1] - k_T[T_1][{}^3O_2] \\ \frac{d[{}^3O_2]}{dt} = U_{O_2} - k_T[T_1][{}^3O_2] + (k_r + k_{nr})[{}^1O_2] \\ \frac{d[{}^1O_2]}{dt} = k_T[T_1][{}^3O_2] - (k_r + k_{nr})[{}^1O_2] \\ \quad - k_{ox}[M][{}^1O_2] - k_{pb}[S_0][{}^1O_2] \\ \frac{d[M]}{dt} = -k_{ox}[M][{}^1O_2] \end{cases} \quad (2)$$

The rate of photon absorption, V_A , depends on the (ground-state) photosensitizer concentration. Conforming to [10], V_A can be expressed as

$$V_A = \frac{\sigma_S \Phi_i}{h\nu_A} \cdot [S_0] \quad (3)$$

where σ_S is the absorption cross section of S_0 , h is the Planck's constant and ν_A is the frequency of the incident light. Therefore, the vector of parameters is given by

$$\Theta^T = (k_f \quad k_p \quad k_T \quad k_{pb} \quad k_{ISC} \quad k_l \quad k_{ox}) \quad (4)$$

with $k_f = k_F + k_{IC}$, $k_p = k_P + k_{TS}$ and $k_l = k_r + k_{nr}$.

The relationship between these parameters and the quan-

tum yield of singlet oxygen production, Φ_Δ , is

$$\Phi_\Delta = \Phi_T \cdot \phi_{en} = \Phi_T \cdot \left(\frac{k_T [{}^3O_2]}{k_P + k_{TS} + k_l [{}^3O_2]} \right) \quad (5)$$

where Φ_T is the quantum yield of triplet formation; ϕ_{en} , efficiency of energy transfer and k_l is the sum of rate constants from the quenching of T_1 by O_2 (knowing that bimolecular reactions such as physical deactivation by molecular oxygen or electron transfer can also compete with energy transfer).

Therefore, determining the parameter values from *in vivo* conditions, we could compare different photosensitizers.

III. PROBLEM STATEMENT

Consider $\mathbf{x}(t, \Theta) \in \mathbb{R}^n$, $y(t, \Theta) \in \mathbb{R}$, $\Theta \in \mathbb{P}$ and $\mathbf{u}(t) \in \mathbb{U}$. \mathbb{P} and \mathbb{U} are two open sets in \mathbb{R}^p and \mathbb{R}^q respectively with $n = 6$, $p = 7$ and $q = 3$. \mathbb{T} is a set of time instants defined by $\mathbb{T} = \{t_j\}$, $j = 0, \dots, N-1$. N , p , q , n denote the numbers of observations, parameters, input variables and state variables respectively. $\mathbf{x}_0 = x(t_0, \Theta)$ is the fixed known initial state. Now suppose that a data set $\{\mathbf{u}(t), y(t)\}$, with $t \in \mathbb{T}$, resulting from one experiment, is available. The classical identification problem consists in estimating Θ from the observed data (\mathbf{u} and y). However, by answering the investigated question, remains to know if whether or not the *in vivo* data $\{\mathbf{u}, y\}$ may be used to estimate the parameters of the model. The associated underlying problem is the practical identifiability of photophysical parameters.

IV. PRACTICAL IDENTIFIABILITY

A. Method

Structural identifiability [11] deals with the possibility to give a unique value to each parameter of a mathematical model structure. The uniqueness of this solution is assessed in an idealized or theoretical framework where the process and the model have identical structures, the data are noise-free, and where the input signals and the measurement times can be chosen at will.

However, in practice, experimental conditions are often subjected to economical and/or technical constraints which can sometimes prevent input design from being applied to the process. Moreover, the number of observations is often limited to a few data points collected at time instants $\{t_j\}$, $j = 0, \dots, N-1$. In such a case, even if a parameter is globally or locally structurally identifiable, it may not be so in practice, due to a lack of information in the available observations. For that reason, D. Dochain and P. Vanrolleghem, in [13], [14], have introduced the notion of practical identifiability. The practical identifiability includes the quality of the data. The main question of the structural and practical identifiability analysis can then be formulated as follows: 'Assume that a certain number of state variables are available for measurements; on the basis of the model structure (theoretical identifiability) or on the basis of the type and quality of available data (practical identifiability), can we expect to obtain unique values for the model parameters?'. The practical identifiability is just a particular case

of the output distinguishability [12] for a finite collection of observations $\{t_j\}$ and a given experiment $(\mathbf{x}_0, \mathbf{u})$. Then, a sufficient condition for the practical identifiability can be stated as follows: given a parametric model structure with given input signals \mathbf{u} and the initial conditions \mathbf{x}_0 ,

$$y(t_j, \Theta, \mathbf{x}_0, \mathbf{u}) = y(t_j, \Theta^*, \mathbf{x}_0, \mathbf{u}) \Rightarrow \Theta_i = \Theta_i^*, \quad (6)$$

$\forall i \in \{1, \dots, p\}$, $\forall t_j \in \mathbb{T}$ and $\forall \Theta \in V(\Theta^*) \subset \mathbb{P}$. If $d\Theta \in V(\Theta^*)$ with $\Theta = \Theta^* + d\Theta$, then a first-order Taylor expansion of $y(t, \Theta^* + d\Theta, \mathbf{x}_0, \mathbf{u})$ is given by

$$y(t, \Theta^* + d\Theta, \mathbf{x}_0, \mathbf{u}) \approx y(t, \Theta^*, \mathbf{x}_0, \mathbf{u}) + \sum_{i=1}^p \left. \frac{\partial y}{\partial \Theta_i} \right|_{\Theta_i^*} d\Theta_i. \quad (7)$$

$V(\Theta^*)$ denotes a parameter neighbourhood. A local approximation of the practical identifiability condition defined in (6) is then given by

$$\sum_{i=1}^p \left. \frac{\partial y}{\partial \Theta_i} \right|_{\Theta_i^*} d\Theta_i = 0 \Rightarrow d\Theta = 0, \quad (8)$$

or

$$\sum_{i=1}^p d\Theta_i \cdot S_y(t, \Theta_i^*, \mathbf{x}_0, \mathbf{u}) = 0 \Rightarrow d\Theta = 0, \quad (9)$$

where $S_y(t, \Theta_i^*, \mathbf{x}_0, \mathbf{u}) = \left. \frac{\partial y}{\partial \Theta_i} \right|_{\Theta_i^*}$ denotes the sensitivity function of the model output y related to the parameter Θ_i . Equation (9) expresses the linear independence of the vectors $S_y(t, \Theta_i^*) \in \mathbb{R}^m$. In other terms, given the input \mathbf{u} and the initial condition \mathbf{x}_0 , the parameters are locally practically identifiable if the mapping $S_y(t, \Theta_i^*, \mathbf{x}_0, \mathbf{u})$, from the parameter space to outputs, is one to one [12].

Let $\mathbf{S}_y(\Theta)$ be the matrix of sensitivity functions,

$$\mathbf{S}_y(\Theta) = (\mathbf{S}_y(\Theta_1), \dots, \mathbf{S}_y(\Theta_p)) \quad (10)$$

$$\mathbf{S}_y(\Theta_i)^T = (S_y(t_1, \Theta_i), \dots, S_y(t_N, \Theta_i)), \quad (11)$$

where $\mathbf{S}_y(\Theta_i) \in \mathbb{R}^{1 \times mN}$ and $\mathbf{S}_y(\Theta) \in \mathbb{R}^{p \times mN}$. The practical identifiability can be numerically implemented as a null-rank test of the matrix $\mathbf{S}_y(\Theta)$. If the rank of $\mathbf{S}_y(\Theta)$ is estimated as significantly null then the model is not locally practically identifiable. The rank of $\mathbf{S}_y(\Theta)$ can be viewed as a practical identifiability degree of a model structure for a given experiment.

B. Sensitivity analysis of block diagrams by computer algebra

Equation (9) also emphasizes the crucial role of sensitivity analysis in the local assessment of the practical identifiability. A symbolic approach which eliminates the drawbacks of the finite-difference approximations and the complexity of the automatic differentiation is proposed and implemented into a software: *Diffedge*[©] (<http://www.appedge.com/>). It combines a computer algebra system and block diagrams to compute the derivatives of a Simulink model with respect to its independent parameters. The derivative model is also represented by a block diagram and can be used like any Simulink model.

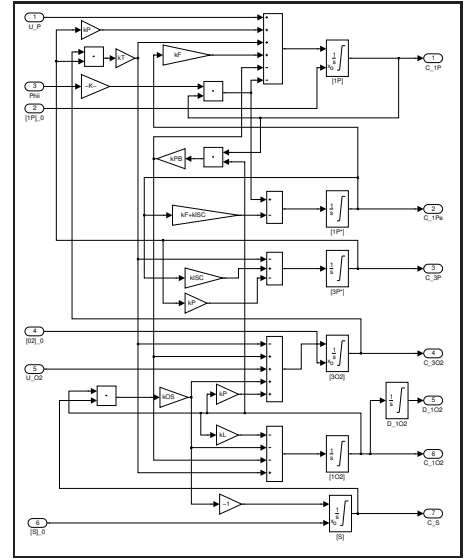


Fig. 1. Block diagram of the photoreactions

C. Application to the photoreaction model

Fig. 1 depicts the block diagram of eq.(2) implemented into the simulation environment Simulink[©]. The initial values of the states have been chosen from literature [10]. Φ_i is a square signal (width: 300 s), $U_P = 0$, and $U_{O_2} = 1.5 \cdot 10^{-4} \text{mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$. Nominal values of the physical parameters in Θ correspond to *in vitro* values obtained in scientific literatures [10], [6]. The numerical rank of $\mathbf{S}_y(\Theta)$ is equal to 7, therefore all the parameters are practically identifiable.

In conclusion, all the considered parameters can be estimated from a realistic *in vivo* experiment.

V. PRACTICAL IDENTIFIABILITY RANKING OF PHOTOPHYSICAL PARAMETERS

Knowing the number of identifiable parameters, it is also interesting to sort out the parameters according to their influence on y and their cross-correlation of their sensitivity functions. Indeed, more a parameter is practically identifiable, more accurate is its estimate.

A. Output sensitivity ranking

The first classification consists in sorting out (in a descending order) the parameters according to their influence on the output variable. The comparison criterion proposed herein can be defined as follows

$$L(\Theta_i) = |S_y(\Theta_i)| = \left| \frac{\partial y(\Theta)}{\partial \Theta_i} \right|, \quad (12)$$

where $S_y(\Theta_i)$ denotes the sensitivity function of $y(\Theta)$ with respect to the model parameter Θ_i . Results are presented in fig. 2 by a logarithmic diagram. It is shown that k_l is the most significant parameter, whereas k_T is the least significant parameter, in the sense of the maximization of L .

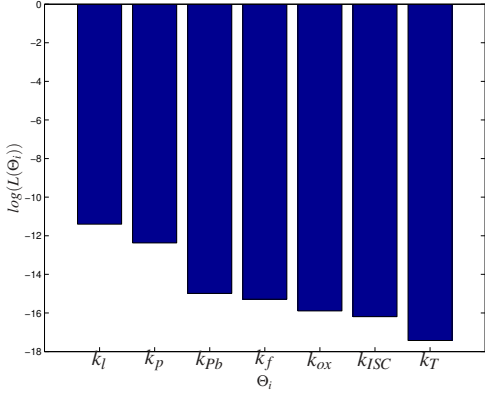


Fig. 2. Output-sensitivity ranking

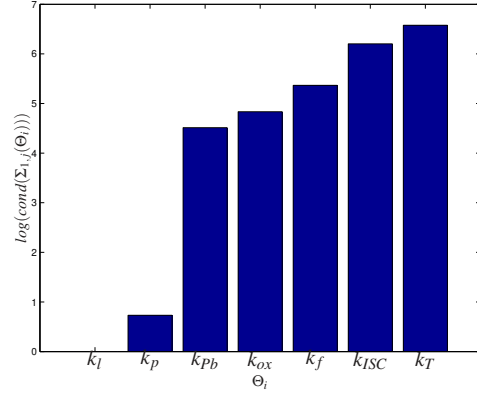


Fig. 3. Condition numbers spectrum of $\Sigma_{1,j}$

B. Condition number ranking

In a second step, a new classification is performed by taking into account the cross-correlation between the sensitivity functions. The vectors $\mathbf{S}_y(\Theta_i) \in \mathbb{R}^N$ are sorted out according to the condition number of a matrix $\Sigma \in \mathbb{R}^{N \times \mu}$ where μ is the number of identifiable parameters. Σ is iteratively built up from its first column $\Sigma_1 = S_y(\Theta_{Lmax})$ such that

$$\Theta_{Lmax} = \arg \max_{\Theta_i} L(\Theta_i) \quad i \in \{1, \dots, \mu\}. \quad (13)$$

In other words, Σ is initialized by the sensitivity function of the most influent parameter on the output, i.e. $S_j(k_l)$ in this case. The other columns of Σ are chosen among the remaining sensitivity functions and are arranged in such an order that

$$\Sigma_j = S_y(\check{\Theta}_i) \quad \text{with:} \quad (14)$$

$$\check{\Theta}_i = \arg \min_{\Theta_i} \text{cond}(\Sigma_{1,j}(\Theta_i)) \quad \forall \Theta_i \neq \Theta_{Lmax} \quad (15)$$

and $j \in \{2, \dots, \mu\}$. Σ_j denotes the j^{th} column of Σ and $\Sigma_{1,j}(\Theta_i) = [\Sigma_1, \dots, \Sigma_{j-1}, S_j(\Theta_i)]$. Hence, Σ_j is selected among a given number of sensitivity functions in order to minimize the condition number of $\Sigma_{1,j}$. The final arrangement of the sensitivity functions is described by a spectrum of the condition number of $\Sigma_{1,j}$ with respect to j . Fig. 3 shows the spectrum of the condition numbers of $\Sigma_{1,j}$ with respect to j . The final ranking of the parameters is given by the x-axis of this spectrum. It appears that k_l is the most identifiable parameter and k_T is the least one. In other terms, the estimation of k_T will be still uncertain than for k_l .

VI. CONCLUSIONS

This article deals with the practical identifiability of the photophysical parameters in PDT. In this study, we have considered realistic *in vivo* constraints: (i) the input signal is a wide square signal and (ii) there is only one measurement, the concentration of intracellular photosensitizer. In these conditions, it is shown that seven photophysical parameters are identifiable. This result opens new perspectives concerning the estimation of photophysical parameters in PDT. The main benefit of the proposed approach is a significant reduction of the experimental cost. Indeed, the seven photophysical

parameters are estimated from one 'dynamic' experiment instead of several 'static' ones as it is usually the case for the estimation of quantum yields. The other main advantage of such an approach is to directly estimate the photophysical parameters in *in vivo* conditions. Future investigations should examine the impact of the input design on the condition number of the sensitivity matrix and new experiments will be handled to estimate some photophysical parameters .

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