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Optimized numerical pharmacokinetics model for optical molecular probes based on diffusion coefficients in matrigel measured using fluorescence imaging

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Abstract- **An original algorithm for measuring diffusion coefficients of optical molecular probes in matrigel from fluorescence data is introduced. The algorithm was developed in Fortran and linked to Graphic User Interface in LabVIEW software that also performs image acquisition and processing. The software models pharmacokinetics of optical molecular probes providing the best fit of experimental data. The paper offers an original way for estimating the diffusion path length through extracellular matrix (ECM) from the rate constants given by the model and from measured diffusion coefficients.**

I. Introduction

MEDICAL imaging provides the possibility that recognizable features be rendered and displayed for clinical applications such as patient diagnosis, surgical intervention planning and optically enhanced surgery, as well as treatment follow-up evaluation. To this end, medical imaging workstations and image-guided therapy workstations have been developed [1, 2]. Near-infrared (NIR) light can penetrate several centimeters of tissue and therefore it can be used to characterize tissue pathology *in vivo*. When optical imaging techniques are coupled with NIR-excitable fluorescent contrast agents molecular-based diagnostic imaging can be developed for *in vivo* targeting and reporting of cancer and other tissue abnormalities [3, 4, 5]. Modeling has been proven to enhance the sensitivity and specificity of medical imaging diagnostic procedures and compartmental models are widely used to this purpose [6, 7]. In our paper we developed a custom compartmental model to describe the pharmacokinetics of the optical molecular probes we used. It was fitted on bio distribution data and rate constants were thus calculated. In order to improve the initial guesses for rate constants in our model, we correlated these values with the actual diffusion coefficients of the optical probes that we determined experimentally.

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II. The Principle of the Method

We have developed a system capable of simultaneous imaging of anatomy (color video) and NIR fluorescence emission. For this study the system was used to characterize the diffusion of the optical molecular probes. Their diffusion in matrigel can be described using Fick's laws. Fick's first law in mathematical form is given by:

$$J = -D\frac{\partial C}{\partial x} \quad . \tag{1}$$

It states that the diffusion flux, J (units: $\text{molecules}/(\text{cm}^2\text{s})$), is proportional to the spatial gradient $\partial C/\partial x$ of the concentration C. The proportionality constant, D, is the diffusion coefficient (units: cm^2/s). The concentration, C, is the amount of substance per unit volume (units: $\text{molecules}/\text{cm}^3$). The mathematical expression of Fick's second law is:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \quad . \tag{2}$$

It states that the time rate of change of the concentration is proportional to the second order derivative of concentration with respect to spatial coordinate. The proportionality constant, D, is the diffusion coefficient, x is the position (cm) and t is the time (s). We assume that the diffusion of the optical molecular probes in a matrigel column of length l is one-dimensional and it is described by equation (2) with boundary conditions:

$$C = \begin{cases} C_1 & \text{for} \quad x = 0; \\ C_2 & \text{for} \quad x = l. \end{cases} \tag{3}$$

$x = 0$ is the spatial coordinate of the bottom of the matrigel column, that is inserted in the solution containing the optical molecular probes and $x = l$ is the spatial coordinate of the top of the matrigel column. The solution of equation (2) with boundary conditions (3) is [8]:

$$\begin{aligned} C(x,t) &= C_1 - (C_1 - C_2)erf\left(x/(2\sqrt{Dt})\right) = \\ &= C_1 erfc\left(x/(2\sqrt{Dt})\right) + C_2 erf\left(x/(2\sqrt{Dt})\right) \end{aligned} \tag{4}$$

erf (X) is the error function and erfc (X) = 1- erf (X).
We assume that the concentration of the diffused substance at the top of the matrigel column is very small, so that it can be approximated by 0. As a result, the concentration profile in the matrigel column is given by:

$$C(x,t) = C_1 erfc\left(x/(2\sqrt{Dt})\right). \tag{5}$$

The normalized concentration profile in the matrigel column will then be given by:

$$\frac{C(x,t)}{C_1} = erfc\left(x/(2\sqrt{Dt})\right) = erfc(X)\,. \qquad (6a)$$

where

$$X = x/(2\sqrt{Dt})\,. \qquad (6b)$$

Equation (6a) makes it possible for us to evaluate the diffusion coefficient, D, using the normalized concentration profile of the optical molecular probe in the matrigel column. It is known that:

$$\text{erfc}\,(X) = 0.15 \text{ for } x/(2\sqrt{Dt}) \cong 1. \qquad (7a)$$

It follows that:

$$x = 2\sqrt{Dt} = (\text{constant}) \times t^{1/2}\,. \qquad (7b)$$

In other words, for Fickian diffusion (diffusion that takes place according to Fick's laws) the diffusion kinetics is parabolic:

$$x^2 = 4Dt = St\,, \qquad (8)$$

and it means that the square of the penetration depth, x, varies linearly with the time, t. We use this relationship to find the diffusion coefficient from the slope S of the curve x^2 versus t:

$$D = S/4\,. \qquad (9)$$

III. In Vitro Experimental Procedure

Matrigel Basement Membrane Matrix was used as diffusion medium. Matrigel columns of about 4 cm were loaded into capillary tubes 1.15 mm in diameter in the cold room at 4°C. Then the capillary tubes containing the matrigel were placed in a dry incubator for about 60 minutes to allow complete transition to gel phase. The dry incubator was set up to 37°C. The optical molecular probes used in this study were IRDye800-CW, and polyethylene- glycol (PEG) conjugates of molecular weights 1 kDa, 5 kDa, 20 kDa and 45 kDa. 10 μM solutions of each optical molecular probe were prepared. The solutions were loaded in vertical vials. The vials were placed on single vial holders. A capillary tube containing matrigel in gel phase was introduced in each vial. Then the vial holder was placed in the incubator at 37°C for the prescribed diffusion time intervals.

At the end every diffusion time interval, the vial holder was taken out of the incubator. The matrigel columns (capillary tubes containing matrigel) in which a certain molecular optical probe was diffused were taken out of the vials, washed in distilled water and dried. The NIR image of each capillary tube was recorded using the NIR fluorescence imaging system. Then the capillary tubes were placed back in the vials and the vial holder was returned to the incubator for another diffusion time interval.

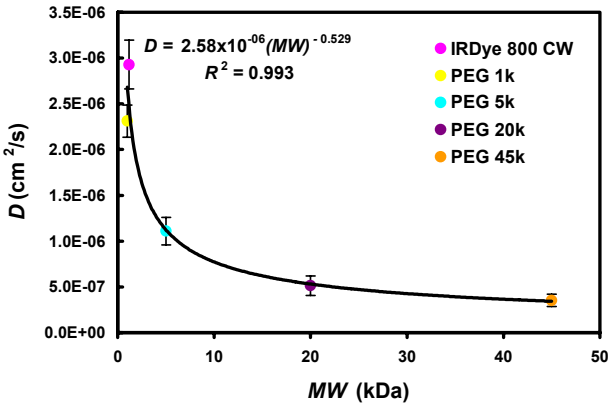
The NIR image was processed using a dedicated LabVIEW software provided the profile of the NIR intensity along the matrigel column at all diffusion times for which the experiment was run. The software performed the normalization of the NIR intensity profiles. We assumed that every normalized NIR intensity profile thus obtained coincides with the normalized concentration profile and is described by equation (6a). The fitting capability of software was used to find the experimental diffusion kinetics curve. If the kinetics is parabolic, equation (9) is used to find the corresponding diffusion coefficient.

IV. *In Vitro* Results

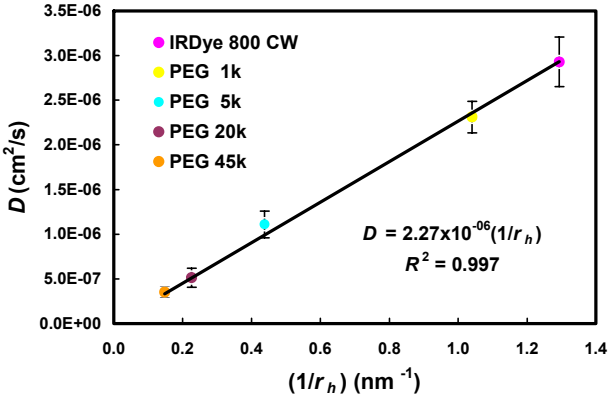
Figure 1 a) shows the dependence of the diffusion coefficient in matrigel on the molecular weight of the optical molecular probes. The experimental data were fitted using a power law and the following dependence was obtained:

$$D = 2.58\times10^{-6}\,(MW)^{-0.529}\,, \qquad (10)$$

where D is the diffusion coefficient (in cm^2/s) in matrigel of the optical molecular probe and MW is its molecular weight (in kDa).



a)



b)

Figure 1 Diffusion coefficient of optical molecular probes in matrigel measured experimentally: a) Diffusion coefficients in matrigel as a function of molecular weight; b) Diffusion coefficients in matrigel as a function of hydrodynamic radius.

It is thus possible to calculate the diffusion coefficient in matrigel of an optical molecular probe for which the molecular mass is known. Furthermore, this semi-empirical law can also be used to find the dependence of the molecular weight of PEG conjugates on their diffusion coefficient in matrigel:

$$MW = \left(2.73\times10^{-11}\right)D^{-1.89}. \quad (11)$$

Based on equation (11) the molecular weight in kDa of a certain PEG conjugate can be found after its diffusion coefficient was measured experimentally. Equation (11) provides a method for validating the functionality of optical molecular probes. Figure 1 b shows the diffusion coefficient in matrigel (in cm^2/s) as a function of the inverse of the hydrodynamic radius (in nm^{-1}). A semi-empirical linear relationship between the diffusion coefficients and the inverse hydrodynamic radius was found – as shown on the graph and in agreement with Stokes – Einstein equation [9]:

$$D = k_B T/(6\pi\eta r_h) = \text{constant}/r_h,$$

where r_h is the hydrodynamic radius.

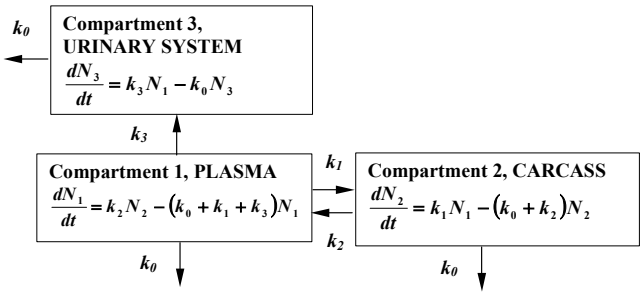


Figure 2 Diagram of compartment model pharmacokinetics

V. *IN VIVO* RESULTS- MATHEMATICAL MODEL AND COMPUTER SIMULATIONS

In the numerical modeling of the optical molecular probe pharmacokinetics we make the following assumptions: (A1) a simplified 3- compartment model can be used to describe the pharmacokinetics for our optical molecular probes; (A2) diffusion is the process controlling the transfer rate between compartments (i.e. rate constants); (A3) matrigel is an adequate model for the extracellular matrix (ECM); (A4) the diffusion coefficient of the ^{99m}Tc – labeled PEG conjugates is very close in value to that for the corresponding PEG conjugate alone.

The plasma pharmacokinetics of ^{99m}Tc – labeled PEG conjugates was determined experimentally by measuring the radioactivity of blood samples collected at different moments after injection. In order to characterize the pharmacokinetics of the PEG conjugates, we used the 3 - compartment model described in Figure 2. The three compartments of this simplified model are plasma (compartment 1), carcass (compartment 2) and urinary system (compartment 3) and consists of the equations [10]:

$$\frac{dN_1}{dt} = k_2N_2 - (k_0 + k_1 + k_3)N_1, \quad (12)$$

$$\frac{dN_2}{dt} = k_1N_1 - (k_0 + k_2)N_2, \quad (13)$$

$$\frac{dN_3}{dt} = k_3N_1 - k_0N_3 \quad (14)$$

and by the initial conditions:

$$N_1 = 100\%\text{ID}; \quad \frac{dN_2}{dt} = 0; \quad \frac{dN_3}{dt} = 0. \quad (15)$$

N_1, N_2 and N_3 are the ^{99m}Tc – labeled PEG conjugate activities in the three compartments, respectively, in units of percentage of initial injected dose (%ID). k_0 is the rate constant that accounts for the radioactive disintegration:

$$k_0 = \ln 2/\left(T_{1/2}\right)_{^{99m}Tc} \cong 0.002\text{min}^{-1}, \quad (16)$$

with $\left(T_{1/2}\right)_{^{99m}Tc}$ =360.6 min is the ^{99m}Tc half-life time. k_1, k_2 and k_3 are the rate constants describing respectively the transfers from compartment 1 to compartment 2, from compartment 2 to compartment 1 and from compartment 1 to compartment 3, in min^{-1}.

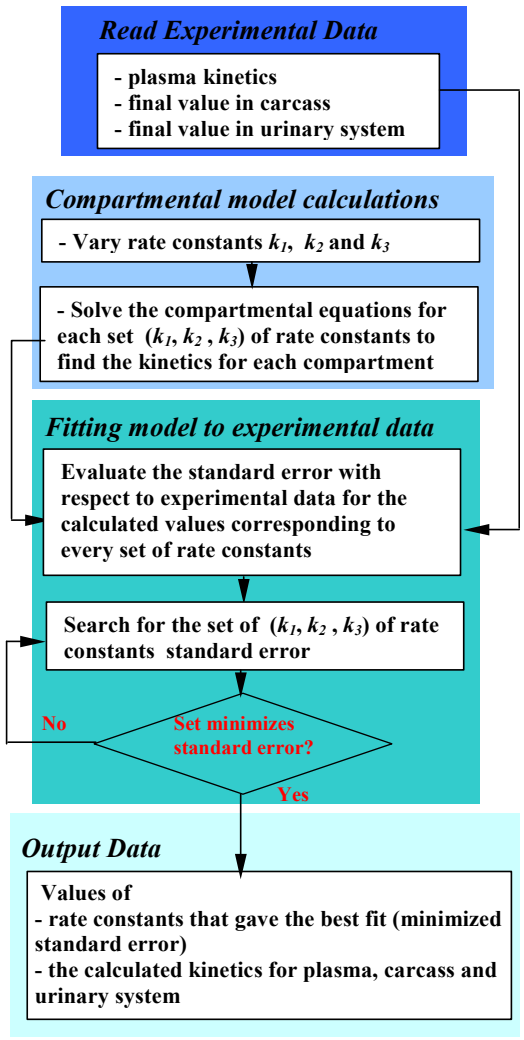


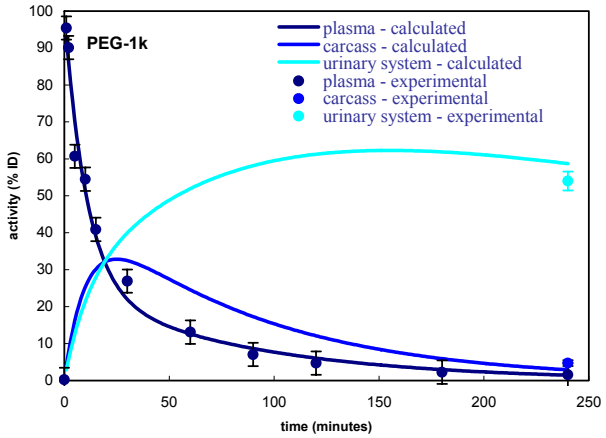
Figure 3 Block diagram of the FORTRAN subroutine

Starting from this compartmental model, we developed a custom Fortran subroutine with the main goal of providing the best fit of the plasma pharmacokinetics experimental data. The block diagram of this Fortran subroutine is shown in Figure 3.

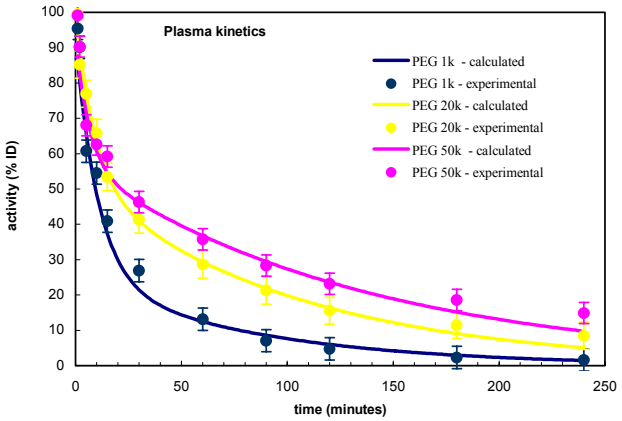
As a consequence of the assumption (A2) of our model, the relationship between the diffusion coefficient and the rate constants is [11]:

$$k = \pi^2 D/(\lambda^2 r^2) \quad (17)$$

where k is the rate constant, D is the diffusion coefficient, λ=1.5 is a factor that accounts for the tortuosity of pathway of the molecule that diffused through the extracellular matrix (ECM) and r is the length of the diffusion path (the ECM thickness, in our case).



a)



b)

Figure 4 Predictions vs *in-vivo* experimental data: a) Pharmacokinetics for PEG 1k; b) Plasma kinetics for PEGs 1k, 20k and 50k

As the diffusion coefficient for a given optical molecular probe in matrigel is part of the input data, the initial guess for the rate constants k_1, k_2 and k_3 is provided by equation (17). The set (12) - (14) of simultaneous equations with initial conditions (15) is solved numerically. The corresponding plasma pharmacokinetics is found and the standard error σ with respect to the experimental values measured is evaluated. This standard error is minimized with respect to all rate constants and thus the optimal fit for the experimental data is obtained, as shown in Figure 4 a and b).

VI. Conclusion

The diffusion coefficient of an unknown molecule measured from fluorescence data can be used as an alternative way of finding the molecular mass of the molecule under investigation. The algorithm for modeling the pharmacokinetics of optical molecular probes can be used to predict the pharmacokinetics for new molecular probes. It can be expanded to describe pharmacokinetics at tumor level, based on experimental data. In a final form, the software will predict the quantity of optical molecular probe needed to be injected in order to provide the required pharmacokinetics at tumor level.

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