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Modeling of Enzyme-FET Biosensor Based on Experimental Glucose-Oxidase Receptor

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***Abstract*— The modeling of biosensors is useful in the design stage. The main device simulator, like Silvaco, has poor software resources for bio-receptors simulations. The modeling is challenging due to the high complexity of the living matter. It requires complementary knowledge from biochemistry, biosensors technology and electronic devices, like FET - Field Effect Transistors. This paper presents an analytical model for the product concentrations versus the time for enzymatic FET based on zero, one or two-order reaction. The mathematical model is confronted with an experimental model tested on a glucose biosensor that uses glucose-oxidase receptor enzyme. The biosensor response time was 36 seconds for enzyme loading of 2µmol/l.**

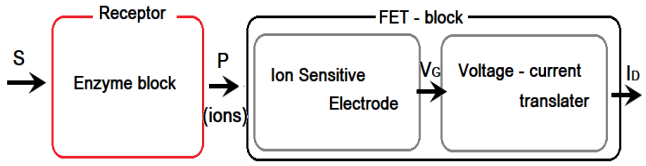
***Clinical Relevance*— The analytical model proposed in this paper represents an analytical tool in the design stage, for any biosensor used in clinical practices. Their modeling is missing.**

I. Introduction

The main purpose of the enzyme kinetics models is to find out the reaction mechanism, in term of consumed substrate, number of moles of each reactant and an experimental-theory confirmation of the predicted kinetic [1]. In those studies, the substrate concentration, the enzyme loading and the reaction rates are the main variable. Michaelis–Menten model offers a dependence between initial reaction rate and substrate concentration, considering an isolated enzyme catalyzed reaction under restricted conditions [2]. Despite above hypotheses, this well-known model is frequently applied to biosensors [3]. The generalized Hill's equation is still reduced to Michaelis–Menten model if the Hill coefficient is equal to 1, [4].

For enzymatic biosensors modeling, we use a key enzyme assisted reactions that was previously well-studied. So, specific data can be available, like the values of Michaelis constant K_M or activation energy E_a. The product concentration P is the main variable in biosensors. Its time dependence allows to establish a Product - Substrate dependence, as a translation function for the first sub-block - Enzyme, Fig. 1a.

A global modeling of a Enzyme-Field Effect Transistor (EN-FET) response to an input stimulus was started few years ago [5] and still continues [6]. The second sub-block Field Effect Transistors (FET) can be easily depicted by a Nerst law [5] for gate voltage translation, plus drain current formalism for FET part [7]. This paper is focused on the modeling of the Enzyme block, so that for a given Analyte input concentration, a Product concentration can be computed, Fig. 1b. In the final part of this paper we check this model on an experimental test-bench, using glucose as analyte, glucose-oxidase as enzyme as receptor and ions as Product.



(a) (b)

Figure 1. Main sub-blocks inside a EN-FET: (a) Enzyme, (b) FET.

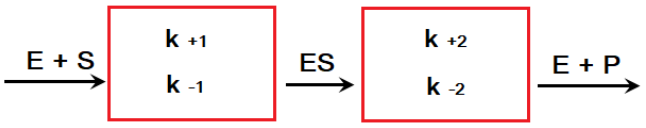


Figure 2. Details of Enzyme block, indicating the reaction rate constants forward and reverse, for each step.

II. Time-Dependent Model for the Enzymatic Kinetics

A. Model proposal - General settings

The product concentration P is an output signal from the Enzyme block and it is an input signal for the transducer block, Fig. 1a-b. Our work hypothesis are in agreement to the Michaelis-Menten formalism: single step reaction, one Substrate, enzyme loading much less than substrate initial concentration, pH temperature and ionic strength are constant and individual rates are $k_{-2} << k_2 < k_{1,-1}$, Fig. 1b. Products of interests are ions able to be detected by FET-part from the second-sub-block, Fig. 1a. Reaction rate expresses the substrate and product concentration variation in time:

$$v = -\frac{dS}{dt} = \frac{dP}{dt} \qquad (1)$$

The following relation results for P concentration:

$$P(t) - P(0) = \int_0^t v(t)dt \qquad (2)$$

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B. Zero-order reaction

For the zero-order reactions, the reaction rate is essentially constant:

$$v = k \cdot S^0(t) \tag{3}$$

where k - reaction rate constant, S(t) is the analyte or substrate concentration versus time, v is the reaction rate.

Using (1) in (3), after each member integration results the time dependence of the substrate concentration:

$$S(t) = S(0) - kt \tag{4}$$

Using (2) and (3), the product concentration results:

$$P(t) = P(0) + kt \tag{5}$$

C. First-order reaction

For the first-order reactions, the reaction rate is proportional to $S^1(t)$:

$$v = k \cdot S^1(t) \tag{6}$$

After integration in each member, the time dependence of the substrate concentration results:

$$S(t) = S(0) \cdot \exp(-kt) \tag{7}$$

The reaction rate results from (6) and (7):

$$v(t) = k \cdot S(0) \cdot \exp(-kt) \tag{8}$$

Integrating the reaction rate, accordingly with (2) we get:

$$P(t) = P(0) + S(0) \cdot \left(1 - e^{-kt}\right) \tag{9}$$

D. Second-order reaction

For the second-order reactions, the reaction rate is dependent to $S^2(t)$:

$$v = k \cdot S^2(t) \tag{10}$$

After integration in each member, the time dependence of the substrate concentration results:

$$S(t) = \frac{1}{kt + \frac{1}{S(0)}} \tag{11}$$

The reaction rate results from (10) and (11):

$$v(t) = \frac{k}{\left(kt + \frac{1}{S(0)}\right)^2} \tag{12}$$

Integrating the reaction rate, accordingly with (2) we get:

$$P(t) = P(0) + S(0) - \frac{1}{kt + \frac{1}{S(0)}} \tag{13}$$

The relations are quite consistent with the limit conditions: $P(t\to 0)=P(0)$, $P(t\to\infty)= P(0) + S(0)$, considering that entire initial substrate S(0) is converted to Product $P(t\to\infty)$ when the time is long enough.

E. Parameters estimation

For a given biosensor, the initial concentrations P(0), S(0) are known. From the biosensor technology we know the enzyme loading E in U.I /mg or in mol/l and we can extract the Michaelis constant K_M.

In Michaelis–Menten equation, the initial reaction rate, v is:

$$v = \frac{v_{max} \cdot S}{K_M + S} \tag{14}$$

$$\text{If } S < K_M \rightarrow v = \frac{v_{max}}{K_M} \cdot S = kS \tag{15}$$

So, the reaction is first-order where $k=v_{max}/K_M$ and n=1.

$$\text{If } S > K_M \rightarrow v = v_{max} \cdot S^0 = kS^0 \tag{16}$$

In this case, the reaction is zero-order where $k=v_{max}$ and n=0.

$$\text{If S is in the vicinity of } K_M \rightarrow v = \frac{v_{max} \cdot S}{K_M + S} = k \cdot S^{i/j} \tag{17}$$

In this case, the reaction is fractional-order and n=i/j and k can be extracted by regression. The product P is in direct contact with ions selective gate electrode of EN-FET, with negligible diffusion process. So, its concentration at transducer level is:

$$P^{trad} = \frac{v_{max} \cdot S}{K_M + S} \cdot t_m + P(0) \tag{18}$$

The $1/v_{max}$ value can be estimated by a Lineweaver–Burk plot, where a line among measured points - 1/v versus 1/S - intercepts the vertical axis.

From v_{max} value and k_2 definition [1], can be estimated:

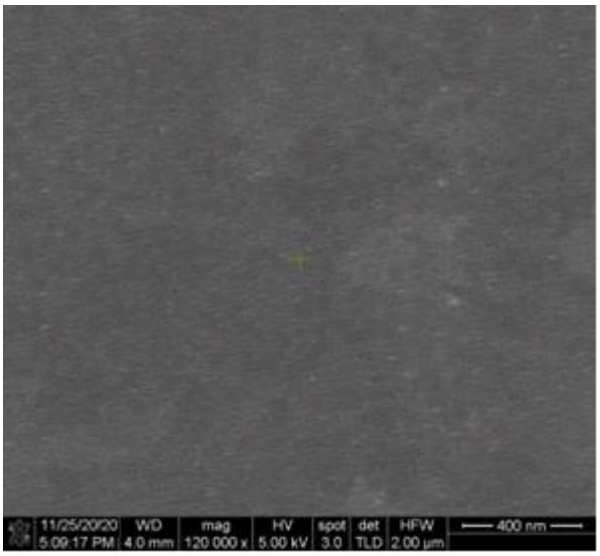
$$v_{max} = k_2 \cdot E \tag{19}$$

In the next paragraph, the previous model is applied to an experimental test-bench.

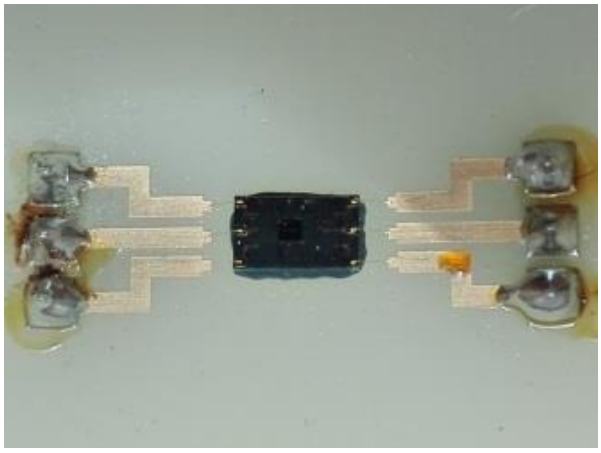
III. Experimental Model of an Enzymatic Biosensor

In order to check the model validity, the current-concentration curves were compared: from a test-bench represented by a glucose biosensor with glucose-oxidase as enzyme receptor and present analytical model.

The glucose-oxidase was entrapped on a silicon wafer, using glutaraldehyde solution (GA) - 2.5% concentration as cross-link agent and serum albumin of bovine provenience (BSA). Details about a complete technology of this glucose biosensor was reported elsewhere, [8]. The estimated enzyme loading is E=2μmol/l. A sample of the covered Si-wafer is available in Fig. 3a.



(a)



(b)

Figure 3. (a) SEM image of the control wafer: glucose-oxidase entrapped in glutaraldehyde/TiO_2/SiO_2/Si-wafer; encapsulated En-FET.

In Fig. 3.b, the fabricated EN-FET with double ports - right and left - is available. The enzyme assisted reaction inside this glucose biosensor is:

$$D-Glucose+O_2 \xrightarrow{Glucose-Oxidase} D-Gluconic\ Acid+ +H_2O_2$$

$$H_2O_2 \rightarrow O_2+2H_+ +2e^-$$

For this biosensor, the parameters $1/v_{max}$ and $1/K_M$ are extracted by interception with Ox, Oy axis, Fig. 4.

From Lineweaver–Burk plot results v_{max} = 1.745mmol/l.s and K_M = 10.5mmol/l. Assuming P(t=0) = 0 and S(t=0) = 2mmol/l, Fig. 5 presents the time dependences of the product concentrations computed accordingly to models: (5), (9), (13), for zero-, one-, and two-order reactions, respectively. For each reaction, the rate reaction constant k is computed with eq. (15)-(18). From this analysis, the response time, t_r, of the biosensor can be extracted: 12 s, 36 s and 149 s, for 0-order, 1-order and 2-order reactions, respectively (Fig. 5). In this experiment, S < K_M, so that in next graphics, a 1-order reaction is treated, accordingly to (15). An analysis of the product concentration with the enzyme loading, E, according to (19) is available in Fig. 6.

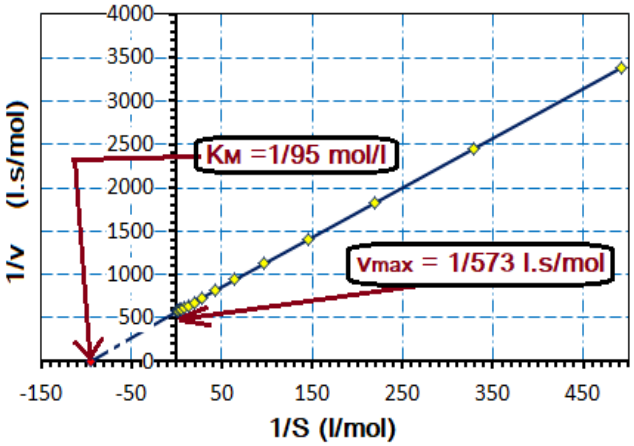


Figure 4. Lineweaver–Burk plot for our experimental points.

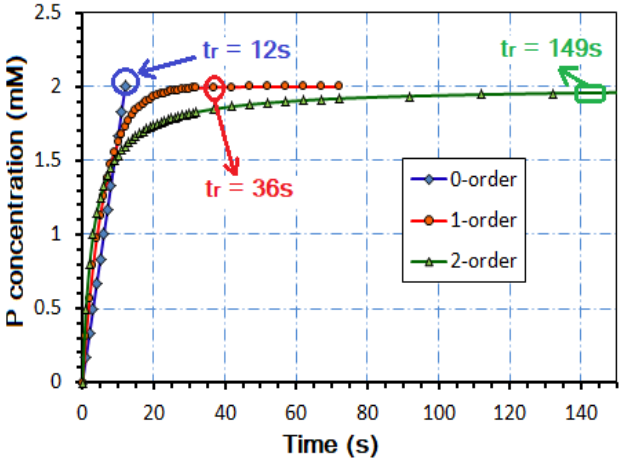


Figure 5. The time dependences of the product concentrations, for an enzyme loading of E=2µmol/l.

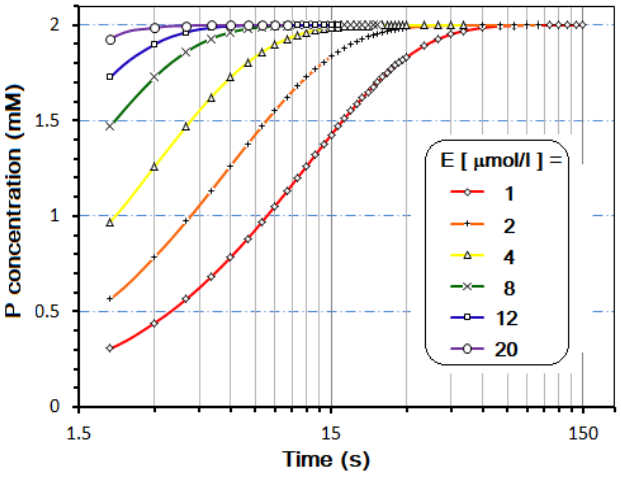


Figure 6. The time dependences of the product concentrations, for different enzyme loading E.

Obviously, increasing the enzyme loading, the reaction rate accelerates.

Consequently, the response time decreases from 150 s for E=1µmol/l up to 9 s when E = 20µmol/l, Fig. 6.

The calibration curve of the biosensor for different substrate concentrations is provided in Fig. 7, accordingly to the actual analytical models and curve of the experimental glucose biosensor from Fig. 3b. The Gate voltage versus the Product P concentrations was computed with the Nerst's law [5]. The Gate voltage - Drain current was modeled by parabolic law, considering a bias operation point of V_{DS}= 1V, V_{GS}=1.1V for the tested transistor.

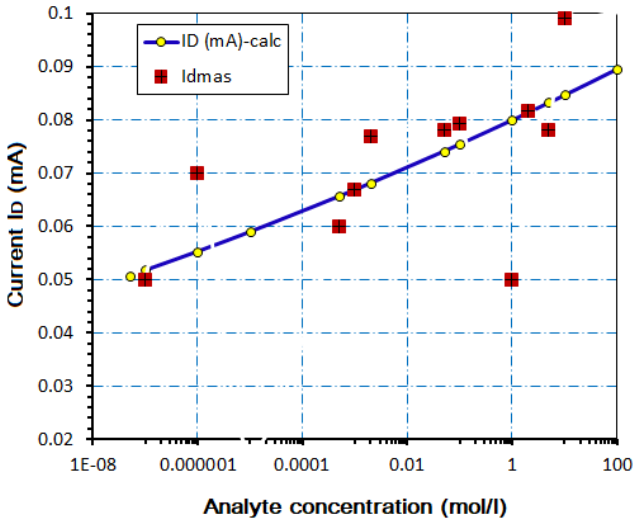


Figure 7. The calibration curve of the glucose biosensor.

IV. Conclusion

This paper reported an analytical model of the product concentration versus time and reaction rate constant expressing on maximal rate, for a biosensor that obeys to the Michaelis-Menten formalism. For each order reaction, the time dependence of the substrate concentration and product concentrations were calculated. The model was confronted with an experimental glucose biosensor with glucose-oxidase enzyme as receptor. The results show that the proposed analytical model is useful in the design stage to estimate the response time, the optimal enzyme loading and the calibration curve.

V. Acknowledgement

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