Jurnal Sains Materi Indonesia
Indonesian Journal of Materials Science

Special Edition on Materials for Sensor 2011, page : 28 - 32
ISSN : 1411-1098

Akreditasi LIPI Nomor : 452/D/2010
Tanggal 6 Mei 2010

NUMERICAL ANALYSIS OF ENZYME MEMBRANE THICKNESS ON THE RESPONSE OF AMPEROMETRIC BIOSENSOR

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ABSTRACT

NUMERICAL ANALYSIS OF ENZYME MEMBRANE THICKNESS ON THE RESPONSE OF AMPEROMETRIC BIOSENSOR. This paper presents a one-dimensional-in-space mathematical model of an amperometric biosensor. The model is based on the reaction-diffusion equations containing a non-linear term related to Michaelis Menten kinetics of the enzymatic reactions. The model is solved numerically by applying the Finite Difference Method (FDM). This model describes the biosensor response to active time of enzymatic reaction in membrane layer. Using numerical solution, the influence of the thickness of enzyme membrane on the biosensor current response was investigated. The numerical results of the biosensor operation showed the monotonous change of the biosensor current response versus the membrane thickness.

Key words : Amperometric biosensor, Reaction-diffusion, Michaelis-Menten kinetics, Finite Difference Method, Membrane thickness

ABSTRAK

ANALISIS NUMERIK DARI PENGARUH KETEBALAN MEMBRAN ENZIM TERHADAP RESPON BIOSENSOR AMPEROMETRIK. Makalah ini memaparkan model matematika satu dimensi dari biosensor amperometrik. Model ini dibuat dengan berbasiskan persamaan difusi reaksi yang mencakup komponen non-linier yang berkaitan dengan Kinetika Michaelis Menten dari reaksi enzim. Model ini diselesaikan secara numerik dengan mengaplikasikan *Finite Difference Method* (*FDM*). Model ini menggambarkan respon dari biosensor terhadap waktu aktif dari reaksi enzim dalam lapisan membran. Dengan menggunakan pemecahan numerik, pengaruh dari ketebalan membran enzim terhadap respon arus dari biosensor dapat dipelajari. Hasil numerik dari operasi biosensor memperlihatkan adanya perubahan monoton dari respon arus dari biosensor terhadap ketebalan membran.

Kata kunci : Biosensor Amperometrik, Difusi reaksi, Kinetika Michaelis Menten, *Finite Difference Method*, Ketebalan membran

NTRODUCTION

Biosensors are devices that combine the ɜlectivity and specificity of a biologically active ɔmpound with a signal transducer and an electronic mplifier [1-4]. The transducer converts the biochemical gnal to an electronic signal. The biosensor signal is roportional to the concentration of measured analyte r a group of analytes. The biosensors are classified ɔcording to the nature of the physical transducer. mperometric biosensors measure the current on an ıdicator electrode due to direct oxidation of the roducts of the biochemical reaction. In case of the nperometric biosensors the potential at the electrode is held constant while the current flow is measured. The amperometric biosensors are reliable, relatively cheap and highly sensitive for environment, clinical and industrial purposes.

Starting from the publication of Clark and Lyons [1], the amperometric biosensors became one of the popular and perspective trends of biochemistry. The understanding of the kinetic regularities of biosensors is of crucial importance for their design. Mathematical models can explain such regularities. The general features of amperometric response were analyzed in the publications of Mell and Maloy [5, 6]. Some later reports

were also devoted to the modeling and investigation of the amperometric biosensor response [7-11].

In this paper, the developed model is based on non-stationary diffusion equations [12], containing a non-linear term related to Michaelis-Menten kinetic of the enzymatic reaction. The numerical method of the biosensor response was carried out using the Finite Difference Method (FDM) [13,14]. The software has been programmed in C language. The program built was employed to investigate the influence of the enzyme membrane thickness, substrate concentration as well as the maximal enzymatic rate on biosensor response.

MATHEMATICAL MODEL

During an enzyme-catalysed reaction

$$S \xrightarrow{E} P \quad \text{..... (1)}$$

the mixture of substrate (S) binds to the enzyme (E) to form enzyme-substrate complex. While it is a part of this complex, the substrate (S) is converted to the product (P). The rate of the reaction is the rate of appearance of the product. This rate is known to depend upon the concentration of substrate.

Let us consider an amperometric biosensor, which can be treated as enzyme electrode, having a layer of enzyme immobilized onto the surface of the probe as presented schematically at Figure 1. Assuming no interaction between analysed substrates (compounds) of the mixture, the symmetrical geometry of the electrode, homogeneous distribution of immobilized enzyme in the enzyme reaction with the diffusion described by Fick's law leads to the following equations :

$$\frac{\partial S}{\partial t} = D_S \frac{\partial^2 S}{\partial x^2} - \frac{V_{\max} S}{K_M + S} \quad 0 < x < d, 0 < t \le T \quad \text{..... (2)}$$

$$\frac{\partial P}{\partial t} = D_P \frac{\partial^2 P}{\partial x^2} + \frac{V_{\max} S}{K_M + S} \quad 0 < x < d, 0 < t \le T \quad \text{..... (3)}$$

where V_{max} is the maximal enzymatic rate of biosensor attainable with that amount of enzyme, when the enzyme is fully saturated with substrate (S), K_M is Michaelis constant, S is the concentration of substrate (S), P is concentration of the reaction product (P), d is thickness of the enzyme layer, t is time, T is full time of biosensor operation to be analysed, D_S and D_P are diffusion coefficients of the substrate S and product P, respectively.

The biosensor operation starts when some substrate appears over the surface of the enzyme layer. This is used in the initial conditions ($t = 0$) :

$$S(x,0) = \begin{cases} 0, & 0 \le x < d, \\ S_0 & x = d, \end{cases} \quad \text{..... (4)}$$

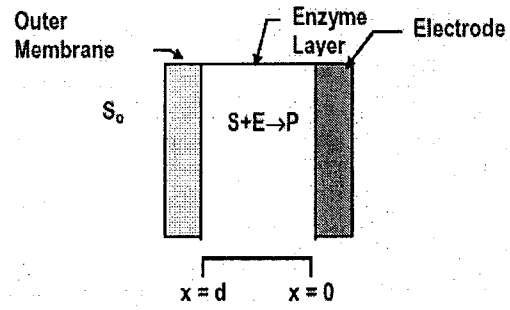

Figure 1. Schematic representation of enzyme-electrode surface in amperometric biosensor

$$P(x,0) = 0, \quad 0 \le x \le d, \quad \text{..... (5)}$$

where S_0 is the concentration of substrate initial over the biosensor.

Because of electrode polarization, the concentration of the reaction product at the electrode surface is being permanently reduced to zero. If the substrate is well-stirred and in powerful motion, then the diffusion layer ($0 < x < d$) will remain at a constant thickness. Consequently, the concentration of substrate as well as product over the enzyme surface (bulk solution/membrane interface) remains constant while the biosensor contact with the substrate. When the analyte disappears, a buffer solution swills the enzyme surface, reducing the substrate concentration at this surface to zero. Because of substrate (analyte) remaining in the enzyme membrane, the mass diffusion as well as the reaction still continues some time even after the disconnected of the biosensor and substrate.

This is used in the boundary conditions ($0 < t \le T$) given by:

$$\left.\frac{\partial S}{\partial x}\right|_{x=0} = 0 \quad \text{..... (6)}$$

$$S(d,t) = \begin{cases} S_0, & t = 0, \\ 0, & t > 0, \end{cases} \quad \text{..... (7)}$$

$$P(0,t) = P(d,t) = 0 \quad \text{..... (8)}$$

The current is measured as a response of a biosensor in a physical experiment. The biosensor current depends upon the flux of reaction product at the electrode surface, i.e., at border $x = 0$. Consequently, density $I(t)$ of the biosensor current, as a results of the reaction of the substrate S with the product P at time t, is proportional to the concentration gradient of the product at the surface of the electrode as described by Faraday's law :

$$I(t) = n_e F D_P \left.\frac{\partial P}{\partial x}\right|_{x=0} \quad \text{..... (9)}$$

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ıere n_e is a number of electrons involved in a charge ınsfer at the electrode surface, and F is Faraday nstant, $F = 96485$ C/mol.

OLUTION OF THE MATHEMATICAL IODEL

Let us assume the problem (2)-(8) formulation for substrate S and reaction product P. Let V_{max} be the aximal enzymatic rate of the modeled biosensor, S is e concentration of substrate S and P is concentration the reaction product P. The problem (2)-(8), formulated for substrate S and reaction product P, was ılved numerically using FDM. To find a numerical ılution of the problem in the domain $[0, d]\times[0, T]$ we troduced an uniform discrete grid $\omega_h \times \omega_\tau$, where :

$$\omega_h = \{x_i : x_i = ih, \quad i = 0,...,N_1; hN_1 = d\},$$

$$\omega_\tau = \{t_j : t_j = j\tau, \quad j = 0,..., \quad N_A,...,N_2; \tau N_A = T_A, \tau N_2 = T\} \quad \text{..... (10)}$$

et us assume the following :

$$S_i^j = S(x_i, t_j), \; P_i^j = P(x_i, t_j) \quad \text{..... (11)}$$

$$i = 0,...,N_1; j = 0, ..., N_2$$

An implicit linear finite difference scheme has een built as a result of the difference approximation. he initial conditions (4) and (5) we approximated s follows :

$$S_i^0 = 0, \quad i = 0,..., N_1 - 1, \quad S_{N_1}^0 = S_0,$$
$$P_1^0 = 0, \qquad i = 0,..., N_1. \quad \text{..... (12)}$$

Differential Equations (2) and (3) were pproximated by scheme :

$$\frac{S_i^{j+1} - S_i^j}{\tau} = D_S \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} - \frac{V_{max} S_i^{j+1}}{K_M + S_i^j}$$

$$i = 1, ..., N1-1, \quad \text{..... (13)}$$

$$\frac{P_i^{j+1} - P_i^j}{\tau} = D_P \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} + \frac{V_{max} S_i^{j+1}}{K_M + S_i^{j+1}}$$

$$j = 1, ..., N2-1, \quad \text{..... (14)}$$

The boundary conditions (6)-(8) were ıpproximated as follows :

$$S_0^j = S_i^j, \quad j = 1,..., N_2,$$
$$S_N^j = S_0, \quad j = 1,..., N_A, \quad \text{..... (15)}$$

$$S_N^j = 0, \quad j = N_A + 1,..., N_2,$$
$$P_0^j = 0, \quad P_{N_1}^j = 0, j = 1,..., N_2. \quad \text{..... (16)}$$

Equations (12) allow to calculate a solution of the problem on the layer $t = t_0 = 0$. When a solution on a layer t_j has been calculated, a solution on the next layer $t = t_{j+1}$ can be calculated in two steps:

[1]. calculate values of S_i^{j+1}, $i = 0, ..., N_1$, solving the system of linear equations (13), (15);

[2]. calculate values of P_i^{j+1}, $i = 0, ..., N_1$, solving the system of linear equations (14), (16) using values of S_i^{j+1}, which have been calculated in step 1.

The system of linear algebraic equations can be solved efficiently in both steps above because of the tridiagonality of matrices of the systems.

Having numerical solution of the problem, the density of biosensor current at time $t = t_j$ is calculated by

$$I(t_j) = \frac{n_e F D_P (P_1^j - P_0^j)}{h}, j = 0, ..., N_2 \quad \text{..... (17)}$$

In step [1], only values of the following parameters: D_S, D_P, V_{max}, and S_0 vary when one computer simulation changes the next one.

DATA SYNTHESIS

The developed computer simulation software was employed to generate data for a calibration of an amperometric biosensor. The mathematical model as well as the numerical solution of the problem was evaluated for different values of the membrane thickness d. The following values of the parameters were constant in the numerical simulation of all the experiments:

$D_S = D_P = 3 \times 10^{-6}$ cm²/s,

$K_M = 1 \times 10^{-7}$ mol/cm³,

$n_e = 2$,

$d = 0.001, 0.0014, 0.0018, 0.0022, 0.0026$ and 0.003 cm.

The evolution of the biosensor currents were characterized by the following value of the maximal enzymatic rate V_{max} :

$V_{max} = 1 \times 10^{-8}$ mol/cm³s.

The value of the substrate concentration S_0 of the evolution of the biosensor currents was employed :

$S_0 = 2 \times 10^{-8}$ mol/cm³.

The active time T_A of enzymatic reaction in membrane layer is :

$T_A = 0.3$ s.

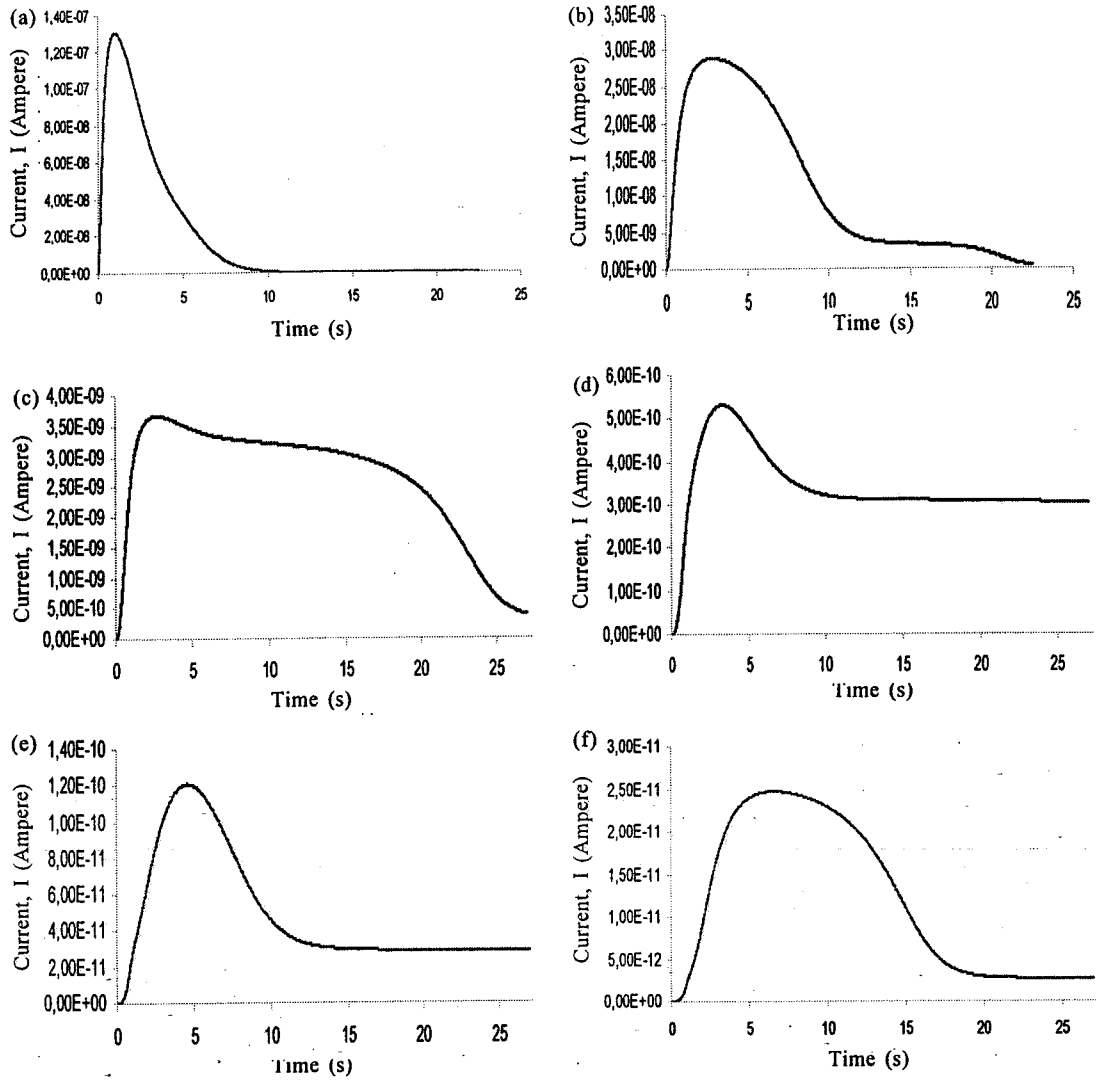

Figure 2. The dynamics of the biosensor current at the maximal enzymatic rate V_{max} = 1x 10^{-7} mol/cm³s and six membrane thickness d : (a). 0.001, (b). 0.0014, (c). 0.0018, (d). 0.0022, (e). 0.0026 and (f). 0.003 cm, S_0 = 2 x10^{-8} mol/cm³.

RESULTS AND DISCUSSION

The evolution of the biosensor current at the maximal enzymatic rate V_{max} of 1 × 10^{-7} mol/cm³s and active time of enzymatic reaction T_A = 0.3 s is presented in Figure 2. The biosensor response was modeled for biosensors having six different membrane thicknesses d = 0.001, 0.0014, 0.0018, 0.0022, 0.0026 and 0.003 cm. One can see in Figure 2 the biosensor current appears with some delay at relatively thick enzyme layers. This delay increases with increase of the enzyme membrane thickness. The biosensor response is notable higher at thinner membrane (d = 0.001 cm) than at other thicker (d=0.0014, 0.0018, 0.0022, 0.0026 and 0.003 cm).

CONCLUSION

The mathematical model (2)-(9) of amperometric biosensor operation can be successfully used to investigate the kinetic regularities of enzyme membrane-based sensors. The biosensor curren a monotonous function of membrane thickness d a value of active time (T_A) of enzymatic reactior membrane layer. Consequently, the maximal biosen current increases with decreasing of the membr thickness d. The greater the membrane thickness d, biosensor response will become minimum and unstal

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