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NUMERICAL MODELLING OF BIOSENSORS WITH PERFORATED AND SELECTIVE MEMBRANES[1]

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Abstract. This paper presents a two-dimensional-in-space mathematical model of amperometric biosensors with perforated and selective membranes. The model is based on the diffusion equations containing a nonlinear term of the Michaelis-Menten enzymatic reaction. The problem was solved numerically using finite-difference technique. Using numerical simulation of the biosensors action, the influence of the geometry of the perforated membrane on the biosensor response was investigated.

Key words: modelling, reaction-diffusion, simulation, biosensor

1. Introduction

A biosensor can be defined as a measuring device that contains a biological entity. The enzyme in the biosensor recognizes the substrate to be measured and specifically converts it into a product of the biochemical reaction [8]. The amperometric biosensors measure the faradic current that arises on a working indicator electrode by direct electrochemical oxidation or reduction of the product. The amperometric biosensors are known to be reliable, cheap and highly sensitive for environment, clinical and industrial purposes [3, 9].

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A practical biosensor contains a multilayer enzyme membrane [1, 8]. The electrode acting as a transducer of the biosensor is covered by selective membrane, following a layer of immobilized enzyme and an outer perforated membrane.

To improve the productivity and efficiency of biosensors design as well as to optimize the biosensors configuration a model of the real biosensors should be build [6]. The modelling of biosensors with perforated membranes has been performed by Schulmeister and Pfeiffer [7]. The model did not take into account the geometry of the holes in the membranes and included the diffusion coefficients having limited physical sense. The task of our investigation was to build a model approaching the practical amperometric biosensor. By changing input parameters the output results were numerically analyzed with special emphasis to the influence of enzyme and membranes parameters to the response of biosensors at steady - state conditions.

2. Modelling Biosensor

We assume that the thickness of the selective membrane as well as of the perforated membrane of a biosensor is much less than its length and width. In the biosensor, the selective membrane is of the uniform thickness. The holes in the perforated membrane were modelled by right cylinders. The holes are of uniform diameter and spacing, forming a hexagonal pattern. Fig. 1a presents the biosensor schematically.

The entire biosensor may be divided into equal hexagonal prisms with regular hexagonal bases. For simplicity, it is reasonable to consider a circle whose area equals to that of the hexagon and to regard one of the cylinders as a unit cell of the biosensor.

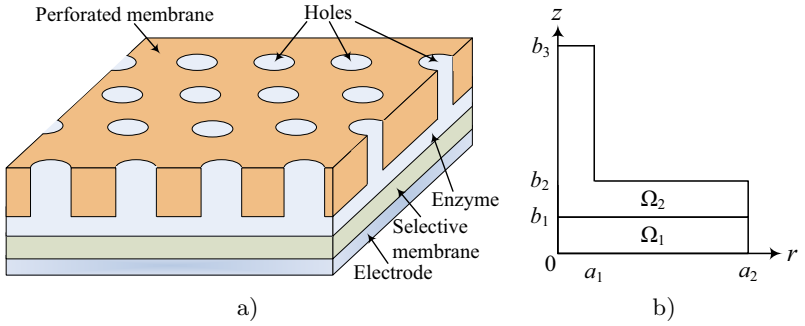

Figure 1. A principal structure of the biosensor and the profile of the unit cell at Y-plane.

Fig. 1 *b* shows the profile of the unit of the biosensor, represented schematically in Fig. 1 *a*. In Fig. 1 *b*, a_2 is the radius of the base of the unit cell, while a_1 is the radius of the holes, a_2 characterizes a density of the holes in the

perforated membrane. b_1 stands for the thickness of the selective membrane, $b_3 - b_2$ is the thickness of the perforated membrane, $b_2 - b_1$ is the thickness of the basic enzyme layer being between the selective and perforated membranes. We also assume that holes are filled with the enzyme.

3. Mathematical Model

Let Ω_1, Ω_2 be open regions (see Fig. 1b) corresponding to the selective membrane and enzyme regions, respectively, and Γ_2 - the upper boundary of the enzyme region,

$$\Omega_1 = (0, a_2) \times (0, b_1), \quad \Gamma_2 = [0, a_1] \times \{b_3\},$$
$$\Omega_2 = ((0, a_2) \times (b_1, b_2)) \cup ((0, a_1) \times [b_2, b_3)).$$

The biosensor action is described by the reaction - diffusion system ($0 < t \leq T$),

$$\frac{\partial P_1}{\partial t} = D_1 \Delta P_1, \ (r, z) \in \Omega_1, \tag{3.1}$$

$$\frac{\partial S}{\partial t} = D_2 \Delta S - \frac{V_{max} S}{K_M + S}, \quad (r, z) \in \Omega_2, \tag{3.2}$$

$$\frac{\partial P_2}{\partial t} = D_2 \Delta P_2 + \frac{V_{max} S}{K_M + S}, \ (r, z) \in \Omega_2,$$

where Δ is the Laplacian in cylindrical coordinates, $S(r, z, t)$ is the concentration of the substrate, $P_i(r, z, t)$ is the concentration of the reaction product in Ω_i, V_{max} is the maximal enzymatic rate, K_M is the Michaelis constant, T is full time of the biosensor operation, $i = 1, 2$.

Let $\overline{\Omega}_i$ be the closed region of the corresponding open region Ω_i, $i = 1, 2$. The initial conditions ($t = 0$) are as follows:

$$S(r, z, 0) = 0, \quad (r, z) \in \overline{\Omega}_2 \setminus \Gamma_2,$$
$$S(r, z, 0) = S_0, \quad (r, z) \in \Gamma_2,$$
$$P_i(r, z, 0) = 0, \quad (r, z) \in \overline{\Omega}_i, \ i = 1, 2.$$

The boundary and matching conditions ($t > 0$) are

$$P_1(r, 0, t) = 0, \quad r \in [0, a_2], \tag{3.3}$$

$$S(r, b_3, t) = S_0, \ P_2(r, b_3, t) = 0, \quad r \in [0, a_1], \tag{3.4}$$

$$\frac{\partial P_1}{\partial r}\Big|_{r=0} = \frac{\partial P_1}{\partial r}\Big|_{r=a_2} = 0, \quad z \in [0, b_1],$$

$$\frac{\partial S}{\partial r}\Big|_{r=0} = \frac{\partial P_2}{\partial r}\Big|_{r=0} = 0, \quad z \in [b_1, b_3],$$

$$\frac{\partial S}{\partial r}\Big|_{r=a_2} = \frac{\partial P_2}{\partial r}\Big|_{r=a_2} = 0, \quad z \in [b_1, b_2], \tag{3.5}$$

$$\frac{\partial S}{\partial r}\Big|_{r=a_1} = \frac{\partial P_2}{\partial r}\Big|_{r=a_1} = 0, \quad z \in [b_2, b_3], \tag{3.6}$$

$$\frac{\partial S}{\partial z}\Big|_{z=b_2} = \frac{\partial P_2}{\partial z}\Big|_{z=b_2} = 0, \quad r \in [a_1, a_2], \tag{3.7}$$

$$\frac{\partial S}{\partial z}\Big|_{z=b_1} = 0, \quad D_1 \frac{\partial P_1}{\partial z}\Big|_{z=b_1} = D_2 \frac{\partial P_2}{\partial z}\Big|_{z=b_1}, \quad r \in [0, a_2], \tag{3.8}$$

$$P_1(r, b_1, t) = P_2(r, b_1, t), r \in [0, a_2], \tag{3.9}$$

The measured current is accepted as a response of the biosensor. The current depends upon the flux of the reaction product at the electrode surface, i.e., at the border $z = 0$. The density $i(t)$ of the current at time t can be obtained explicitly from Faraday's and Fick's laws

$$i(t) = n_e F D_1 \frac{1}{\pi a_2^2} \int_0^{2\pi} \int_0^{a_2} \frac{\partial P_1}{\partial z}\Big|_{z=0} r dr d\varphi, \tag{3.10}$$

where n_e is a number of electrons involved in a charge transfer, F is Faraday constant and φ is the third cylindrical coordinate.

We assume, that the system (3.1)-(3.9) approaches an equilibrium or steady - state when $t \to \infty$, $i_\infty = \lim\limits_{t\to\infty} i(t)$.

4. Solution of the Problem

Definite problems arise when solving analytically nonlinear partial differential equations in domain of a complex geometry. Because of this the problem (3.1)-(3.9) was solved numerically using the finite difference technique [4].

Using alternating direction method a semi-implicit linear finite difference scheme has been built as a result of the difference approximation. The resulting system of linear algebraic equations was solved efficiently because the matrix of the system is tridiagonal.

In the digital simulation, the main problem is an overload of calculation due to the boundary conditions and permissible conditions: $a_1 \ll a_2$ and $b_2 \ll b_3$. To have an accurate and stable result it was required to use very small step size in z direction at the boundaries $z = 0$ and $z = b_3$. Because of the concavity of an angle at the point (a_1, b_2) we used very small step size in both space directions: r and z at the boundaries $r = a_1$ and $z = b_3$. Due to the matching conditions (3.8), (3.9), we used also small step size at the boundary $z = b_1$. We assume, that farther from all these peculiar boundaries, step size may increase in both space directions: r and z. Consequently, in the direction r, an exponentially increasing step size was used to both sides from a_1: to a_2 and to 0. In the direction z, an exponentially increasing step size was used form 0 to $b_1/2$, from b_1 down to $b_1/2$, from b_1 to $(b_1 + b_2)/2$, from b_2 down to $(b_1 + b_2)/2$, from b_2 to $(b_2 + b_3)/2$, and from b_3 down to $(b_2 + b_3)/2$.

The step size in the direction of time is restricted due to the non-linear reaction term in (3.2), boundary conditions and the geometry of the domain. In order to achieve accurate and stable solution of the problem, at the beginning of the reaction-diffusion process we employed the restriction condition, which is usually used for fully explicit schemes. Since the biosensor action obeys the steady-state assumption when $t \to \infty$, it is reasonable to apply an increasing step size in the time direction. The final step size was in a few orders of magnitude higher than the fist one.

5. Results and Discussion

Using computer simulation we have investigated the dependence of the steady-state biosensor current on the geometry of the membrane perforation. The radius a_1 of the holes was expressed through the radius a_2 of the unit cell, $a_1 = (1 - \theta)a_2$, and the biosensor response was calculated at various values of a_1 and a_2. The dimensionless degree θ expresses the level of covering of the surface of the perforated membrane. The case when $\theta = 0$ ($a_1 = a_2$) corresponds to a biosensor having no perforated membrane.

The biosensor current is very sensitive to changes of the maximal enzymatic rate V_{max} and the substrate concentration S_0 [2, 5, 8]. Because of this, the steady - state current of the biosensor having perforated membrane upon the enzyme layer was normalized with respect to the steady - state current of the corresponding biosensor having no perforated membrane,

$$i_N(\theta) = \frac{i_\infty(\theta)}{i_\infty(0)}, \quad \theta = 1 - \frac{a_1}{a_2}, \quad 0 \le \theta \le 1. \tag{5.1}$$

Results of calculations are depicted in Fig. 2.

One can see in Fig. 2, the behaviour of the biosensor response significantly depends on the enzymatic activity V_{max}. At low enzymatic activity (V_{max} $= 10^{-9}\,\mathrm{mol/cm^3 s}$) the steady - state current of the biosensor having perforated membrane can generate the steady - state current which is more than 10 times higher than the current if the biosensor would be without the perforated membrane ($\theta = 0$). This feature of biosensors with perforated and selective membranes can be applied in design of novel highly sensitive biosensors. Selecting the geometry of perforated membrane allows increasing the sensitivity of the biosensors.

The parameter a_2 characterizes a density of the holes in the perforated membrane. Fig. 2 shows, that the absolute values of the radius a_1 of holes as well as the half-distance a_2 between adjacent holes have only weak influence on the biosensor response. The biosensor current depends mainly on the join factor θ of the geometry of perforation.

6. Conclusions

The mathematical model (3.1)-(3.9) of amperometric biosensors with perforated and selective membranes can be used to investigate the kinetic pecu-

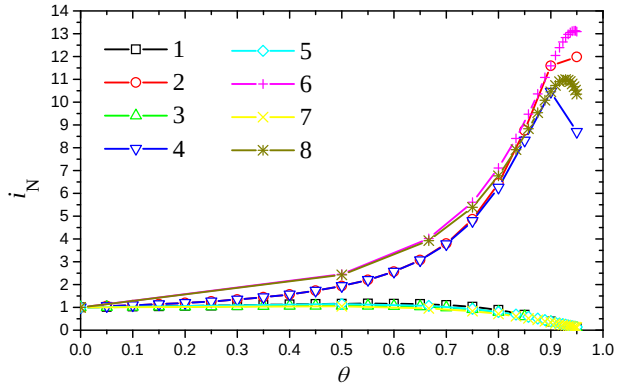

Figure 2. The normalized steady-state current i_N versus the covering degree θ, $a_2 = 1$ (1-4), $a_1 = 0.1$ (5-8), $b_1 = 2$, $b_2 = 4$, $b_3 = 14$ μm, S_0: 100 (1, 2, 5, 6), 1 (3, 4, 7, 8) μM, V_{max}: 1000 (1, 3, 5, 7), 1 (2, 4, 6, 8) μM/s, $D_1 = 1\,\mu\text{m}^2/\text{s}$, $D_2 = 300\,\mu\text{m}^2/\text{s}$, $K_M = 100\,\mu$M, $n_e = 2$.

liarities of the biosensor response. The computer simulation of the biosensors can be used as a tool in design of novel highly sensitive biosensors.

The sensitivity of biosensors can be increased by selecting of the appropriate geometry of perforated membrane (Fig. 2). The level of the possible increase of the sensitivity highly depends on the maximal enzymatic rate V_{max}. Significant gain in sensitivity can be achieved at low values of V_{max} only.

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