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ORIGINAL PAPER

Mathematical modeling of biosensor action in the region between diffusion and kinetic modes

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Abstract We describe the action of electrochemical enzyme-based biosensor by applying mathematical modeling. We consider two types of biosensors: a biosensor containing a single heterogeneous enzyme layer and biosensor containing an additional protecting polymer-based layer. The initial parameters of the biosensor were selected on the basis of typical immobilized glucose oxidase-based electrochemical biosensor. A phenomenon of the accumulation of the electrochemically active product inside the biocatalytic layer was evaluated. It was shown that accumulation of the product can increase sensitivity of the biosensor no more than 2.6 times. Due to the asymmetric distribution of the electrochemically active product inside the enzyme-containing membrane and asymmetric diffusion of the substrate, it was shown that the thickness of the membrane possesses an optimal value. For the selected set of initial parameters, the optimal thickness of the enzyme-containing layer was 2.9–4.5 μm. Real profiles of the impact of the thickness of the membranes were evaluated. A method for the evaluation of acceptable fluctuations of the membrane diffusion parameters on biosensor response was created, and the profiles of the dependence were calculated. These dependencies can be used for development of the software for biosensor monitoring.

Keywords Biosensor · Mathematical modeling · Bioelectrochemistry · Enzyme

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1 Introduction

Biosensors nowadays are common instruments in the number of analytical systems. Fundamentals of biosensors are well reported in a number of reviews [1–4]. Biosensors found interest even in defense technologies [5,6]. Traditionally, biosensors are applied in microbiology [7] and food industry [8]. However, the widest field of biosensor applications lies in medicine. Nowadays, biosensors for the medicine applications are constructed as microelectrodes and microchips [9,10]. Recently, a lot of nanomaterials have been applied for the biosensor design [11]. A list of applied new enzymes, enzyme complexes, and recombinant enzymes increases rapidly. We applied PQQ containing dehydrogenases for creating oxygen-free biosensors for a number of metabolites [12, 13] and newly synthesized carbon materials for the electrochemical monitoring of the urease activity [14].

Increasing the fields of biosensor applications increases requirements to the reliability of the biosensor response. The majority of the biosensors created is based on the enzymes or enzymatic complexes. This is due to the exclusive selectivity of the enzyme and uniform mechanism of the biosensor action.

Behavior of enzyme in heterogeneous system very often differs from their action in solution. In the case of the heterogeneous process, the product can accumulate inside the enzymatic layer of the biosensor. This should lead to the increase of the biosensor response (if the product will be registered). This phenomenon in general was reported previously [15]. Especially, this phenomenon should be pronounced in the case of big charged mediators, such as ferrocene or phenazine derivatives often used in biosensors. On the other hand, the structure of a biosensor very often is not a stable construction. Biosensors implanted into the body can be "glued up" by the adsorbed antibodies due to the inflammation process. Biosensors in the reactors can also be glued up by sorption of proteins, fats, and other components of the microbiological content of the reactor. In both cases, this process will lead to a decreased accessibility for the substrate and increased concentration of the product inside the sensor. A biosensor operating in flow-through systems can be affected by fluctuations of flow speed and pressure. These factors can change the thickness of diffusion layer of the biosensor and thereby affect the response. Accumulation of the product in enzyme media can decrease the activity of the enzyme due to reversible nature of the enzyme action and/or possible inhibition of the enzyme activity by product. Both processes can affect the biosensor response in opposite directions and can be a source of mistakes in evaluation of biosensor response.

This problem can be partially simplified when a biosensor operates in deep diffusion mode of action. This can be observed in the case of low catalytic activity. However, when a biosensor operates at low concentration of substrate in the border between kinetic and diffusion regimes, the problems arise with correct interpretation of the signal. The lower limit of the biosensor action is mostly important for the biosensor application because of the technological requirements to determine the substrates in the bulk as sensitive as possible. Evaluation of the limiting conditions of the biosensor action is a crucial point in the creation of the algorithm of the monitoring of the analytical instrument.

The purpose of this paper is determination of the influence profiles of the thickness and diffusion parameters of the membranes on the biosensor response. The limiting levels of the biosensor response are evaluated, and the optimal parameters of the biosensor construction are elucidated. The novelty of the research lies in the calculation of the biosensor behavior taking into account the possibility to trap the signal formatting product.

The paper is organized as follows. In Sect. 2, the mathematical models are presented. In Sect. 3, we discuss the numerical results. Conclusions and remarks are presented in Sect. 4.

2 Biosensor

2.1 Biosensor construction

Let us consider a biosensor as a flat electrode with enzyme-containing layer deposited on the surface of the electrode (Fig. 1a).

The thickness of the biocatalytic layer is a_e. In special cases, this thickness can be reduced up to the monomolecular (several nm) layer, but usually the thickness of the polymer-containing immobilized enzyme layer is about 1–10 μm. Let the substrate (S) diffuse from the bulk into the enzyme-containing layer. Let us consider the substrate as a small molecule (molecular weight about 100–300 Da), like glucose. We suppose that diffusion of the substrate into the enzyme-containing layer is much slower than in the bulk. Substrate is recognized by enzyme, and catalytical conversion to the product (P) is preceded:

$$\mathrm{S} \xrightarrow{\mathrm{ENZYME}} \mathrm{P} \tag{1}$$

We suppose that P is electrochemically active and can be converted (for example, oxidized) on the surface of the electrode:

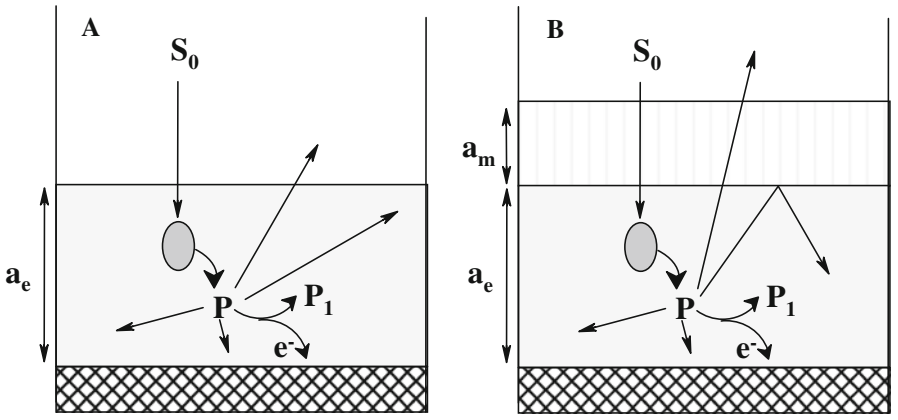

Fig. 1 Principal scheme of the biosensor. **a** Biosensor without outer membrane. **b** Biosensor with outer membrane

$$\mathrm{P} \xrightarrow{\text{ELECTRODE}} \mathrm{P}_1 + n \text{ electrons} \tag{2}$$

In this case, the anodic current of the electrode (I) will correlate with the initial concentration of the substrate in the bulk (S_0). A lot of biosensors can operate according to the following scheme [3]:

$$\begin{aligned} \text{Glucose} + \mathrm{O_2} &\xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + \mathrm{H_2O_2} \\ \mathrm{H_2O_2} &\xrightarrow{\text{ELECTRODE}} \mathrm{O_2} + 2\mathrm{H^+} + 2 \text{ electrons} \end{aligned} \tag{3}$$

Hundreds of glucose biosensors have been reported, operating according to this scheme. In such a glucose biosensor, glucose oxidase can be replaced by lactate oxidase (lactate biosensor), ethanol oxidase (ethanol biosensor), etc. The same scheme can be applied in a more general manner to a number of enzymes where proper electron acceptor (M) can be applied (the substrate is oxidized, and the anodic current is recorded), as was shown for PQQ-dehydrogenases [12]:

$$\begin{aligned} \mathrm{S_0} + \mathrm{M_{Ox.}} &\xrightarrow{\text{Enzyme}} \text{Product} + \mathrm{M_{Red.}} \\ \mathrm{M_{Red.}} &\xrightarrow{\text{ELECTRODE}} \mathrm{M_{Ox.}} + n \text{ electrons} \end{aligned} \tag{4}$$

or the substrate is reduced, and the cathodic current is recorded, as was shown for PQQ-dehydrogenases on Prussian Blue matrices [13]:

$$\begin{aligned} \mathrm{S_0} + \mathrm{M_{Red.}} &\xrightarrow{\text{Enzyme}} \text{Product} + \mathrm{M_{Ox.}} \\ \mathrm{M_{Ox.}} + n \text{ electrons} &\xrightarrow{\text{ELECTRODE}} \mathrm{M_{Red.}} \end{aligned} \tag{5}$$

The same scheme can be applied for the electrochemical detection of urease catalyzed products of urea hydrolysis [14], and even in a number of optical biosensors.

As was mentioned above, the product (P) produced inside the enzyme-containing layer can diffuse to the surface of the electrode and be electrochemically converted. However, part of the product can diffuse out from the enzyme-containing layer to the bulk and will be lost.

We suppose that substrate recognition and biocatalytic conversion of the substrate is expressed as a Michaelis–Menten process. We also suppose that the substrate concentration in the bulk is very low, much lower than the value of k_M. (Application of the Michaelis–Menten kinetics to the heterogeneous processes is complicated, but it becomes linear at low substrate concentrations.) Finally, we suppose that the consumption rate of P on the electrode surface is very high. This means that we may expect (1) a linear response dependence on substrate concentration and (2) that the limiting step of the biosensor action is a biocatalytic process or diffusion process of the substrate, but not an electrochemical conversion of the product.

2.2 Differential equations

The mathematical model contains two key parameters of the biosensor. One is a substrate (analyte), and the other is an electrochemically active recordable product of the enzymatic conversion of the substrate. We consider the substrate and product concentrations $S(t, x)$ and $P(t, x)$ as functions of two variables, the coordinate variable x (the distance to the biosensor electrode) and time variable t. The values $0 < x < a_e$ correspond to the enzyme-containing layer, and the values $a_e < x < a_e + a_m$ correspond to the points inside the outer membrane. For $0 < x < a_e + a_m$ and $t > 0$, the substrate and product kinetics are described by the following nonlinear reaction–diffusion equations:

$$\frac{\partial S}{\partial t} = \frac{\partial}{\partial x}\left(D_S(x)\frac{\partial S}{\partial x}\right) - \alpha(x)\frac{V_{\max} S}{k_M + S}, \tag{6}$$

$$\frac{\partial P}{\partial t} = \frac{\partial}{\partial x}\left(D_P(x)\frac{\partial P}{\partial x}\right) + \alpha(x)\frac{V_{\max} S}{k_M + S}, \tag{7}$$

where the functions $D_S(x)$, $D_P(x)$, and $\alpha(x)$ are defined as follows:

$$D_S(x) = \begin{cases} D_{S_e}, & 0 < x \le a_e, \\ D_{S_m}, & a_e < x \le a_e + a_m, \end{cases} \tag{8}$$

$$D_P(x) = \begin{cases} D_{P_e}, & 0 < x \le a_e, \\ D_{P_m}, & a_e < x \le a_e + a_m, \end{cases} \tag{9}$$

$$\alpha(x) = \begin{cases} 1, & 0 < x \le a_e, \\ 0, & a_e < x \le a_e + a_m. \end{cases} \tag{10}$$

Note that no enzymatic process takes place inside the outer membrane; therefore, $\alpha(x) = 0$ for $a_e < x < a_e + a_m$, and Eqs. (6) and (7) become linear.

Suppose that the concentration of the substrate in the solution S_0 remains stable during all process time. At the beginning of process ($t = 0$), there is neither substrate nor product inside the enzyme-containing layer and outer membrane:

$$\begin{aligned} S(0, x) &= \begin{cases} 0, & 0 \le x < a_e + a_m, \\ S_0, & x = a_e + a_m, \end{cases} \\ P(0, x) &= 0, \quad 0 \le x < a_e + a_m. \end{aligned} \tag{11}$$

The rate of electrochemical conversion of the product is very high in comparison with enzymatic reaction rate. In our model, the substrate is an electrochemically inactive substance. Hence, on the biosensor electrode ($x = 0$), the following boundary conditions are satisfied:

$$\left.\frac{\partial S}{\partial x}\right|_{x=0} = 0, \quad P(t, 0) = 0, \quad t > 0. \tag{12}$$

The second boundary condition defines the value of S at the biosensor boundary with the buffer solution ($x = a_e + a_m$):

$$S(t, a_e + a_m) = S_0, \quad t > 0. \tag{13}$$

The steady-state current density (I) is used as a response of biosensor:

$$I = \lim_{t\to\infty} i(t), \quad i(t) = n_e F D_{P_e} \frac{\partial P}{\partial x}\Big|_{x=0}, \tag{14}$$

where n_e is the number of electrons involved in the charge transfer at the electrode surface, and F is the Faraday constant (F=96,485 C/mol). The numerical value of I was calculated by using the following formula:

$$I \approx i(T), \quad T = \min_{i(t)>0}\left\{t : \frac{1}{i(t)}\left|\frac{di(t)}{dt}\right| < \delta\right\}, \tag{15}$$

where δ is the given dimensionless decay rate (for calculations, we used $\delta = 10^{-3}$).

2.3 Models of biosensor operation

In this paper, we investigate three limiting models of biosensor action.

Model A A biosensor without outer membrane ($a_m = 0$), as shown in Fig. 1a. An electrochemically active product (P) can diffuse both to the electrode and out from the membrane to the bulk. This is a common situation for the most of biosensors. The boundary conditions in this case are as follows:

$$P(t, a_e) = 0, \quad t = 0. \tag{16}$$

The current of a biosensor of such a type, when $S_0 \ll k_M$, can be approximately expressed by the simplified formula [16,17]

$$I^A = n_e F D_{S_e} \frac{S_0}{a_e}\left(1 - \frac{1}{\cosh\sigma}\right), \tag{17}$$

where σ is the dimensionless diffusion module (Damköhler number)

$$\sigma = \sqrt{\frac{V_{\max}}{k_M D_{S_e}}}\, a_e. \tag{18}$$

It is known [16,17] that I^A achieves the maximum at the enzyme layer thickness $a_{e,\max} = \sigma_{\max}\sqrt{k_M D_{S_e}/V_{\max}}$, where $\sigma_{\max} \approx 1.5055$. The parameters of the biosensor can be varied by varying the thickness and permeability of the enzymatic layer, as well as the enzymatic activity.

Model B A biosensor without outer membrane ($a_m = 0$) with no flux of the product through the boundary ($x = a_e$). Then the boundary condition is as follows:

$$\left.\frac{\partial P}{\partial x}\right|_{x=a_e} = 0, \quad t > 0. \tag{19}$$

An electrochemically active product cannot leave the enzyme-containing layer. In this case, the flow of the product P is toward the electrode. The current of the electrode when $S_0 \ll k_M$ can be expressed by the simplified formula

$$I^B = n_e F D_{S_e} \frac{S_0}{a_e} \sigma \tanh \sigma. \tag{20}$$

This model is rather theoretical. Such a situation can be realized when the outer surface of the enzyme-containing membrane is charged and the product has the charge of the same sign. The permeability of such a membrane for the product will be very low, and thereby, almost all products will be trapped inside the biosensor.

Model C A biosensor shown in Fig. 1b ($a_m > 0$). The product can diffuse out through the inner and outer membranes. This is a common situation for the biosensors containing protecting membranes. This implies the boundary condition

$$P(t, a_e + a_m) = 0, \quad t = 0. \tag{21}$$

The current of the electrode when $S_0 \ll k_M$ can be expressed by the simplified formula [15,18]

$$\begin{aligned} I^C = {} & n_e F D_{P_e} \frac{S_0}{a_e + a_m} \left(a_e + a_m \frac{D_{S_m} - \sigma D_{S_e} \tanh \sigma}{D_{S_m} + \sigma (a_m/a_e) D_{S_e} \tanh \sigma} \right) \\ & \times \left[\sigma (a_m/a_e) D_{S_e} \tanh \sigma + \frac{D_{S_e} D_{P_m}}{D_{P_e}} \left(1 - \frac{1}{\cosh \sigma} \right) \right] \\ & \times \left(D_{P_m} a_e + D_{P_e} a_m \right)^{-1}. \end{aligned} \tag{22}$$

This situation can be additionally controlled by the thickness and permeability of the outer membrane.

2.4 Initial parameters

The model of the biosensor was adapted to the glucose biosensor containing glucose oxidase. In this case, the Michaelis–Menten constant $k_M = 0.23$ mM. We took a small initial concentration of the substrate (S_0), $S_0 = 0.023$ mM, much lower than the k_M of the enzyme to fit into linear diapason of biosensor action. The maximal activity of the immobilized biocatalyzer is very difficult to predict. From our experience we conjecture that inactivation of the enzyme during covalent immobilization leads to a 10–100 times higher inactivation. In this case, we selected the activity of the enzyme $V_{\max}$ in the range from 0.11 to 1.1 mM/s. The thickness of the enzymatic

layer a_e and the thickness of the outer membrane a_m can vary in the range from 1 to 10 μm. The diffusion coefficient for substrate in the enzyme containing layer, $D_{S_e} \in [35 \times 10^{-13}; 35 \times 10^{-12}]\,\mathrm{m}^2/\mathrm{s}$, was taken almost 200 times lower in comparison with the diffusion coefficient for oxygen, to be sure that the limiting step will be diffusion of substrate, but not of oxygen or other small mediators. These coefficients were selected and corrected according to the real biosensor designed for this purpose. In this case, glucose oxidase (obtained from Sigma) and bovine serum albumin water solution were deposited on the flat polished Pt electrode (3-mm diameter) and shifted with glutaraldehyde. As an outer membrane, we used a cellulose acetate membrane possessing different permeability. Time/response curves were used to correct the mathematical model and diffusion coefficients.

In numerical simulations, the product diffusion coefficient, D_{P_e}, in the enzyme-containing layer and the substrate diffusion coefficient, D_{S_m}, in the outer membrane vary from 35×10^{-13} to $35 \times 10^{-12}\,\mathrm{m}^2/\mathrm{s}$. The product diffusion coefficient in the outer membrane was changed from 0 to $35 \times 10^{-12}\,\mathrm{m}^2/\mathrm{s}$.

3 Results and discussion

3.1 Theoretical maximal response

To calculate the current profiles, we can apply the simplified formulas mentioned above only at very low concentrations of substrate. Complete evaluation of the biosensor response profile can be obtained only by full calculation of the biosensor model.

Accumulating the product inside the heterogeneous enzyme-containing layer should lead to an increase of the biosensor sensitivity. Let us consider this phenomenon in more detail in the theoretically limiting case: what maximal current can be obtained by increasing the catalytic activity of the biosensor ($V_{\max}$) when the other parameters of the biosensor (D_{S_e}, D_{P_e}, D_{S_m}, D_{P_m}, a_e, and a_m) are optimal (inside the decided intervals)? This situation is represented in Fig. 2. Curve *B* indicates the maximal sensitivity of the biosensor that can be expected if we trap the product inside the enzymatic layer and any additional restrictions for substrate are eliminated (absence of outer membrane).

However, this is only a theoretical case. In real situation, permeability of the enzyme-containing layer for the product takes place in all directions. Curve *A* indicates a most common situation where the permeability of the layer is the same for the product and substrate. As can be seen, the maximal sensitivity of the biosensor is reduced almost 2.6 times. In both cases, the maximal current is achieved when $D_{S_e} = D_{P_e} = 35 \times 10^{-12}\,\mathrm{m}^2/\mathrm{s}$. In both cases, the sensitivity of the biosensor does not depend linearly on the catalytical activity of the biosensor. As can be seen, the slopes of curves *B* and *C* in Fig. 2 are different. This means that increasing the possibility for the product to leave the heterogeneous catalytic layer, the influence of the catalytic activity on the biosensor response decreases. Taking into account that the main parameter of the biosensor stability is the stability of the catalytic process, we can conclude that increasing the sensitivity of the biosensor by trapping the product inside the biosensor, we decrease the overall stability of the biosensor.

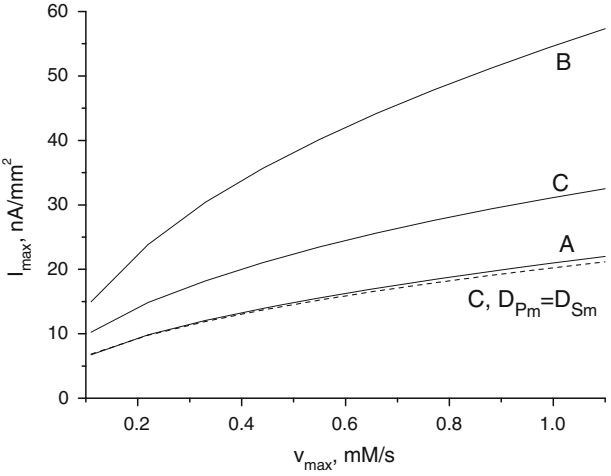

Fig. 2 Dependence of the maximal current of biosensor on the biocatalytic activity of the enzymatic layer. For curves *A* and *B* (models *A* and *B*), biosensor configuration as shown in Fig. 1a, $D_{S_e} = D_{P_e} = 35 \times 10^{-12}\,\mathrm{m}^2/\mathrm{s}$. For curve *C*, biosensor configuration as shown in Fig. 1b, *solid line*— model *C* with $D_{S_e} = D_{P_e} = D_{S_m} = 35 \times 10^{-12}$, $D_{P_m} = 35 \times 10^{-13}\,\mathrm{m}^2/\mathrm{s}$, *dashed line*—model *C* with $D_{S_m} = D_{P_m}$, $D_{S_e} = D_{P_e} = D_{S_m} = D_{P_m} = 35 \times 10^{-12}\,\mathrm{m}^2/\mathrm{s}$

If we apply an additional inert membrane, maximally permeable for the substrate and minimally permeable for the product (Fig. 2, curve *C*), the amplification effect will be lower due to the diffusion restrictions for the substrate. The maximal I^C was achieved at $D_{S_e} = D_{P_e} = D_{S_m} = 35 \times 10^{-12}$ and $D_{P_m} = 35 \times 10^{-13}\,\mathrm{m}^2/\mathrm{s}$. The response of the biosensor increases by increasing the permeability of the both membranes for substrate, increasing the permeability of the biocatalytic layer for the product, and decreasing the permeability of the outer membrane for the product. However, practically, it is quite difficult to keep such a big difference in diffusion parameters for the product and substrate. It can be achieved in several cases where the charge of the substrate and product are different and a charged outer membrane is applied. Such a case can be observed when artificial mediators are used. A number of factors can change the charge of the membrane: fluctuations of pH, concentration of salts, etc. If the charge of the outer membrane were changed, the response of the biosensor would change as well.

Due to the asymmetric nature of the substrate and product diffusion in the membrane, biosensors possess the optimal thickness of the catalytic layer. In Fig. 3, this feature is described in detail.

In the case where the diffusions of the substrate and product are of the same rate (about $35 \times 10^{-12}\,\mathrm{m}^2/\mathrm{s}$), the maximal current is achieved at the thickness of the catalytic layer 4.1 μm (Curve 1). The dot on the curve indicates that the diffusion mode of the biosensor action swathes to the kinetic mode at thinner membranes, and the maximal activity of the biosensor is achieved in the diffusion mode of the biosensor action. When the catalytic activity of the biosensor decreases (Curve 2), both the biosensor mode swathing point and optimal thickness of the membrane move

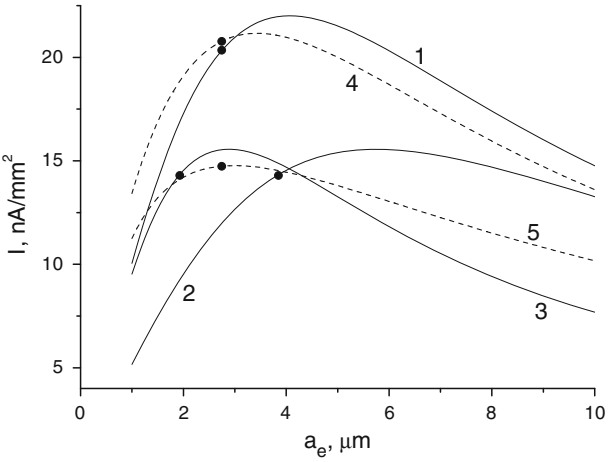

Fig. 3 Dependence of the current of the biosensor on the thickness of the catalytic layer. *Curve 1*—model *A*, $D_{S_e} = D_{P_e} = 35 \times 10^{-12}\mathrm{m}^2/\mathrm{s}$, $V_{\max} = 1.1\,\mathrm{mM/s}$; *Curve 2*—the same as *Curve 1*, but $V_{\max} = 0.55\,\mathrm{mM/s}$; *Curve 3*—the same as *Curve 1*, but $D_{S_e} = D_{P_e} = 17.5 \times 10^{-12}\mathrm{m}^2/\mathrm{s}$; *Curve 4*—model *C*, $D_{S_e} = D_{P_e} = D_{S_m} = D_{P_m} = 35 \times 10^{-12}\mathrm{m}^2/\mathrm{s}$, $a_m = 1\,\mu\mathrm{m}$; *Curve 5*—the same as *Curve 4*, but $a_m = 5\,\mu\mathrm{m}$. *Filled circle* indicates the values when the Damköhler number is equal to 1

to the thicker membrane (5.8 μm). Decreasing the permeability of the catalytic layer (Curve 3) decreases the optimal thickness of the biocatalytic layer as well (2.9 μm). We can conclude that fluctuations of the catalytic membrane thickness will impact on the response of the biosensor at very thin layers more than in the case of thick membranes. A biosensor with thin membrane possesses fast response; however, such a biosensor is more sensitive to the fluctuations of the thickness of the biocatalytic layer. A biosensor is most stable at the optimal thickness of the biocatalytic membrane. Another important feature is that decreasing the catalytic activity of the biosensor increases the stability of the biosensor action.

Application of outer membrane in general decreases the impact of the thickness fluctuations and improves the biosensor stability. Comparing Curves 1 and 4, we can conclude that in the case of very thin biocatalytic membrane, an additional thin outer membrane can improve the sensitivity of the biosensor. This means that natural inactivation of the immobilized enzyme can be compensated by gluing the surface of the electrode by proteins and cells from the bulk. Let us describe this phenomena in more detail. The role of the outer membrane during the biosensor aging is very important. During exploitation of the biosensor, the enzyme is inactivating, and the sensitivity of the biosensor is decreasing. Usually, the decreased $V_{\max}$ is considered as the main reason of the reduced biosensor sensitivity. At the constant thickness of the catalytic layer, the increased thickness of the diffusion layer should increase the product trapping effect and thereby increase the sensitivity of the biosensor. Suppose that the diffusion parameters of substrate and product are equal ($D_{S_e} = D_{P_e} = D_{S_m} = D_{P_m} = 35 \times 10^{-12}\mathrm{m}^2/\mathrm{s}$). In this case, we can calculate how the thickness of outer membrane should change if, keeping the current of the biosensor constant, we would decrease its catalytical activity. Such a situation is shown in Fig. 4 (see also Table 1).

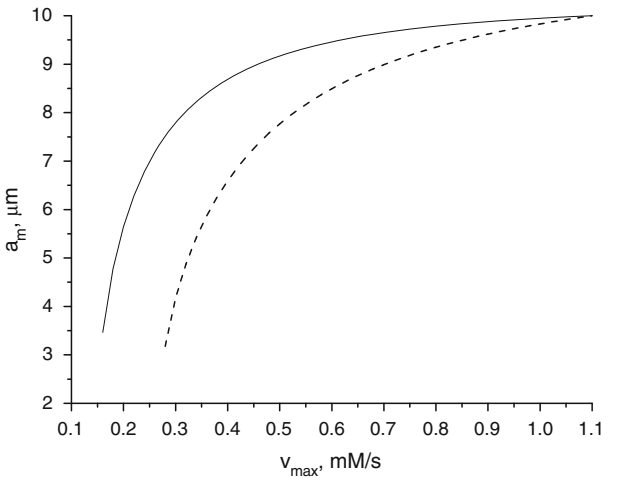

Fig. 4 Dependence of the thickness of the outer membrane on the activity of the enzyme when the response of biosensor I is constant. *Solid line*—$a_e = 10\,\mu$m. *Dashed line*—$a_e = 5\,\mu$m

Table 1 Necessary thickness of the outer membrane (a_e) to keep the same current of the biosensor when the catalytic capacity of the biosensor ($V_{\max}$) decreases. $D_{S_e} = D_{P_e} = D_{S_m} = D_{P_m} = D$

$V_{\max}$, mM/s	D, m^2/s					
	35×10^{-12}		17.5×10^{-12}		35×10^{-13}	
	a_e, μm		a_e, μm		a_e, μm	
	10	5	10	5	10	5
1.1[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]
0.6	9.46	8.5	9.86	9.37	10	9.96
0.3	7.79	4.18	9.27	7.69	10	9.8

[a] Initial values

The lines indicate that it is possible to keep the same sensitivity of the biosensor when both the thickness of the outer membrane and the catalytic activity of the biosensor decrease. Such phenomena can be observed when the outer membrane of the biosensor is slowly degrading or the pressure or flow speed are increasing.

The thicker the outer membrane, the deeper the diffusion mode of the biosensor action and the lower the impact of the inactivation of the biosensor. However, increasing the thickness of the membranes increases the response time of the biosensor. The same is observed when the diffusion coefficient decreases.

3.2 Impact of fluctuations of the diffusion coefficient of the biocatalytic layer on the response

When the outer membrane changes its thickness, it usually shrinks or swells. In this case, the diffusion coefficient (D_{S_m} and/or D_{P_m}) changes. On the other hand, when the charge of the membrane changes, the permeability of the membrane to the charged

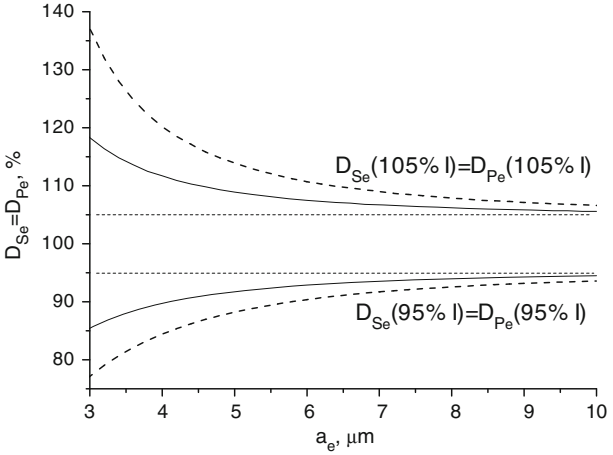

Fig. 5 Impact of fluctuations of the diffusion coefficient of the biocatalytic layer on the response of the biosensor. Biosensor model as shown in Fig. 1a. *Solid line*—$V_{\max} = 1.1\,\text{mM/s}$. *Dashed line*—$V_{\max} = 0.55\,\text{mM/s}$. Initial $D_{S_e} = D_{P_e} = 35 \times 10^{-12}\text{m}^2/\text{s}$

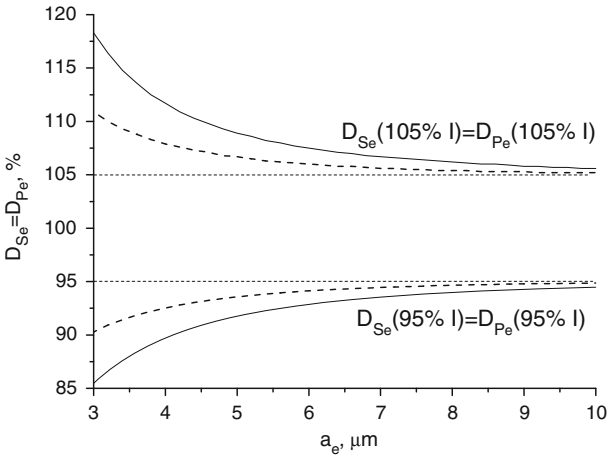

Fig. 6 Impact of fluctuations of the diffusion coefficient of the biocatalytic layer on the response of the biosensor. Biosensor model as shown in Fig. 1a. $V_{\max} = 0.55\,\text{mM/s}$. *Solid line*—$D_{S_e} = D_{P_e} = 35 \times 10^{-12}\text{m}^2/\text{s}$. *Dashed line*—$D_{S_e} = D_{P_e} = 17.5 \times 10^{-12}\text{m}^2/\text{s}$

product also changes. How fluctuations of the diffusion coefficient will impact the stability of the biosensor response? The data are shown in the next figures. Figures 5, 6, 7 show the range of allowed fluctuations of the diffusion coefficient that would change the current of the biosensor no more than 5 %. (Typical acceptable variation of the measurement in biomedical experiments is 5 %). The area between curves indicates the range of acceptable fluctuations of the diffusion coefficient. Figures 5 and 6 correspond to the biosensors containing only one catalytic layer as shown in

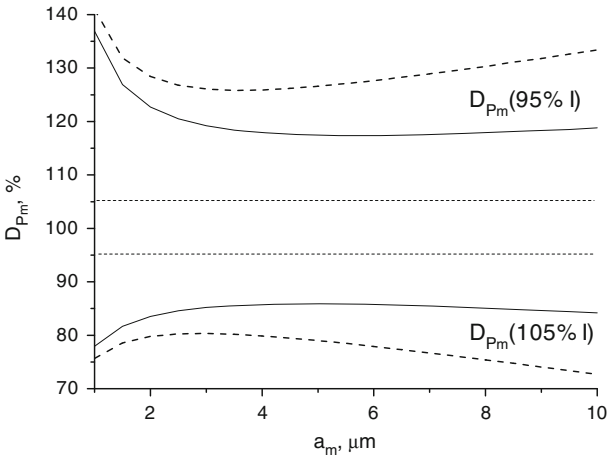

Fig. 7 Impact of fluctuations of the diffusion coefficient of the outer membrane on the response of the biosensor. Biosensor model as shown in Fig. 1b. *Solid line*—$a_e = 10\,\mu$m. *Dashed line*—$a_e = 5\,\mu$m. Initial $D_{S_e} = D_{P_e} = D_{S_m} = D_{P_m} = 35 \times 10^{-12}\text{m}^2/\text{s}$

Fig. 1a, and Fig. 7 corresponds to the biosensors containing two layers as shown in Fig. 1b.

In the case of thick biocatalytic layer, fluctuations of the diffusion parameter (thickness) of the membrane directly affect the response of the biosensor. However, reducing the thickness of biocatalytic layer reduces the biocatalytic capacity of the biosensor, and the biosensor slowly switches to the kinetic mode of action. This means that the impact of the diffusion parameters on the biosensor action decreases. The diffusion module σ decreases from 3.5 at $a_e = 10\,\mu$m to 1.1 at $a_e = 3\,\mu$m. The lower the initial activity of the biocatalytic layer, the lower the impact of the diffusion parameters on the metrological parameters of the biosensor (σ decreases from 2.6 at $a_e = 10\,\mu$m to 0.8 at $a_e = 3\,\mu$m).

Decreasing the initial permeability of the membrane increases the impact of the diffusion parameters of the biosensor, as can be seen in Fig. 6. When $D_{S_e} = D_{P_e} = 17.5 \times 10^{-12}\,\text{m}^2/\text{s}$, the biosensor response is fully controlled by the diffusion parameters. The diffusion module σ decreases from 11.7 at $a_e = 10\,\mu$m to 3.5 at $a_e = 3\,\mu$m. This means that biosensor with higher diffusion restrictions is more sensitive to the fluctuations of the diffusion coefficient. Biosensor specialists usually apply high diffusion restrictions with the purpose of extension of the range of the biosensor action.

In the case of outer membrane (Fig. 7), the impact of the fluctuations of the diffusion coefficient of outer membrane is less expressed than those of the inner biocatalytic membrane (compare data of Figs. 6 and 7). Decreasing the thickness of biocatalytic membrane (which means reducing the catalytical capacity of the biocatalytic layer) reduces the impact of the diffusion parameters of the outer membrane due to the increased weight of the kinetic factor of the biosensor action. Due to asymmetric nature of the substrate diffusion, the thickness of the outer membrane possesses a local optimum.

4 Conclusions

Increasing the sensitivity of the biosensor by trapping the product inside the biosensor decreases the overall stability of the biosensor due to the increased impact of the catalytic activity on the response.

Application of outer membrane diffusion restrictions on the substrate and product not always reduces the sensitivity of the biosensor. It is possible to select an optimal combination of parameters of both membranes to keep the current of the biosensor constant.

The effectiveness of the biosensor depends on the ratio of the diffusion parameters of substrate and product rather than on the thickness of the catalytic layer.

The response of the biosensor with thicker catalytic layer is less sensitive to the fluctuations of outer membrane.

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