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The Influence of the Enzyme Membrane Thickness on the Response of Amperometric Biosensors

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Abstract: A mathematical model of amperometric biosensors has been developed. The model is based on non-stationary diffusion equations containing a non-linear term related to Michaelis-Menten kinetics of the enzymatic reaction. Using digital simulation, the influence of the thickness of enzyme membrane on the biosensor response was investigated. The digital simulation of the biosensor operation showed the non-monotonous change of the maximal biosensor current versus the membrane thickness at the various maximal enzymatic rates. Digital simulation was carried out using the finite difference technique. Results of the numerical simulation was compared with known analytical solutions. This paper presents a framework for selection of the membrane thickness, ensuring the sufficiently stable sensitivity of a biosensor in a required range of the maximal enzymatic rate.

Keywords: Amperometric biosensor, enzyme membrane, diffusion, modelling, simulation.

Introduction

Biosensors are analytical devices that are based on the direct coupling of an immobilised biologically active compound with a signal transducer and an electronic amplifier [1-3]. Starting from the publication of Clark and Lyons in 1962 [1], the amperometric biosensors became one of the popular and perspective trends of biosensorics [2]. The amperometric biosensors measure the changes of the current of indicator electrode by direct electrochemical oxidation or reduction of the products of the biochemical reaction [4-6]. In amperometric biosensors the potential at the electrode is held

constant while the current is measured. The amperometric biosensors are known to be reliable, cheap and highly sensitive for environment, clinical and industrial purposes.

The understanding of the kinetic peculiarities of the biosensors is of crucial importance for their design. Because it is not generally possible to measure the concentration of substrate inside enzyme membranes, starting from seventies various mathematical models of amperometric biosensors have been developed and used as an important tool to study and optimise analytical characteristics of actual biosensors [7-9]. A comprehensive study of the mathematical modelling of amperometric biosensors is given in [10]. The goal of this investigation is to make a model allowing an effective computer simulation of membrane biosensor as well as to investigate the influence of the physical and kinetic parameters on the response of the biosensors. The developed model is based on non-stationary diffusion equations, containing a non-linear term related to the enzymatic reaction [11-13].

One of the most critical characteristic of biosensors is their stability [14]. The operational stability of a biosensor response may vary considerably depending on geometry and method of sensor preparation, a transducer use and some other parameters. Furthermore it is strongly depend upon the response rate limiting factor, i.e. substrate diffusion and enzymatic reaction rate. In this paper the influence of the biosensor geometry on the biosensor stability is investigated. A framework for selection of the enzyme membrane thickness, ensuring the sufficiently stable biosensor response in a required range of the enzymatic rate has been described.

In this investigation, digital simulation of the biosensor response was carried out using the implicit finite difference scheme [15-18]. The software has been programmed in C language [19]. The program built was employed to investigate the influence of the enzyme membrane thickness, the substrate concentration as well as the maximal enzymatic rate on the biosensor response. The program was used also for the numerical investigation of the kinetics of the biosensors response taking place during phenols detection in waste waters [20].

Mathematical Model

Consider an enzyme-catalysed reaction

$$\mathrm{S} \xrightarrow{\mathrm{E}} \mathrm{P}. \tag{1}$$

In this scheme the substrate (S) binds to the enzyme (E) and converts to the product (P).

The biosensor can be considered as an enzyme electrode, having a layer of enzyme immobilised onto the surface of the probe. Let us assume the symmetrical geometry of the electrode and homogeneous distribution of immobilised enzyme in the enzyme membrane. Coupling the enzyme-catalysed reaction in enzyme layer with the one-dimensional-in-space diffusion, described by Fick's law, leads to the following equations:

$$\frac{\partial S}{\partial t} = D_{\mathrm{S}} \frac{\partial^2 S}{\partial x^2} - \frac{V_{\max} S}{K_{\mathrm{M}} + S}, \quad 0 < x < d, \quad 0 < t \le T, \tag{2}$$

$$\frac{\partial P}{\partial t} = D_{\mathrm{P}} \frac{\partial^2 P}{\partial x^2} + \frac{V_{\max} S}{K_{\mathrm{M}} + S}, \quad 0 < x < d, \quad 0 < t \le T, \tag{3}$$

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

Let $x = 0$ represents the electrode surface, while $x = d$ represents the bulk solution/membrane interface. The operation of biosensor starts when some substrate appears over the surface of the enzyme layer. The initial conditions ($t = 0$) are

$$S(x,0) = 0, \quad 0 \le x < d, \qquad S(d,0) = S_0, \tag{4}$$

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

where S_0 is the concentration of substrate in the bulk solution.

In case of amperometric biosensors, due to electrode polarisation the concentration of the reaction product at the electrode surface is being permanently reduced to zero. The substrate does not react at the electrode surface. 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 during the biosensor operation. This is used in the boundary conditions ($0 < t \le T$) given by

$$\left.\frac{\partial S}{\partial x}\right|_{x=0} = 0, \qquad S(d,t) = S_0, \tag{6}$$

$$P(0,t) = P(d,t) = 0. \tag{7}$$

The measured current is accepted as a response of a biosensor in a physical experiment. The current depends upon the flux of the reaction product at the electrode surface, i.e. at border $x = 0$. Consequently, the density $i(t)$ of the anodic current at time t can be obtained explicitly from Faraday's law and Fick's low using the flux of the product concentration at the surface of the electrode

$$i(t) = n_e F D_P \left.\frac{\partial P}{\partial x}\right|_{x=0}, \tag{8}$$

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

Digital Simulation

Definite problems arise when solving analytically the non-linear partial differential equations with complex boundary conditions [12,16]. To obtain an approximate analytical solution, approximation and classification of each different condition are usually needed. On the other hand, the digital simulation to obtain a numerical solution can be applied almost to any case. Consequently, the problem (2)-(7) was solved numerically.

The finite difference technique was applied for discretization of the mathematical model [15]. We introduced an uniform discrete grid in both: x and t directions [21]. An implicit linear finite difference scheme has been built as a result of the difference approximation of Eqs. (2)-(7). The resulting system

of linear algebraic equations was solved efficiently because of the tridiagonality of the matrices of the systems. Having a numerical solution of the problem (2)-(7), the density of the biosensor current $i(t)$ can be calculated easily.

The mathematical model as well as the numerical solution of the problem was evaluated for different values of the maximal enzymatic rate V_{max}, substrate concentration S_0, as well as the membrane thickness d. The following values of the parameters were constant in the numerical simulation of all the experiments:

$$D_S = D_P = 3.0\times10^{-6}\,\mathrm{cm}^2/\mathrm{s},$$
$$K_M = 1.0\times10^{-7}\,\mathrm{mol/cm}^3, n_e = 2. \qquad (9)$$

The evolution of the biosensor current at the maximal enzymatic rate V_{max} of 10^{-7} mol/cm^{3}s is presented in Fig. 1. The biosensor response was modelled for biosensors having four different membrane thickness d: 0.001, 0.0015, 0.01, 0.015 cm. One can see in Fig. 1 the biosensor current appears with some delay at relatively thick enzyme layers. This delay increases with the increase of the enzyme membrane thickness. Comparing the evolution of the biosensor current (Fig. 1) in two cases of relatively thin (d = 0.001 and 0.0015 cm) membrane, one can see that the biosensor response is notable higher at thicker membrane (d = 0.0015 cm) than at thinner one (d = 0.001 cm). However, comparing the biosensor responses in other two cases of ten times thicker (d = 0.01 and 0.015 cm) membranes, we see the opposite tendency: the biosensor of thicker (d = 0.015 cm) membrane generates lower response than thinner one (d = 0.01 cm). We discuss the effect of the membrane thickness on the biosensor response in details.

The maximal biosensor current i_{max} (the biosensor response) as well as the time moment of occurrence of the maximal current (response time) were assumed and analyzed as ones of the most important characteristics of a biosensor.

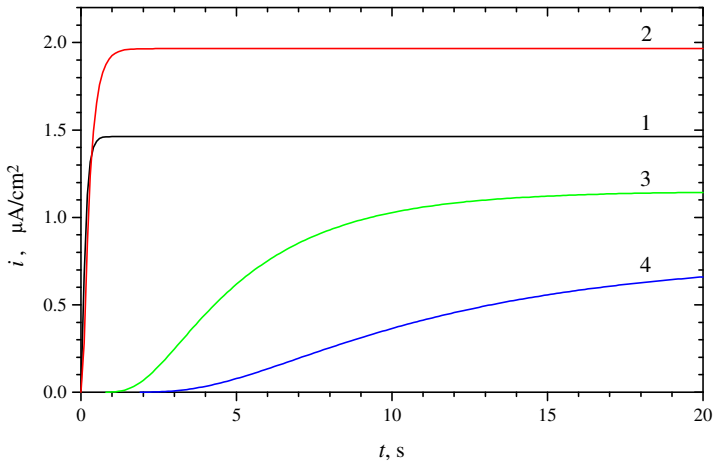

Figure 1. The dynamics of the biosensor current i at the maximal enzymatic rate V_{max} = 10^{-7} mol/cm^{3}s and four membrane thickness d: 0.001 (1), 0.0015 (2), 0.01 (3), 0.015 (4) cm, S_0 = 2×10^{-8} mol/cm^3.

In digital simulation, the biosensor response time was assumed as the time when the absolute current slope value falls below a given small value normalised with the current value. In other words, the time needed to achieve a given dimensionless decay rate ε is used

$$T_{\mathrm{R}} = \min_{i(t)>0}\left\{t : \frac{1}{i(t)}\left|\frac{\mathrm{d}i(t)}{\mathrm{d}t}\right| < \varepsilon\right\}. \tag{10}$$

Consequently, the maximal biosensor current $i_{\max}$ was assumed as the current at the biosensor response time T_{R}. We employed $\varepsilon = 10^{-6}$. However, the response time T_{R} as an approximate steady-state time is very sensitive to the decay rate ε, i.e. $T_{\mathrm{R}} \to \infty$, when $\varepsilon \to 0$. Because of this we investigate the change of a half of steady-state time [12]. The resultant relative output signal function $i^*(t)$ can be expressed as:

$$i^*(t) = \frac{i_R - i(t)}{i_R}, \quad i_{\mathrm{R}} = i(T_{\mathrm{R}}), \quad i_{\max} = i_{\mathrm{R}}, \tag{11}$$

where $i(t)$ is the output current density at time t as defined in (8), i_{R} is assumed as the steady-state current. Let us notice, that $0 \le i^*(t) \le 1$ at all $t \ge 0$, $i^*(0) = 1$ and $i^*(T_{\mathrm{R}}) = 0$. Let $T_{0.5}$ be the time at which the reaction-diffusion process reaches the medium, called half time of steady-state or, particularly, half of the time moment of occurrence of the maximal current, i.e. $i^*(T_{0.5}) = 0.5$.

Results and Discussion

Using computer simulation we have investigated the dependence of the maximal biosensor current on the thickness of the enzyme membrane. The maximal biosensor current $i_{\max}$ was assumed as steady-state current i_∞, calculated at the response T_{R} time defined by formula (10), $i_{\max} = i_\infty = i_{\mathrm{R}}$. The investigation was carried out at the following values of $V_{\max}$: 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/cm^{3}s to get results for a wide range of values of the maximal enzymatic rate. Fig. 2 shows the maximal current while Fig. 3 presents the half time $T_{0.5}$ of the maximal current versus the thickness d of the enzyme membrane. Fig. 2 presents also values of the stationary current i_∞ [22],

$$i_\infty = \lim_{t\to\infty} i(t) = n_{\mathrm{e}} F D_{\mathrm{S}} S_0 \frac{1}{d}\left(1 - \frac{1}{\cosh(\sigma)}\right) \tag{12}$$

where σ dimensionless diffusion modulus, Damkoehler number,

$$\sigma^2 = \frac{V_{\max} d^2}{D_{\mathrm{S}} K_{\mathrm{M}}}. \tag{13}$$

Formula (12) is valid at substrate concentrations significantly lower than Michaelis constant, $S_0 << K_{\mathrm{M}}$. In Fig. 2, values of i_∞ obtained by (12) are depicted as a function of the membrane thickness d. Due to the assumption of $i_{\max} = i_\infty = i_{\mathrm{R}}$ and substrate concentration $S_0 = 0.2K_{\mathrm{M}} < K_{\mathrm{M}}$, employed in the calculations above, the analytical solution (12) compares sufficiently well with the numerical solution of the model (2)-(7) at different enzymatic rates $V_{\max}$ and membrane thickness d.

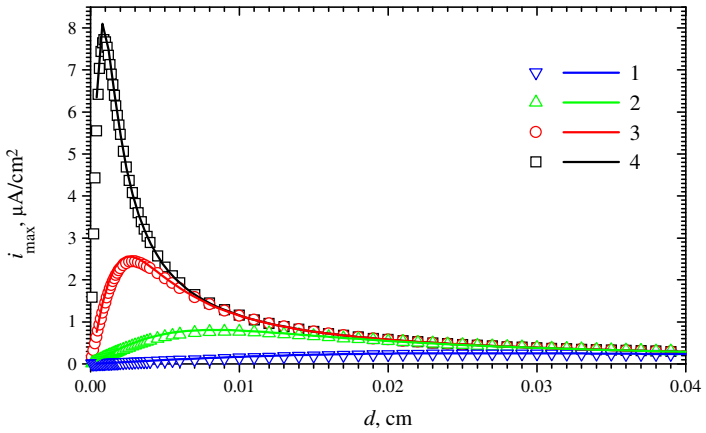

Figure 2. The dependence of the maximal biosensor current i_{max} on the thickness d of the enzyme membrane at four maximal enzymatic rates V_{max}: 10^{-9} (1), 10^{-8} (2), 10^{-7} (3) and 10^{-6} (4) mol/cm^{3}s, $S_0 = 2\times10^{-8}$ mol/cm^3. Symbols are numerical solutions, while lines are analytical ones (formula 12).

One can see (Fig. 2) that the maximal biosensor current i_{max} is a non-monotonous function of d at all values of the maximal enzymatic rate V_{max}. The higher maximal enzymatic rate V_{max} corresponds to the greater maximal value of i_{max}.

From the results, obtained by digital simulation, we determine, that the maximum of i_{max} equals to about 7.72 μA/cm^2 at $V_{max} = 10^{-6}$ mol/cm^{3}s, while $i_{max} \approx 2.45$ μA/cm^2 at $V_{max} = 10^{-7}$ mol/cm^{3}s. The higher maximum of i_{max} corresponds to thinner enzyme membrane. In the case of $V_{max} = 10^{-6}$ mol/cm^{3}s, the maximum of $i_{max}(d)$ is gained at $d \approx 0.0009$ cm, while in the case of $V_{max} = 10^{-7}$ mol/cm^{3}s, the maximum of i_{max} is gained at $d \approx 0.0028$ cm.

Using (12) we find analytically the membrane thickness d, at which the state-state current i_∞ gains the maximum at given n_e, D_S, S_0, V_{max}, K_M and $S_0 << K_M$. At first, we calculate a derivative of $i_\infty(d)$ with the respect to the thickness d

$$\frac{\partial i_\infty(d)}{\partial d} = n_e F D_S S_0 \frac{-\cosh^2(\sigma)+\cosh(\sigma)+\sigma\sinh(\sigma)}{d^2\cosh^2(\sigma)} \qquad (14)$$

Then we look for σ at which that derivative gets zero

$$-\cosh^2(\sigma)+\cosh(\sigma)+\sigma\sinh(\sigma) = 0\,. \qquad (15)$$

Eq. (15) was solved numerically. A single solution $\sigma = \sigma_{max} \approx 1.5055$ was obtained. Consequentially, i_∞ gains the maximum at the membrane thickness d_{max}, where

$$d_{max} = \frac{1}{\sigma_{max}}\sqrt{\frac{D_S K_M}{V_{max}}}, \quad \sigma_{max} = 1.5055\,. \qquad (16)$$

Accepting (9), we find, that $d_{max} \approx 0.000825$, $i_{max} \approx 8.1$ at $V_{max} = 10^{-6}$; $d_{max} \approx 0.00261$ cm, $i_{max} \approx 2.56$ μA/cm^2 at $V_{max} = 10^{-7}$ mol/cm^{3}s etc. These values compare sufficiently well with the corresponding

values obtained by the numerical simulation of the biosensor operation. Corresponding values of the maximal current i_{max} as well as thickness d varies by about 5%. That variation in values appears because of substrate concentration $S_0 = 0.2K_M$. The analytical solution (12) is valid at $S_0 << K_M$ only, while the numerical one does not have such kind of restrictions at all. Because of this, values of d_{max}, calculated using the model (2)-(7) are more accurate than analytical ones at $S_0 = 20$ nmol/cm^3 and (9).

Using formula (12) we find that the maximal biosensor current as a function of the membrane thickness d gains the maximum when the diffusion modulus σ equals to $\sigma_{max} = 1.5055$. According to (13) and (15) σ_{max} does not depend on the substrate concentration S_0. Nevertheless, using the numerical simulation we have calculated values of σ_{max} at some more values of S_0. We obtained the following values: $\sigma_{max} \approx 1.51$ at $S_0 = 2\times10^{-11}$, $\sigma_{max} \approx 1.55$ at $S_0 = 2\times10^{-9}$ mol/cm^3 and $\sigma_{max} \approx 2.5$ at $S_0 = 2\times10^{-7}$ mol/cm^3. The modulus σ_{max} is approximately constant at $S_0 << K_M$, so that it is about coincident with the value obtained from the analytical solution (12). σ_{max} increases with increase of substrate concentration S_0. The increase is especially notable at substrate concentrations $S_0 > K_M$. The dependence of σ_{max} on V_{max} is practically insignificant: σ_{max} varies by less than 3.5% while V_{max} changes from 10^{-9} to 10^{-6} mol/cm^{3}s at any concrete S_0.

The stability of the response is one of the most critical characteristics of biosensors [14]. It is very important to have biosensors keeping their analytical capability for a long period. Usually the maximal enzymatic rate V_{max} decreases permanently due to enzyme inactivation. In general, the biosensor response is sensitive to changes of V_{max}. Fig. 2 shows, that the maximal biosensor current can differ by some dozens, changing V_{max}. The variation is especially notable in cases of relatively thin enzyme membranes. In case of relatively thick enzyme membrane, i_{max} practically does not vary by changing V_{max}. Consequently, a biosensor containing thicker enzyme layer gives more stable response than a biosensor with thinner layer. However, the thick membrane-based biosensors have very durable response time (Fig. 3). It is possible to notice (Fig. 3), that the half time $T_{0.5}$ of the maximal biosensor current is about 18.5 s when the membrane thickness d equals to 0.02 cm and $V_{max} = 10^{-6}$ mol/cm^{3}s. The half time is even more durable at thicker enzyme membrane as well as lower enzymatic rate, so that biosensors of such thickness is of limited applicability in flow injection systems, which are widely used for determination of various compounds [23].

Thus, a problem of the membrane thickness optimisation arises. The task is to find the thickness of membrane so small as possible, ensuring the stability of the biosensor response at a range of V_{max} as wide as possible. Let V_1 and V_2 be two values of the maximal enzymatic rate ($V_1 < V_2$) such as we need to have stable biosensor response to substrate of concentration of S_0. Then we describe the minimal membrane thickness $d_\delta(V_1, V_2, S_0)$, at which the relative difference $R(d, V_1, V_2, S_0)$ between the biosensor response (the maximal biosensor current i_{max}) at $d = d_\delta$, $V_{max} = V_1$ and another one response at $d = d_\delta$, $V_{max} = V_2$ is less than dimensionless decay rate δ

$$R(d,V_1,V_2,S_0) = \frac{\left|i_{max}(d,V_1,S_0) - i_{max}(d,V_2,S_0)\right|}{i_{max}(d,V_1,S_0)}, \tag{17}$$

$$d_\delta(V_1,V_2,S_0) = \min_{d>0}\left\{d : R(d,V_1,V_2,S_0) < \delta\right\} \tag{18}$$

where $i_{max}(d, V_{max}, S_0)$ is the maximal biosensor current at the membrane thickness of d, maximal enzymatic rate V_{max} and substrate concentration S_0.

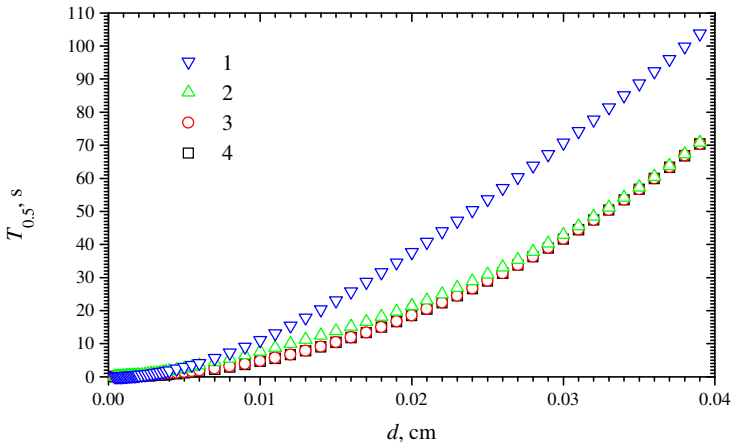

Figure 3. The dependence of the half time $T_{0.5}$ of the maximal biosensor current on the membrane thickness d at four maximal enzymatic rates V_{max}: 10^{-9} (1), 10^{-8} (2), 10^{-7} (3) and 10^{-6} (4) mol/cm^{3}s, $S_0 = 2\times10^{-8}$ mol/cm^3.

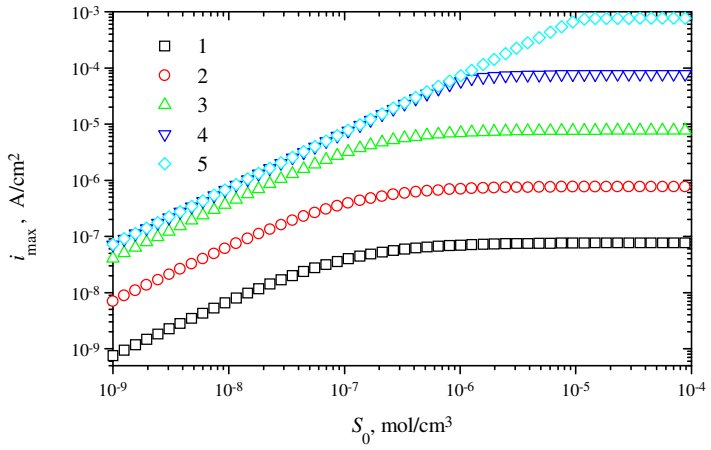

Figure 4. The dependence of the maximal biosensor current i_{max} on the substrate concentration S_0 at five maximal enzymatic rates V_{max}: 10^{-10} (1), 10^{-9} (2), 10^{-8} (3), 10^{-7} (4) and 10^{-6} (5) mol/cm^{3}s, $d = d_\delta(10^{-7}, 10^{-6}, 2\times10^{-8}) = 0.008$ cm, calculated by formula (18) assuming $\delta = 0.02$.

Let us assume $S_0 = 20$ nmol/cm^{3}s, $V_1 = 10^{-7}$, $V_2 = 10^{-6}$ mol/cm^{3}s and $\delta = 0.02$. From the numerical results, presented in Fig. 2, we found $d_\delta \approx 0.008$ cm. We have calculated the response of a biosensor, based on the membrane of thickness $d = d_\delta(V_1, V_2, S_0) = 0.008$ cm, at wide range of the substrate concentration S_0 to evaluate the biosensor stability at that range. Fig. 4 shows i_{max} versus S_0 at five values of V_{max}: 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol/cm^{3}s. No notable difference (Fig. 4) is observed between values of i_{max}, calculated at two values of V_{max}: 10^{-7} and 10^{-6} mol/cm^{3}s, when the substrate concentration S_0 is less than about 10^{-6} mol/cm^3. Fig. 4 expressively shows the stable response of the

biosensor, based on the enzyme membrane of thickness d = 0.008 cm, when the maximal enzymatic rate reduces ten times: from 10^{-6} to 10^{-7} mol/cm^{3}s. Although the membrane thickness d_δ was calculated at the substrate concentration $S_0 = 2\times10^{-8}$ mol/cm^3, the biosensor response is sufficiently stable to the substrate of concentration being up to about 10^{-6} mol/cm^3. The dependence of d_δ on the substrate concentration was noticed before. The biosensor response is very sensitive to changes of V_{max} at high concentration of substrate. Fig. 4 shows that the response of the biosensor of thickness of 0.008 cm is approximately constant at the concentration higher than about 10^{-5} mol/cm^3. Because of this, such biosensor is practically unuseful to determinate larger substrate concentration.

Fig. 5 presents an effect of substrate concentration S_0 on the half time $T_{0.5}$ of the maximal biosensor current. The thickness d of the enzyme membrane is the same as above, i.e. $d = d_\delta$ = 0.008 cm. One can see in Fig. 5, $T_{0.5}$ is a monotonous decreasing function of S_0 at $V_{max} = 10^{-10}$, 10^{-9} and 10^{-8} mol/cm^{3}s, and $T_{0.5}$ is a non-monotonic function of S_0 at $V_{max} = 10^{-7}$ and 10^{-6} mol/cm^{3}s. The effect of non-monotonous behaviour of the half time of maximal biosensor current versus substrate concentration has been discussed recently for the cases when the biosensor response is under diffusion control [21]. However, the most important feature for this investigation is the sufficiently short time of the biosensor response. One can see, $T_{0.5}$ does not exceed 8 s. The biosensor, based on enzyme membrane of thickness of 0.008, gives very stable response in a sufficiently short time when V_{max} is between 10^{-7} and 10^{-6} mol/cm^{3}s as well as the substrate concentration S_0 is less than about 10^{-6} mol/cm^3.

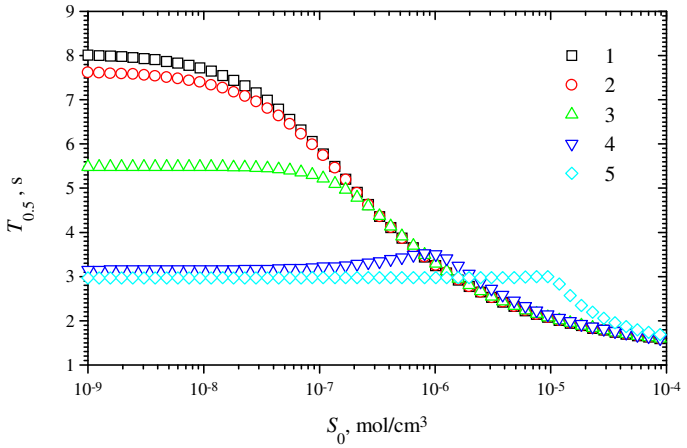

Figure 5. The dependence of halftime $T_{0.5}$ of the maximal biosensor current on the substrate concentration S_0 at five maximal enzymatic rates V_{max}: 10^{-10} (1), 10^{-9} (2), 10^{-8} (3), 10^{-7} (4) and 10^{-6} (5) mol/cm^{3}s, other parameters are the same as in Fig. 4.

The concept of the minimal membrane thickness $d_\delta(V_1, V_2, S_0)$, at which the relative difference $R(d, V_1, V_2, S_0)$ of the biosensor response is less than the decay rate δ, can be considered as a framework to be used for determination of the membrane thickness in a design of biosensors producing highly stable response to the substrate of concentration S_0 while the enzymatic rate changes from V_1 to V_2. In this case the minimal thickness d_δ needs to be calculated at the concrete characteristics of biosensor operation: the diffusion coefficients D_S, D_P, number of electrons n_e, Michaelis constant K_M and the substrate concentration S_0 approximate to expected one. Rather often the concentration of analyte to be

analysed varies within a known interval. Since the biosensor response is usually more stable at lower concentrations of the substrate (Fig. 4) than at higher concentrations, a larger value of the range of expected concentrations should be employed in calculation of d_δ to ensure the stable response in the entire interval of the expected concentrations. In cases when $S_0 << K_M$, the i_{max} may be calculated analytically from (12), otherwise the model (2)-(7) is preferable for calculation of $i_{max}(d, V_{max}, S_0)$, used in the framework, expressed by formulas (17), (18).

To be sure, that the framework, based on definition (17) and (18), really helps to find the membrane thickness at which the biosensor gives relatively stable response, we calculate the biosensor response also in a case of significantly thinner membrane. Fig. 6 shows i_{max} versus S_0 at the same values of V_{max} as in Fig. 4, however the enzyme membrane is eight times thinner, d = 0.001cm. One can see in Fig. 6, the biosensor response is very sensitive to changes of V_{max}. For example, in a case of $S_0 = 10^{-9}$ mol/cm^3, the maximal current i_{max} at $V_{max} = 10^{-6}$ mol/cm^{3}s is about 4.7 times higher than i_{max} at $V_{max} = 10^{-7}$ mol/cm^{3}s (Fig. 6), while the corresponding values of i_{max} are approximately the same in the case when the membrane is of thickness $d_\delta(10^{-7}, 10^{-6}, 2\times10^{-8}) = 0.008$ cm (Fig. 4).

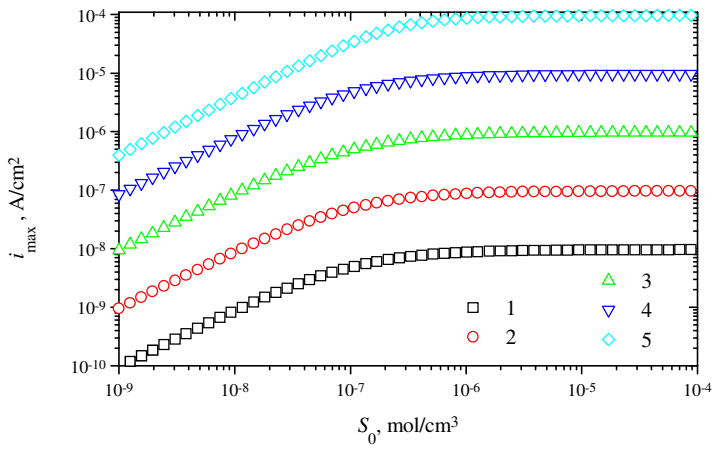

Figure 6. The dependence of the maximal biosensor current i_{max} on the substrate concentration S_0 at five maximal enzymatic rates V_{max}: 10^{-10} (1), 10^{-9} (2), 10^{-8} (3), 10^{-7} (4) and 10^{-6} (5) mol/cm^{3}s, d = 0.001cm.

Let us notice (Fig. 4), that at d = 0.008 cm, the relative difference R (formula 17) between i_{max} at $V_{max} = 10^{-8}$ and another one i_{max} at $V_{max} = 10^{-7}$ mol/cm^{3}s is about 0.86 when $S_0 = 2\times10^{-8}$ mol/cm^3. This difference keeps approximately unchanged at all S_0 less than about 10^{-7} mol/cm^3. Let us reduce that difference. Using definition (18) and results, presented in Fig. 4, we find $d_\delta(V_1, V_2, 2\times10^{-8})$ to be equal to about 0.017 cm when $V_1 = 10^{-8}$, $V_2 = 10^{-6}$ mol/cm^{3}s, assuming $\delta = 0.1$.

Fig. 7 plots i_{max} versus S_0 at d = 0.017 and the same values of V_{max} as above. No notable difference is observed between values of i_{max}, calculated at three values of V_{max}: 10^{-8}, 10^{-7} and 10^{-6} mol/cm^{3}s, when the substrate concentration S_0 is less than about 5×10^{-7} mol/cm^3. Fig. 7 presents the stable response of the biosensor, based on the enzyme membrane of thickness d = 0.017 cm, when the maximal enzymatic rate reduces 100 times: from 10^{-6} to 10^{-8} mol/cm^{3}s while analysing substrate of concentration less than 5×10^{-7} mol/cm^3.

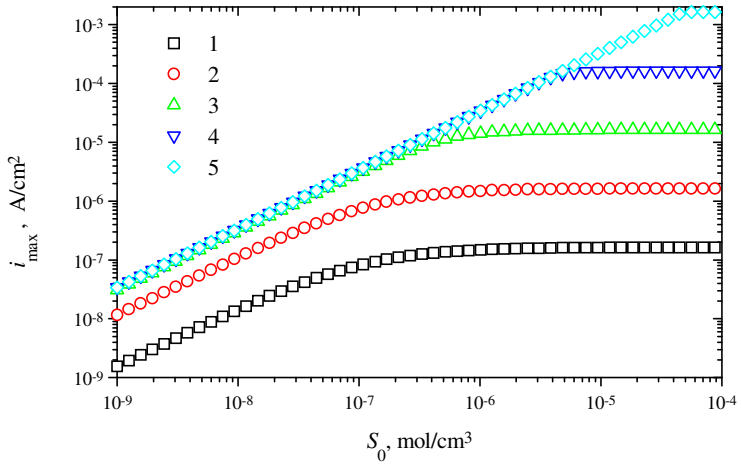

Figure 7. The dependence of the maximal biosensor current i_{max} on the substrate concentration S_0 at five maximal enzymatic rates V_{max}: 10^{-10} (1), 10^{-9} (2), 10^{-8} (3), 10^{-7} (4) and 10^{-6} (5) mol/cm^{3}s, $d = d_\delta(10^{-8}, 10^{-6}, 2\times10^{-8}) = 0.017$ cm, calculated by formula (18) accepting $\delta = 0.1$.

In the high substrate concentration case, $S_0 >> K_M$, the stationary current can be expressed as follows [25],

$$i_\infty = \frac{n_e F V_{max} d}{2}. \qquad (19)$$

In the all cases of the investigation of the effect of the substrate concentration on the biosensor response, values of i_{max}, obtained by digital simulation at $S_0 = 10^{-4}$ mol/cm^3, were compared with the corresponding values, calculated by formula (19). The difference between two corresponding values varies less than 0.1%. Consequentially, in the high substrate concentration case, $S_0 >> K_M$, the maximal biosensor current can be successfully calculated from formula (19), while (12) may be used in the low substrate concentration case, $S_0 << K_M$. However, the digital simulation, based on the model (2)-(7), may be successfully applied in the entire domain of substrate concentration, and the simulation is especially reasonable in the middle substrate concentration case, $S_0 \approx K_M$.

The sensitivity is one of the most important characteristic of biosensors. The sensitivity B_S (Acm/mol) of a biosensor can be expressed as a gradient of the maximal biosensor current density i_{max} (A/cm^2) with respect to the substrate concentration S_0 (mol/cm^3)

$$B_S = \frac{\partial i_{max}(S_0)}{\partial S_0}. \qquad (20)$$

Fig. 8 shows the biosensor sensitivity B_S versus the substrate concentration S_0 at the same five maximal enzymatic rates as above in the case of membrane thickness d of 0.017 cm. No notable difference is observed between the sensitivity B_S, calculated at two values of V_{max}: 10^{-7} and 10^{-6} mol/cm^{3}s, when the substrate concentration is less than about 2×10^{-6} mol/cm^3. The biosensor sensitivity at $V_{max} = 10^{-8}$ mol/cm^3 is about 10% less than in two cases of higher V_{max}: 10^{-7} and 10^{-6} mol/cm^{3}s. Let us remind, that the membrane thickness of 0.017 cm has been calculated requiring the

relative difference $R(d, 10^{-8}, 10^{-6}, 2\times10^{-8})$ be less than $\delta = 0.1$, i.e. 10%. Because of a large scale that minimal difference (0.1) is not practically notable in Fig. 7. However, this easy seems in Fig. 8, which represents the biosensor sensitivity. Comparing Figs. 7 and 8 we see direct relation between the maximal biosensor current i_{max} as a function of S_0 and the function B_S of S_0.

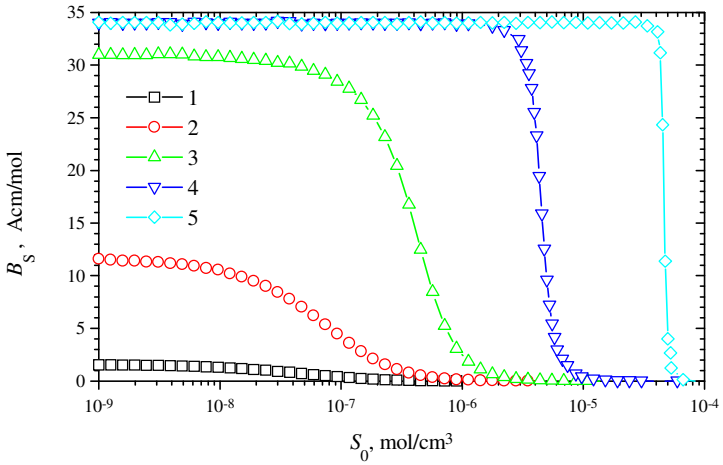

Figure 8. The dependence of the biosensor sensitivity B_S (formula 20) on the substrate concentration S_0 at five maximal enzymatic rates V_{max}: 10^{-10} (1), 10^{-9} (2), 10^{-8} (3), 10^{-7} (4) and 10^{-6} (5) mol/cm^{3}s. Other parameters are the same as in Fig. 7.

Using formula (12) we can calculate also the derivative of the stationary current i_∞ with respect to S_0. In that way we obtain a constant biosensor sensitivity B_S for $S_0 << K_M$. This stagnancy of B_S can be also noticed in Fig. 8. One can see in Fig. 8, at high enzymatic rates, e.g. $V_{max} = 10^{-7}$ and 10^{-6} mol/cm^{3}s, the biosensor sensitivity remains approximately constant even at S_0 greater than K_M. However at low enzymatic rates ($V_{max} = 10^{-10}$ and 10^{-9} mol/cm^{3}s), the sensitivity starts to decrease notable already at $S_0 < K_M$.

In the high substrate concentration case, $S_0 >> K_M$, value of B_S can be obtained also from the formula (19) as $B_S = 0$, which compares favourably with the results of digital simulation (Fig. 8).

Fig. 2 shows the significant influence of the membrane thickness on the biosensor response. However, the significance of the influence is different at the different membrane thickness. We introduce a resistance B_R of the membrane-based biosensors to changes of membrane thickness. The resistance B_R (A/cm^3) of a biosensor is expressed as a gradient of the maximal biosensor current density i_{max} (A/cm^2) with respect to the membrane thickness d (cm)

$$B_R = \frac{\partial i_{max}(d)}{\partial d}. \qquad (21)$$

Fig. 9 plots the biosensor resistance B_R versus the membrane thickness d. The substrate concentration S_0 as well as other parameters are the same as in Fig. 2. Since the resistance B_R varies in orders of magnitude, B_R was normalised with V_{max}. So, Fig. 9 shows the resistance B_R divided by V_{max}. versus the membrane thickness d. In Fig. 9, symbols are numerical solutions of the model (2)-(7), while lines are analytical ones (formula 14). One can see (Fig. 9) the interval (from –2.5 to 19.2

mAs/mol) of variation of B_R/V_{max} is approximately the same at all four values of the maximal enzymatic rate V_{max}. It means that the maximal as well as minimal biosensor resistance B_R is directly proportional to V_{max}. Since the shape of curves of the normalised resistance considerably differs (Fig. 9), the dependence of B_R on V_{max} is non-linear in entire domain of d. The relative difference between numerical solutions and analytical ones reaches about 20%. The largest difference is notable at thinnest enzyme membranes.

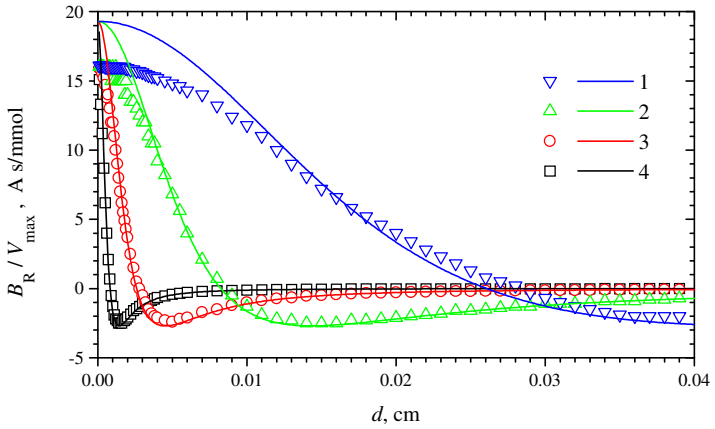

Figure 9. The biosensor resistance B_R (formula 21), normalised with the maximal enzymatic rate V_{max}, versus the membrane thickness d. Symbols are numerical solutions, while lines are analytical ones (formula 14). All parameters are the same as in Fig 2.

Conclusions

The mathematical model (2)-(7) of amperometric biosensor operation can be successfully used to investigate the kinetic regularities of enzyme membrane-based sensors.

The maximal biosensor current i_{max} is a non-monotonous function of membrane thickness d at various values of the maximal enzymatic rate (Fig. 2). When the substrate concentration S_0 is significantly less than the Michaelis constant K_M, $S_0 << K_M$, the function $i_{max}(d)$ gains the maximum at the membrane thickness d of which the diffusion modulus σ equals to 1.5055. The diffusion modulus, maximising $i_{max}(d)$, increases with increase of S_0. Consequently, the maximal current i_{max} increases with increase of the membrane thickness d when the enzyme kinetics predominate in the biosensor response, while i_{max} decreases when the response is significantly under diffusion control. The higher maximal enzymatic rate V_{max} corresponds to a greater maximum of $i_{max}(d)$.

In the high substrate concentration case, $S_0 >> K_M$, the maximal biosensor current can be successfully calculated from formula (19), while formula (12) may be used in the low substrate concentration case, $S_0 << K_M$. However, the digital simulation, may be successfully applied in the entire domain of substrate concentration. The simulation is especially reasonable in the intermediate case of the substrate concentration, $S_0 \approx K_M$.

The mathematical model (2)-(7) together with definition (17) and (18) describe a way for selection of the membrane thickness, ensuring the stable biosensor response. In cases when $S_0 << K_M$, the

maximal current i_{max} to be used in formula (17), may be calculated analytically from (12), otherwise the model (2)-(7) is preferable for the calculation.

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Samples Availability: Available from the authors.