Nonlinear Analysis: Modelling and Control, 2003, Vol. 8, No. 1, 3–18

Computer Simulation of the Response of Amperometric Biosensors in Stirred and non Stirred Solution [1]

R. Baronas[1], F. Ivanauskas[2], J. Kulys[3]

[1]Vilnius University, Naugarduko 24, 2600 Vilnius, Lithuania
romas.baronas@maf.vu.lt

[2]Vilnius University, Naugarduko 24, 2600 Vilnius, Lithuania
Institute of Mathematics and Informatics, Akademijos 4, 2600 Vilnius, Lithuania
feliksas.ivanauskas@maf.vu.lt

[3]Institute of Biochemistry, Mokslininkų 12, 2600 Vilnius, Lithuania
jkulys@bchi.lt

Received: 26.02.2003
Accepted: 13.03.2003

Abstract. A mathematical model of amperometric biosensors has been developed to simulate the biosensor response in stirred as well as non stirred solution. The model involves three regions: the enzyme layer where enzyme reaction as well as mass transport by diffusion takes place, a diffusion limiting region where only the diffusion takes place, and a convective region, where the analyte concentration is maintained constant. Using computer simulation the influence of the thickness of the enzyme layer as well the diffusion one on the biosensor response was investigated. The computer simulation was carried out using the finite difference technique.

Keywords: reaction-diffusion, modelling, biosensor.

1 Introduction

Biosensors are analogue devices that are based on the direct coupling of an immobilised biologically active compound with a signal transducer and an electronic amplifier [1]–[4]. The biosensors yield a signal, which is proportional to the concentration of measured analyte or a group of analytes. The biosensors are classified according to the nature of the physical transducer.

[1]This work was supported by EU financed RTD project, contract No. QLK3-CT-2000-01481

Amperometric biosensors measure the current on an indicator electrode due to direct oxidation of the products of the biochemical reaction [5]. In case of the amperometric biosensors the potential at the electrode is held constant while the current flow is measured. The amperometric biosensors are reliable, relatively cheap and highly acceptable for environment, clinical and industrial purposes [6].

The modern concept of biosensors has been evolved in publication of Clark and Lyons [1]. From the publication the amperometric membrane 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 was analysed in the publications of Mell and Maloy [7, 8]. Some later reports were also devoted to the modelling and investigation of the amperometric biosensor response [9]–[14].

The goal of this investigation is to make a model allowing an effective computer simulation of amperometric biosensor response in a stirred as well as non stirred analyte. The developed model is based on non-stationary diffusion equations [15], containing a non-linear term related to Michaelis-Menten kinetic of the enzymatic reaction. The model involves three regions: the enzyme layer where enzyme reaction as well as mass transport by diffusion takes place, a diffusion limiting region where only a mass transport by diffusion takes place, and a convective region, where the analyte concentration is maintained constant. The intensity of stirring is expressed by the thickness of the diffusion limiting layer. The thickness of the diffusion layer is inversely proportional to the intensity of stirring. The more intensive stirring relates to the thinner diffusion layer. The digital simulation of the biosensor response was carried out using the semi-implicit finite difference scheme [16, 17].

The developed software was employed to investigate the influence of the thickness of the enzyme layer as well as the diffusion on the biosensor response. The maximal biosensor response as well as the time of the maximal response were investigated as functions of the dimensionless ratio of the thickness of entire diffusion domain to the thickness of the enzyme layer.

2 Mathematical Model

During an enzyme-catalysed reaction

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

the substrate S is converted to product P. The rate of the appearance of the product depends on the concentration of the substrate.

In the simplest case, when the diffusion of substrate as well as product molecules is neglected and steady-state conditions are assumed for the enzyme reaction, the mathematical model of enzyme kinetics is given by Michaelis-Menten equation

$$v = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{V_{max}S}{K_M + S} \tag{2}$$

where $v = v(S)$ is the rate of the enzymatic reaction, 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, S is the substrate concentration, P is concentration of the reaction product, and t is time. V_{max} corresponds to relative activity of substrate [2]. However, the mass transport by diffusion is a first-order reaction with respect to substrate concentration [7]–[10].

We consider a membrane amperometric biosensor which can be treated as an enzyme electrode, having a layer of enzyme immobilised onto the surface of the probe. If the bulk solution is well-stirred and in powerful motion, then the diffusion layer remains at a constant thickness. The concentration of substrate as well as product over the enzyme surface (bulk solution/membrane interface) remains constant while the biosensor keeps in touch with the substrate. However, if the bulk solution is not stirred, then the concentration of the substrate as well as product over the enzyme surface depends on the diffusion of the species in bulk solution. Because of this the model consists of three regions: the enzyme layer where enzyme reaction as well as mass transport by diffusion takes place, a diffusion limiting region where only mass transport by diffusion takes place, and a convective region, where the analyte concentration is maintained constant.

To formulate the corresponding mathematical model we assume the symmetrical geometry of the electrode and uniform distribution of the immobilised enzyme in the enzyme membrane. This allows to formulate the model in one spatial dimension. The dynamics of the considered biosensor system can be described by the reaction-diffusion system

$$\frac{\partial S_e}{\partial t} = D_{Se}\frac{\partial^2 S_e}{\partial x^2} - \frac{V_{max} S_e}{K_M + S_e}, \quad 0 < x < l,\ 0 < t \le T, \tag{3}$$

$$\frac{\partial P_e}{\partial t} = D_{Pe}\frac{\partial^2 P_e}{\partial x^2} + \frac{V_{max} S_e}{K_M + S_e}, \quad 0 < x < l,\ 0 < t \le T, \tag{4}$$

$$\frac{\partial S_b}{\partial t} = D_{Sb}\frac{\partial^2 S_b}{\partial x^2}, \quad l < x < L,\ 0 < t \le T, \tag{5}$$

$$\frac{\partial P_b}{\partial t} = D_{Pb}\frac{\partial^2 P_b}{\partial x^2}, \quad l < x < L,\ 0 < t \le T, \tag{6}$$

where S_e, S_b (P_e, P_b) are the concentration of the substrate (reaction product) in the enzyme and in the bulk solution, respectively, l is thickness of the enzyme layer, L is the boundary (consequently, $L - l$ is the thickness) of the diffusion layer, T is full time of biosensor operation to be analysed, D_{Se} (D_{Pe}) is the diffusion coefficient of substrate (reaction product) in the enzyme layer and D_{Sb} (D_{Pb}) is diffusion coefficient in the bulk solution.

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

$$S_e(x,0) = 0, \quad P_e(x,0) = 0, \quad 0 \le x < l, \tag{7}$$

$$S_e(l,0) = S_0, \quad P_e(l,0) = 0, \tag{8}$$

$$S_b(x,0) = S_0, \quad P_b(x,0) = 0, \quad l \le x \le L, \tag{9}$$

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

The boundary conditions ($0 < t \le T$) are

$$\left.\frac{\partial S_e}{\partial x}\right|_{x=0} = 0, \tag{10}$$

$$S_b(L,t) = S_0, \tag{11}$$

$$D_{Se}\left.\frac{\partial S_e}{\partial x}\right|_{x=l} = D_{Sb}\left.\frac{\partial S_b}{\partial x}\right|_{x=l}, \tag{12}$$

$$S_e(l,t) = S_b(l,t), \tag{13}$$

$$P_e(0,t) = P_b(L,t) = 0, \tag{14}$$

$$D_{Pe}\frac{\partial P_e}{\partial x}\Big|_{x=l} = D_{Pb}\frac{\partial P_b}{\partial x}\Big|_{x=l}, \tag{15}$$

$$P_e(l,t) = P_b(l,t) = 0. \tag{16}$$

Consequently, concentration S of the substrate S and concentration P of the reaction product P can be defined for the entire domain $0 \leq x \leq L$ as follows:

$$S(x,t) = \begin{cases} S_e(x,t), & 0 \leq x \leq l,\ t \geq 0, \\ S_b(x,t), & l < x \leq L,\ t \geq 0, \end{cases} \tag{17}$$

$$P(x,t) = \begin{cases} P_e(x,t), & 0 \leq x \leq l,\ t \geq 0, \\ P_b(x,t), & l < x \leq L,\ t \geq 0. \end{cases} \tag{18}$$

Let us notice, that because of conditions (13) and (16) both functions: S and P are continuous in entire domain: $0 \leq x \leq L$.

The diffusion layer $l < x < L$ may be treated as a Nernst diffusion layer, which is widely used in modelling of the electrochemical reactions [18]. According to Nernst approach, a layer of thickness $\delta_N = L - l$ (the Nernst diffusion layer) remains stagnant. Away from it the solution is in motion and uniform in concentration. The thickness of the layer remains unchanged with time. The thickness of the Nernst diffusion layer is inversely proportional to the intensity of stirring. No exact analytical expression is available for stirred solutions. δ_N can be estimated experimentally by measuring the electrode response at given bulk concentration. Furthermore, δ_N depends on the type of stirring. The more intensive stirring is, the thinner the Nernst diffusion layer is.

In case of extremely intensive stirred bulk solution the Nernst diffusion layer may be neglected, i.e., $L = l$. In such a case, assuming $L = l$, equations (5), (6), (9), (12) and (15) may be removed from the model (3)–(16) while four boundary conditions: (11), (13), (14) and (16) reduced to two only: $S_e(l,t) = S_0$, $P_e(0,t) = P_e(l,t) = 0$. The reduced model is identical to the model presented in [14]. The model (3)–(16) generalises the earlier model [14] of the amperometric biosensors, operating in batch analysis.

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

$$I(t) = n_e F D_{Pe} \frac{\partial P_e}{\partial x}\Big|_{x=0}, \tag{19}$$

where n_e is a number of electrons involved in a charge transfer at the electrode surface, and F is Faraday constant, $F \approx 9.65 \times 10^4$ C/mol. Having a numerical solution of the problem (3)–(16), the density $I(t)$ of the biosensor current can be calculated easily.

3 Solution of the Problem

The problem (3)–(16) was solved numerically using the finite difference technique [15, 16]. To find a numerical solution of the problem in the domain $[0, L] \times [0, T]$ we introduced an uniform discrete grid $\omega_h \times \omega_\tau$, where

$$\begin{aligned} \omega_h &= \{x_i \colon x_i = ih,\ i = 0, \ldots, N_1, \ldots, N;\ hN_1 = l,\ hN = L\}, \\ \omega_\tau &= \{t_j \colon t_j = j\tau,\ j = 0, \ldots, M;\ \tau M = T\}. \end{aligned} \tag{20}$$

We assume the following

$$S_i^j = S(x_i, t_j),\ P_i^j(x_i, t_j),\ I_j = I(t_j),\ i = 0, \ldots, N;\ j = 0, \ldots, M. \tag{21}$$

A semi-implicit linear finite difference scheme has been built as a result of the difference approximation. Assuming (17) and (18) the initial conditions (7)–(9) we approximated as follows

$$\begin{aligned} S_i^0 &= 0, \quad i = 0, \ldots, N_1 - 1, \\ S_i^0 &= S_0, \quad i = N_1, \ldots, N, \\ P_i^0 &= 0, \quad i = 0, \ldots, N. \end{aligned} \tag{22}$$

Differential equations (3), (4) were approximated by the scheme

$$\frac{S_i^{j+1} - S_i^j}{\tau} = D_{Se} \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} - \frac{V_{max} S_i^{j+1}}{K_M + S_i^j}, \tag{23}$$

$$\frac{P_i^{j+1} - P_i^j}{\tau} = D_{Pe}\frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} - \frac{V_{max}S_i^{j+1}}{K_M + S_i^{j+1}}, \tag{24}$$
$$i = 1, \ldots, N_1 - 1,\ j = 1, \ldots, M.$$

Differential equations (5), (6) were approximated by the scheme

$$\frac{S_i^{j+1} - S_i^j}{\tau} = D_{Sb}\frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2}, \tag{25}$$
$$\frac{P_i^{j+1} - P_i^j}{\tau} = D_{Pb}\frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2}, \tag{26}$$
$$i = N_1 + 1, \ldots, N,\ j = 1, \ldots, M.$$

The boundary conditions (10)–(16) were approximated as follows:

$$\begin{aligned} &S_0^j = S_1^j, \quad S_N^j = S_0, \\ &D_{Se}(S_{N_1}^j - S_{N_1-1}^j) = D_{Sb}(S_{N_1+1}^j - S_{N_1}^j), \end{aligned} \tag{27}$$

$$\begin{aligned} &P_0^j = 0, \quad P_N^j = 0, \\ &D_{Pe}(P_{N_1}^j - P_{N_1-1}^j) = D_{Pb}(P_{N_1+1}^j - P_{N_1}^j), \\ &j = 1, \ldots, M. \end{aligned} \tag{28}$$

Equations (22) 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}, $i = 0, \ldots, N$, solving the system of linear equations (23), (25) and (27);

2) calculate values of P_i^{j+1}, $i = 0, \ldots, N$, solving the system of linear equations (24), (26) and (28) using values of S_i^{j+1}, which have been calculated in step 1.

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

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

$$I(t_j) = n_e F D_{P_e}(P_1^j - P_0^j)/h, \quad j = 0, \ldots, M. \tag{29}$$

4 Results and Discussion

The mathematical model (3)–(16) as well as the numerical solution of the model was evaluated for different values of the maximal enzymatic rate V_{max} as well as substrate concentration S_0. The following values of the parameters were constant in the numerical simulation of all the experiments:

$$\begin{aligned} &D_{Se} = D_{Pe} = 3.0 \times 10^{-6}\mathrm{cm}^2/\mathrm{s}, \\ &D_{Sb} = 2D_{Se}, \quad D_{Pb} = 2D_{Pe}, \\ &K_M = 1.0 \times 10^{-7}\mathrm{mol/cm}^3, \quad n_e = 2. \end{aligned} \tag{30}$$

Figure 1 shows the profile of substrate as well as product concentrations

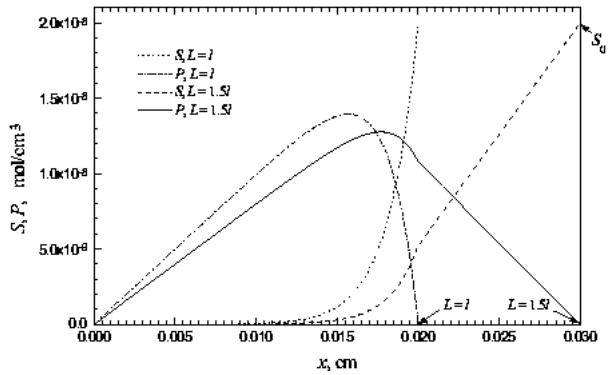

Fig. 1. Profile of substrate and product concentrations at steady-state and thickness $l = 0.02$ cm of enzyme layer when solution is well stirred ($L = l$) and weak stirred ($L = 1.5\,l$), $V_{max} = 10^{-7}\mathrm{mol/cm}^3\mathrm{s}$, $S_0 = 2 \times 10^{-8}\mathrm{mol/cm}^3$

at thickness $l = 0.02$ cm of enzyme layer in both regimes of stirring: well and weak. Here we assume $L = l$ for well stirred regime of analysis, while $L = 1.5\,l$ for weak one. The profile shows the concentration at time $T = 130$ s in well stirred regime and $T = 195$ s in weak one when the maximal biosensor response is reached. The maximal biosensor response was achieved faster in well stirring regime in comparison to the weak one. The computer simulation was carried out at the maximal enzymatic rate $V_{max} = 10^{-7}$ mol/cm^{3}s and the substrate concentration $S_0 = 2 \times 10^{-8}$ mol/cm^3. As it is possible to notice in Fig. 1, the gradient of the reaction product at electrode surface ($x = 0$) is

higher when bulk solution was well stirred. It means that in well stirring regime of analysis the maximal current is higher than in weak one. These preliminary properties were reviewed in wide range of thickness of the enzyme layer at different maximal enzymatic rates.

The thickness of the enzyme layer of the biosensor can usually be measured precisely enough. However, the thickness of the diffusion one (Nernst layer) can not, especially when the bulk solution is in weak motion. Because of this we calculate the biosensor response at different thickness of the Nernst layer to investigate the influence of the intensity of the solution stirring on the biosensor response.

The maximal biosensor current and the time of the maximal current are the main characteristics of the biosensor response. As it was mentioned above, the model (3)–(16) expresses biosensor action in batch analysis [14], when a biosensor remains immersed in the bulk solution of infinite volume and during long time. In a case of the batch analysis the maximal biosensor current I_{max} is the steady-state current I_∞. In the computer simulation, the biosensor response was checked every 0.1s if the steady-state current reached. The calculation was terminated when the relative difference of two values of the current is less than $10^{-5}\%$.

Since the steady-state time is very sensitive to the accuracy of calculation of the maximal current, we investigate the evolution of half of steady-state time [15]. The resultant relative output signal $I^*(t)$ of an amperometric biosensor can be expressed as follows:

$$I^*(t) = \big(I_{\max} - I(t)\big)/I_{\max}, \quad I_{\max} = I_\infty = \lim_{t\to\infty} I(t), \tag{31}$$

where $I(t)$ is the output current density at time t as defined in (19), I_∞ is the density of the steady-state as well as the maximal current. The half $T_{0.5}$ of the steady-state time is the time when the reaction-diffusion process reaches the medium, i.e., $I^*(T_{0.5}) = 0.5$.

Multiple computer simulation of the biosensor response at different values of the thickness of the enzyme layer as well as the diffusion layer showed that I_∞ and $T_{0.5}$ depends mainly on the relative thickness of the pure diffusion layer. Because of this we investigate the dependence of the maximal biosensor response and the time of the maximal response on the dimensionless ratio k of

the thickness L of the entire layer of analysis to the thickness l of the enzyme layer, $k = L/l$.

The maximal biosensor current is very sensitive to the thickness of the enzyme layer. The maximal current varies even in orders of magnitude. Because of this we normalise the maximal biosensor current to evaluate the effect of relative thickness $k = L/l$ on the maximal response. Let $I(l, L, t)$ be the output current density at time t, as defined in (19), at given thickness l of the enzyme layer and thickness L of the entire domain. We express the normalised maximal biosensor current I_{Nmax} as the maximal current of the biosensor divided by the maximal current in well (extremely) stirred solution

$$\begin{aligned} I_{Nmax}(l,k) &= I_\infty(l,L)/I_\infty(l,l),\ k = L/l \geq 1, \\ I_\infty(l,L) &= \lim_{t\to\infty} I(l,L,t), \end{aligned} \tag{32}$$

where $I_\infty(l, L)$ is the density of the steady-state current. The normalised maximal biosensor current $I_{N_{max}}$ is a function of the thickness l of enzyme layer and the dimension less ratio k of the thickness L of the entire domain to l. According to the definition (32), $I_{N_{max}}(l, 1) = 1$ for all values of $l > 0$.

Assuming $T_{0.5}(l, L)$ as the halftime of the maximal biosensor response at given thickness l of the enzyme layer and thickness L of the entire domain, we introduce normalised halftime $T_{N,0.5}$ as follows

$$T_{N,0.5}(l,k) = T_{0.5}(l,L)/T_{0.5}(l,l), \quad k = L/l \geq 1. \tag{33}$$

The influence of the dimensionless ratio $k = L/l$ on the maximal biosensor current has been investigated at two values of the maximal enzymatic rate V_{max}: 10^{-7} and 10^{-8} mol/cm^3 s and two values of the substrate concentration S_0: 2×10^{-8} and 2×10^{-7} mol/cm^3. Results of calculation are depicted in Figs. 2–7. Since the behaviour of the biosensor response at $V_{max} > K_M$ is practically the same as in the case of $V_{max} = K_M$ [14], we employed no values of V_{max} less than K_M. The maximal biosensor response is usually linear proportional to substrate concentration S_0 at values of the concentration significantly less than K_M [3, 14]. On the other hand, the maximal biosensor response is independent from the substrate concentration S_0 when $S_0 \gg K_M$. Because of this we employed only that two values of the substrate concentration.

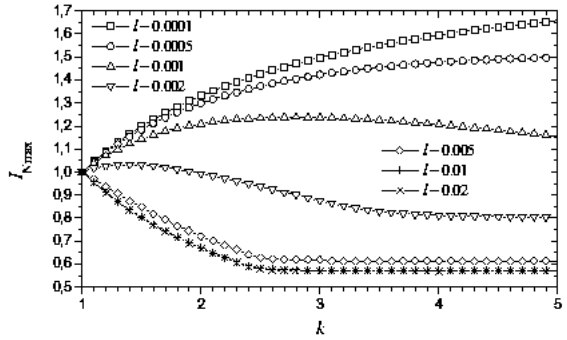

Fig. 2. The normalised maximal biosensor current I_{Nmax} versus ratio k=L/l at different values of l (cm), $V_{max} = 10^{-7}$ mol/cm^{3}s, $S_0 = 2 \times 10^{-8}$ mol/cm^3

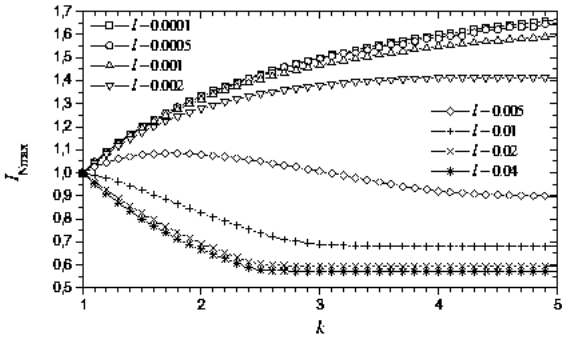

Fig. 3. The normalised maximal biosensor current I_{Nmax} versus ratio k=L/l at different values of l (cm), $V_{max} = 10^{-8}$ mol/cm^{3}s, $S_0 = 2 \times 10^{-8}$ mol/cm^3

Figure 2 shows the dependence of the normalised maximal biosensor current I_{Nmax} on the ratio k at $V_{max} = 10^{-7}$ while Fig. 3 shows one at $V_{max} = 10^{-8}$ mol/cm^{3}s and $S_0 = 2 \times 10^{-8}$ mol/cm^3. In Fig. 4, I_{Nmax} versus k is presented at $V_{max} = 10^{-7}$ mol/cm^{3}s and $S_0 = 2 \times 10^{-8}$ mol/cm^3. As it is possible to notice in these figures, the shape of the normalised current I_{Nmax} (as well as the non-normalised one I_{max}) is very sensitive to the thickness l of the enzyme layer. I_{Nmax} is monotonous decreasing function of the ratio k for relatively thick biosensors ($l > \approx 0.01$ cm). In such cases the maximal biosensor response decreases significantly for $k < \approx 2.5$ only. More distant increase of the thickness of the Nernst diffusion layer unchanges the maximal biosensor response. Because of this, if the enzyme layer of a biosensor is rather thick, then it is enough to restrict the domain of analysis to $L = 2.5l$ in the

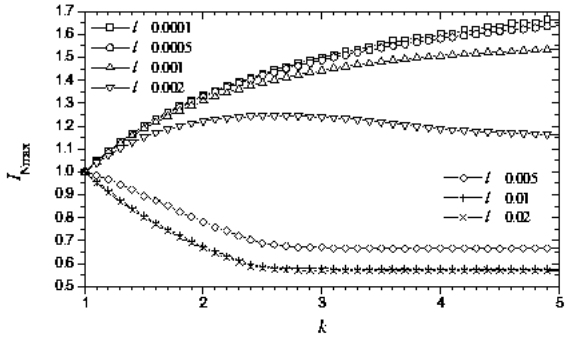

Fig. 4. The normalised maximal biosensor current I_{Nmax} versus ratio k=L/l at different values of l (cm), $V_{max} = 10^{-7}$ mol/cm^{3}s, $S_0 = 2 \times 10^{-7}$ mol/cm^3

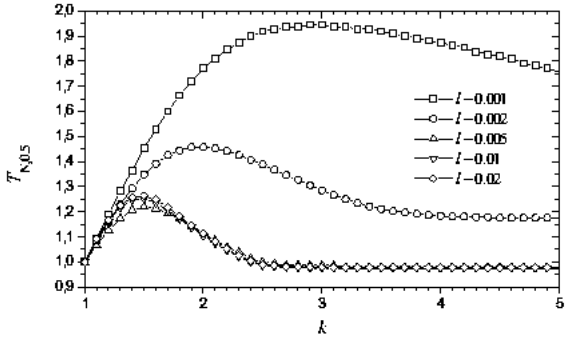

Fig. 5. The normalised halftime $T_{\mathrm{N},0.5}$ of the maximal biosensor response versus ratio $k = L/l$ at different values of l (cm), $V_{max} = 10^{-7}$ mol/cm^{3}s, $S_0 = 2 \times 10^{-8}$ mol/cm^3

model (3)–(16) to simulate biosensor action accurately in non stirred solution. This is valid for both values of the maximal enzymatic rate V_{max}: 10^{-7} and 10^{-8} mol/cm^{3}s as well as both substrate concentrations: 2×10^{-8} and 2×10^{-7} mol/cm^3. The conception of the "thick enzyme layer" depends on V_{max} and S_0. However, according to Figs. 2–4 this dependence is slight.

In case of thin ($l <\approx 0.001$) enzyme layer, I_{Nmax} is a monotonous increasing function of the ratio k. If the enzyme layer is especially thin (e.g., $l = 0.0001$ cm), the increase of the maximal current is notable even at $k = 5$. It means, that in the case of very thin enzyme layers, even slight stirring can have an effect on the maximal response.

Figures 2–4 show that the intensity of stirring can change the maximal

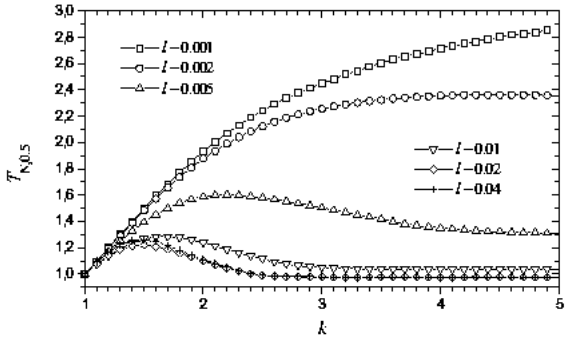

Fig. 6. The normalised halftime $T_{\mathrm{N},0.5}$ of the maximal biosensor response versus ratio $k = L/l$ at different values of l (cm), $V_{max} = 10^{-8}$ mol/cm^{3}s, $S_0 = 2 \times 10^{-8}$ mol/cm^3

biosensor current up to about 2 times. However, in some cases the maximal biosensor current varies slightly only. It appears when l is about 0.002 cm at $V_{max} = 10^{-7}$ mol/cm^{3}s, and l is about 0.005 cm at $V_{max} = 10^{-8}$ mol/cm^{3}s.

The biosensor response is known to be under mass transport control if the enzymatic reaction in enzyme layer is faster than the transport process. The concentration of substrate reaches zero inside the enzyme layer at close proximity to $x = l$ when the dimensionless parameter σ^2 is much greater than unity, where

$$\sigma^2 = \frac{V_{max} l^2}{D_{Se} K_M}. \tag{34}$$

This parameter essentially compares the rate of enzyme reaction (V_{max}/K_M) with the diffusion through the enzyme layer (l^2/D_{Se}). If $\sigma^2 < 1$, enzyme kinetics predominate. The response is under diffusion control if $\sigma^2 > 1$. Since D_{Se}, and K_M are constant in all our numerical experiments as defined in (30), we express the thickness l_1 of the enzyme layer through V_{max} at $\sigma = 1$ as follows:

$$l_1 = \sigma \times \sqrt{\frac{D_{Se} K_M}{V_{max}}} = \sqrt{\frac{3 \times 10^{-13}}{V_{max}}} = \begin{cases} 0.00173, \; V_{max} = 10^{-7} \frac{\mathrm{mol}}{\mathrm{cm^3 s}}, \\ 0.00548, \; V_{max} = 10^{-8} \frac{\mathrm{mol}}{\mathrm{cm^3 s}}. \end{cases} \tag{35}$$

Comparing these values of $l = l_1$ with the values discussed above, we notice that behaviour of the normalised maximal biosensor current I_{Nmax}

favourably depends on that either the enzyme kinetics or the diffusion predominates. Figures 2–4 show, that in a case when the enzyme kinetics distinctly predominates ($l \ll l_1$), I_{Nmax} is a monotonous increasing function of the ratio k. I_{Nmax} is a monotonous decreasing function of k if the biosensor response is distinctly under diffusion control ($l \gg l_1$). However, I_{Nmax} is under the small influence also of the substrate concentration S_0.

The dependence of the normalised halftime $T_{N,0.5}$ of the maximal biosensor response on the dimensionless ratio $k = L/l$ is presented in Figs. 5–7. Comparing Fig. 5 with Fig. 6 we see the influence of the maximal enzymatic rate V_{max} on the behaviour of $T_{N,0.5}$ versus k, while comparing Fig. 5 with Fig. 7 we can estimate the influence of the substrate concentration S_0 on the behaviour of $T_{0.5}$ versus k. Since the halftime $T_{0.5}$ does not reach even 0.1 s when the thickness l of the enzyme layer is very thin, results are depicted for $l \geq 0.001$ cm only.

Figures 5–7 show very similar shape of $T_{N,0.5}$ versus k for biosensors, having relatively thick enzyme layer: $l > 0.002$ cm at $V_{max} = 10^{-7}$ and $l > 0.005$ cm at $V_{max} = 10^{-8}$ mol/cm^{3}s. If biosensors are distinctly under diffusion control ($l \gg l_1$), then the halftime $T_{0.5}$ of maximal response increases with increase of k up $k \approx 1.5$, further the halftime $T_{0.5}$ decreases up to $k \approx 2.5$, so that in well stirred solution $T_{0.5}$ is approximately the same as in non-stirred one.

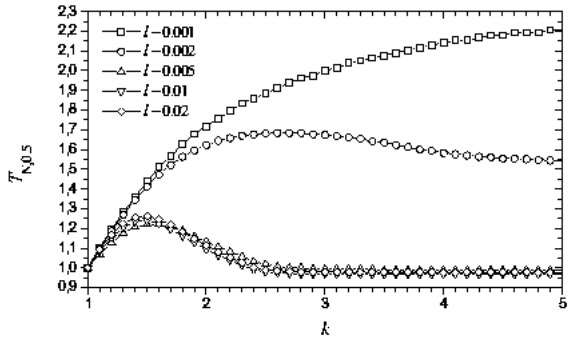

Fig. 7. The normalised halftime $T_{N,0.5}$ of the maximal biosensor response versus ratio $k = L/l$ at different values of l (cm), $V_{max} = 10^{-7}$ mol/cm^{3}s, $S_0 = 2 \times 10^{-7}$ mol/cm^3

As it is possible to notice in Figs. 5–7, if the enzyme kinetics distinctly

predominates ($l \ll l_1$) in biosensors action, then variation of halftime $T_{0.5}$ is much more significant than in the case when the diffusion controls the biosensor action. $T_{0.5}$ can be even more than 3 times greater when biosensor operates in non-stirred solution than in well stirred one. Figures 5–7 show also that the normalised halftime $T_{N,0.5}$ increases with increase of l.

5 Conclusions

The mathematical model (3)–(16) of operation of an amperometric biosensor can be used to investigate regularities of the biosensor response in stirred and non stirred analytes.

The maximal biosensor current is a monotonous decreasing function of dimensionless ratio k of the thickness of entire diffusion domain to the thickness of the enzyme layer if the biosensor response is distinctly under diffusion control. In the case when the enzyme kinetics distinctly predominates the maximal biosensor current increases with increase of the ratio k, i.e., with increase of the thickness of the pure diffusion layer. The intensity of stirring can change the maximal biosensor current up to about 2 times.

A non-monotonous evolution of the halftime of the maximal biosensor current versus the ratio k is observed when the biosensor response is distinctly under diffusion control. If the enzyme kinetics distinctly predominates in the biosensor operation, then the increase of the halftime up to 3 times is observed in numerical experiments while increasing the thickness of the pure diffusion layer (ratio k), i.e., decreasing intensity of stirring.

If the enzyme layer of a biosensor is rather thick, i.e., the biosensor response is distinctly under diffusion control, then it is enough to restrict the entire domain of analysis to $L = 2.5l$ in model (3)–(16) to simulate biosensor action accurately in weak stirred as well as non stirred solution.

References

1. Clarc L.C., Loys C. "Electrode system for continuous monitoring in cardiovascular surgery", *Ann. N.Y. Acad.Sci.*, **102**, p. 29–45, 1962

2. Turner A.P.F., Karube I., Wilson G.S. *Biosensors: Fundamentals and Applications*, Oxford University Press, Oxford, 1987

3. Scheller F., Schubert F. *Biosensors*, Elsevier, Amsterdam, **7**, 1992

4. Chaubey A., Malhotra B.D. "Mediated biosensors", *Biosens. Biolectron.*, **17**, p. 441–456, 2002

5. Calvo E.J., Danilowicz C. "Amperometric enzyme electrodes", *J. Braz. Chem. Soc.*, **8**(6), p. 563–574, 1997.

6. Rogers K.R. "Biosensors for environmental applications", *Biosens. Biolectron.*, **10**, p. 533–541, 1995

7. Mell C.D., Maloy J.T. "A model for the amperometric enzyme electrode obtained through digital simulation and applied to the glucose oxidase system", *Anal. Chem.*, **47**, p. 299–307, 1975

8. Mell C.D., Maloy J.T. "Amperometric response enhancement of the immobilized glucose oxidase enzyme electrode", *Anal. Chem.*, **48**, p. 1597, 1976

9. Kulys J.J., Sorochinski V.V., Vidziunaite R.A. "Transient response of bienzyme electrodes", *Biosensors*, **2**, p. 135–146, 1986

10. Schulmeister T. "Mathematical modelling of the dynamics of amperometric enzyme electrodes", *Selective Electrode Rev.*, **12**, p. 260–303, 1990

11. Sorochinskii V.V., Kurganov B.I. "Steady-state kinetics of cyclic conversions of substrate in amperometric bienzyme sensors", *Biosens. Bioelectron.*, **11**(3), p. 225–238, 1996

12. Yokoyama K., Kayanuma Y. "Cyclic voltammetric simulation for electrochemically mediated enzyme reaction and determination of enzyme kinetic constants", *Anal. Chem.*, **70**(16), p. 3368–3376, 1998

13. Baronas R., Ivanauskas F., Kulys J. "Modelling a biosensor based on the heterogeneous microreactor", *J. Math. Chem.*, **25**(2-3), p. 245–252, 1999

14. Baronas R., Ivanauskas F., Kulys J. "Modelling dynamics of amperometric biosensors in batch and flow injection analysis", *J. Math. Chem.*, **32**(2), p. 225–237, 2002

15. *Crank J. The mathematics of diffusion*, 2nd ed., Clarendon Press, Oxford, 1975

16. Samarskii A. *Difference Scheme Theory*, Nauka, Moskow, 1983 (in Russian)

17. Britz D. *Digital simulation in electrochemistry*, 2nd ed., Springer-Verlag, Berlin, 1988

18. Gueshi T., Tokuda K., Matsuda H. "Voltammetry at partially covered electrodes", *J. Electroanal. Chem.*, **89**, p. 247–260, 1978