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The effect of diffusion limitations on the response of amperometric biosensors with substrate cyclic conversion

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A mathematical model of amperometric biosensors in which chemical amplification by cyclic substrate conversion takes place in a single enzyme membrane has been developed. 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 thicknesses of the enzyme layer and the diffusion region on the biosensor response was investigated. This paper deals with conditions when the mass transport in the exterior region may be neglected to simulate the biosensor response in a well-stirred solution. The digital simulation was carried out using the finite difference technique.

KEY WORDS: reaction–diffusion, modelling, biosensor, amplification

AMS subject classification: 35K57, 65M06, 76R50, 92C45

1. Introduction

Biosensors are analytical devices which convert a biological response into an electrical signal [1–3]. The biosensors yield a signal, which is proportional to the concentration of measured analyte. The biosensors are classified according

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to the nature of the physical transducer. In cases of 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 [4–6]. The detection limit of the biosensors depends upon sensitivity [7–9]. The biosensors, sensitivity can be increased significantly by cyclic conversion of the substrate [10–12].

In the literature, mathematical models have been widely employed to investigate the kinetic peculiarities of the amperometric biosensors including the biosensors with cyclic conversion of the substrate [13–17]. Models coupling the enzyme-catalysed reaction with the diffusion in an enzyme layer (membrane) are usually used [18,19]. In cases when the analyte is assumed to be well stirred and in powerful motion, the mass transport by diffusion outside the enzyme membrane is usually neglected.

The goal of this investigation is to make a mathematical model of amperometric biosensors in which chemical amplification by cyclic substrate conversion takes place in a single enzyme membrane [20]. The model involves three regions: the enzyme layer where enzyme reaction as well as mass transport by diffusion take 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 [15,21,22].

Using computer simulation the influence of the thickness of the enzyme membrane as well the diffusion layer on the biosensor response was investigated. This paper analyses conditions when the mass transport outside the enzyme membrane may be neglected to simulate the biosensor response accurately in a well-stirred solution. The computer simulation was carried out using the finite difference technique [21,23].

2. Mathematical model

A membrane biosensor may be considered as an electrode, having a layer of enzyme applied onto the electrode surface. We consider a scheme of substrate (S) electrochemical conversion to a product (P) following catalysed with enzyme (E) product conversion to substrate [20].

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

Assuming the symmetrical geometry of the electrode and homogeneous distribution of immobilised enzyme in the membrane, the dynamics of the biosensor

can be described by the reaction–diffusion system ($t > 0$)

$$\begin{aligned}
\frac{\partial S_e}{\partial t} &= D_{S_e}\frac{\partial^2 S_e}{\partial x^2} + \frac{V_{\max} P_e}{K_M + P_e}, \\
\frac{\partial P_e}{\partial t} &= D_{P_e}\frac{\partial^2 P_e}{\partial x^2} - \frac{V_{\max} P_e}{K_M + P_e}, \quad x \in (0, d),
\end{aligned} \tag{2}$$

$$\begin{aligned}
\frac{\partial S_b}{\partial t} &= D_{S_b}\frac{\partial^2 S_b}{\partial x^2}, \\
\frac{\partial P_b}{\partial t} &= D_{P_b}\frac{\partial^2 P_b}{\partial x^2}, \quad x \in (d, d + \delta),
\end{aligned} \tag{3}$$

where x and t stand for space and time, respectively, $S_e(x, t)$, $S_b(x, t)$ ($P_e(x, t)$, $P_b(x, t)$) are the substrate (reaction product) concentrations in the enzyme and bulk solution, respectively, d is the thickness of the enzyme membrane, δ is the thickness of the diffusion layer, D_{S_e}, D_{S_b}, D_{P_e}, D_{P_b} are the diffusion coefficients, $V_{\max}$ is the steady-state enzymatic rate and K_M is Michaelis constant.

Let $x = 0$ represent the electrode surface, while $x = d$ represents the boundary layer between the analysed solution and enzyme membrane. The operation of the biosensor starts when the substrate appears over the surface of the enzyme membrane. This is used in the initial conditions ($t = 0$)

$$\begin{aligned}
&S_e(x, 0) = 0, \quad P_e(x, 0) = 0, \quad x \in [0, d], \\
&S_e(d, 0) = S_0, \quad P_e(d, 0) = 0, \\
&S_b(x, 0) = S_0, \quad P_b(x, 0) = 0, \quad x \in [d, d + \delta],
\end{aligned} \tag{4}$$

where S_0 is the concentration of the substrate to be analysed.

In the scheme (1) the substrate is electro-active substance. The electrode potential is chosen to keep zero concentration of the substrate at the electrode surface. During the electrochemical conversion the product is generated. The rate of the product generation at the electrode is proportional to the rate of conversion of the substrate. Consequently, the boundary and matching conditions are ($t > 0$)

$$\begin{aligned}
&S_e(0, t) = 0, \quad S_e(d, t) = S_b(d, t), \quad S_b(d + \delta, t) = S_0, \\
&D_{P_e}\frac{\partial P_e}{\partial x}\bigg|_{x=0} = -D_{S_e}\frac{\partial S_e}{\partial x}\bigg|_{x=0}, \\
&P_e(d, t) = P_b(d, t), \quad P_b(d + \delta, t) = 0,
\end{aligned} \tag{5}$$

$$D_{S_e}\frac{\partial S_e}{\partial x}\Big|_{x=d} = D_{S_b}\frac{\partial S_b}{\partial x}\Big|_{x=d},$$
$$D_{P_e}\frac{\partial P_e}{\partial x}\Big|_{x=d} = D_{P_b}\frac{\partial P_b}{\partial x}\Big|_{x=d}. \tag{6}$$

The concentration S of the substrate S and the concentration P of the reaction product P can be defined in entire domain $x \in [0, d+\delta]$ as follows ($t \geq 0$):

$$S(x,t) = \begin{cases} S_e(x,t), & x \in [0,d], \\ S_b(x,t), & x \in (d, d+\delta], \end{cases}$$
$$P(x,t) = \begin{cases} P_e(x,t), & x \in [0,d], \\ P_b(x,t), & x \in (d, d+\delta]. \end{cases} \tag{7}$$

Both concentration functions: S and P are continuous in the entire domain $x \in [0, d+\delta]$.

The measured current is accepted as a response of a biosensor in a physical experiment. The current depends upon the flux of the electro-active substance (substrate) at the electrode surface, i.e. at the border $x = 0$. Consequently, a density $I(t)$ of the biosensor current at time t can be obtained explicitly from Faraday's law and Fick's law

$$I(t) = n_e \mathrm{F}\ \mathrm{D}_{S_e}\frac{\partial S_e}{\partial x}\Big|_{x=0} = -n_e \mathrm{F}\ \mathrm{D}_{P_e}\frac{\partial P_e}{\partial x}\Big|_{x=0}, \tag{8}$$

where n_e is the number of electrons involved in a charge transfer, F is Faraday constant, $F \approx 9.65 \times 10^4$ C/mol.

We assume, that the system (3)–(6) approaches a steady-state as $t \to \infty$

$$I_\infty = \lim_{t\to\infty} I(t). \tag{9}$$

I_∞ is assumed as the steady-state biosensor current.

3. Computer simulation

The problem (3)–(6) was solved numerically using the finite difference technique [21,23]. To simulate the biosensor action for $t \in [0, T]$ we introduce a uniform discrete grid $\omega_h \times \omega_\tau$, where

$$\omega_h = \{x_i : x_i = ih, i = 0, \ldots, N_d, \ldots, N;\ hN_d = d,\ hN = d+\delta\},$$
$$\omega_\tau = \{t_j : t_j = j\tau, j = 0, \ldots, M;\ \tau M = T\}. \tag{10}$$

We assume the following

$$S_i^j = S(x_i, t_j),\ P_i^j = P(x_i, t_j),\ I_j = I(t_j), \quad i = 0, \ldots, N;\ j = 0, \ldots, M. \tag{11}$$

We use an implicit difference scheme where the differential equations (3) and (4) are replaced with the following difference equations:

$$\begin{aligned}
&\frac{S_i^{j+1} - S_i^j}{\tau} = D_{S_e} \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} + \frac{V_{\max} P_i^j}{K_M + P_i^j}, \\
&\frac{P_i^{j+1} - P_i^j}{\tau} = D_{P_e} \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} - \frac{V_{\max} P_i^j}{K_M + P_i^j}, \\
&i = 1, \ldots, N_d - 1, \quad j = 1, \ldots, M,
\end{aligned} \tag{12}$$

$$\begin{aligned}
&\frac{S_i^{j+1} - S_i^j}{\tau} = D_{S_b} \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2}, \\
&\frac{P_i^{j+1} - P_i^j}{\tau} = D_{P_b} \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2}, \\
&i = N_d + 1, \ldots, N - 1, \quad j = 1, \ldots, M.
\end{aligned} \tag{13}$$

The initial conditions (4) are approximated by

$$\begin{aligned}
S_i^0 &= 0, \quad i = 0, \ldots, N_d - 1, \\
S_i^0 &= S_0, \quad i = N_d, \ldots, N, \\
P_i^0 &= 0, \quad i = 0, \ldots, N.
\end{aligned} \tag{14}$$

The boundary and matching conditions (5) and (6) are approximated as follows:

$$\begin{aligned}
&S_0^j = 0, \quad S_N^j = S_0, \\
&D_{S_e}(S_{N_d}^j - S_{N_d-1}^j) = D_{S_b}(S_{N_d+1}^j - S_{N_d}^j), \quad j = 1, \ldots, M.
\end{aligned} \tag{15}$$

$$\begin{aligned}
&D_{P_e}(P_1^j - P_0^j) = -D_{S_e}(S_1^j - S_0^j), \\
&D_{P_e}(P_{N_d}^j - P_{N_d-1}^j) = D_{P_b}(P_{N_d+1}^j - P_{N_d}^j),\ P_N^j = 0, \quad j = 1, \ldots, M.
\end{aligned} \tag{16}$$

The resulting systems of linear algebraic equations are solved efficiently because of the tridiagonality of their matrices.

Having a numerical solution of the problem, the density of the biosensor current at time $t = t_j$ can be calculated easily by

$$I(t_j) = n_e F D_{S_e}(S_1^j - S_0^j)/h, \quad j = 0, \ldots, M. \tag{17}$$

In digital simulation, the biosensor response time $T = T_R$ 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

$$T_R = \min_{I(t_j)>0} \left\{ t_j : \frac{1}{I(t_j)} \frac{|I(t_j) - I(t_{j-1})|}{\tau} < \epsilon\,,\ j = 1, \dots \right\} \tag{18}$$

needed to achieve a given dimensionless decay rate ϵ is used. In calculation, we employ $\epsilon = 10^{-5}$. The current $I(T_R)$ was assumed as an approximation of the steady-state current I_∞, $I_\infty \approx I(T_R)$.

The digital simulator has been programmed in C language [24].

4. Results and discussion

Using numerical simulation, the influence of the thickness of both layers: the enzyme and diffusion on the steady-state current was investigated. The following values of the parameters were constant in the simulation of all the experiments discussed below:

$$\begin{aligned} &D_{S_e} = D_{P_e} = 3.0 \times 10^{-6}\,\mathrm{cm^2/s}, \\ &D_{S_b} = 2D_{S_e}, \quad D_{P_b} = 2D_{P_e}, \\ &K_M = 10^{-7}\,\mathrm{mol/cm^3}, \quad S_0 = 2 \times 10^{-8}\mathrm{mol/cm^3}, \quad n_e = 2. \end{aligned} \tag{19}$$

The thickness d of the enzyme membrane of a biosensor can usually be measured physically rather precisely. The thickness δ of the diffusion layer depends upon the stirring of the buffer solution. The thickness δ is inversely proportional to the intensity of the stirring (rotation speed of the electrode). The more intensive the stirring is, the thinner the diffusion layer is. No exact analytical expression of δ is available for stirred solutions. δ can be estimated experimentally by measuring the electrode response at a given bulk concentration. Furthermore, δ depends upon the type of stirring.

4.1. The dynamics of the biosensor current

We calculate the biosensor current at an ordinary thickness $d = 0.01\,\mathrm{cm}$ of the biosensor membrane. The biosensor response was simulated at different thickness δ of the diffusion layer to simulate the biosensor action at different conditions of analyte stirring. Figure 1 shows the biosensor current at the steady-state enzymatic rate $V_{\max} = 10^{-8}\,\mathrm{mol/cm^3 s}$.

One can see in figure 1 that the steady-state current decreases slightly with increase of the thickness δ of the diffusion layer. The time of the steady-state current significantly increases with increase of δ.

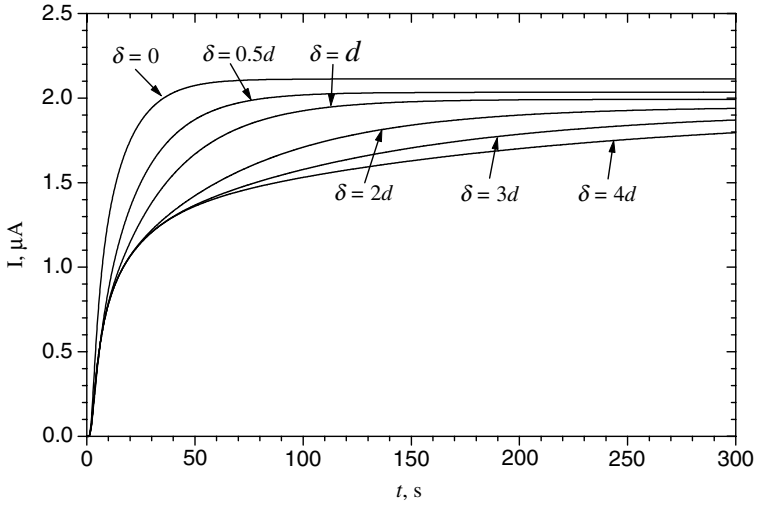

Figure 1. The dynamics of the biosensor current I at the membrane thickness $d = 0.01\,\text{cm}$ and different thickness δ of the diffusion layer, $V_{\max} = 10^{-8}\,\text{mol/cm}^3\text{s}$.

Four parameters: $V_{\max}, K_M, d$ and D_{S_e} are among the parameters significantly influencing the behaviour of biosensors [13,19]. The biosensor response is known to be under mass transport control if the enzymatic reaction in the enzyme layer is faster than the transport process. The diffusion modulus (Damköhler number) σ^2 essentially compares the rate of enzyme reaction $(V_{\max}/K_M)$ with the diffusion through the enzyme layer (D_{S_e}/d^2)

$$\sigma^2 = \frac{V_{\max} d^2}{D_{S_e} K_M}. \tag{20}$$

If $\sigma^2 < 1$, the enzyme kinetics controls the biosensor response. The response is under diffusion control when $\sigma^2 > 1$. At values of D_{S_e} and K_M given in (19), $d = 0.01\,\text{cm}$, and $V_{\max} = 10^{-8}\,\text{mol/cm}^3\text{s}$ the diffusion modulus σ^2 equals approximately 3.3. Consequently, figure 1 shows the biosensor behaviour in a case when the response is under diffusion control.

We calculate the biosensor current at 10 times thinner membrane $d = 0.001\,\text{cm}$ and the same $V_{\max} = 10^{-8}\,\text{mol/cm}^3\text{s}$ as above, $\sigma^2 \approx 0.33 < 1$, to investigate the dynamics of the current in a case when the enzyme kinetics controls the biosensor response. Results of calculations are presented in figure 2. One can see in figure 2 that the steady-state current decreases with increase of the thickness δ. This decrease is more significant than in the previous case of $d = 0.01\,\text{cm}$ when the biosensor response is under diffusion control. In the case when the enzyme kinetics controls the biosensor response (figure 2), the time of the steady-state current increases with increase of δ similar to the thicker

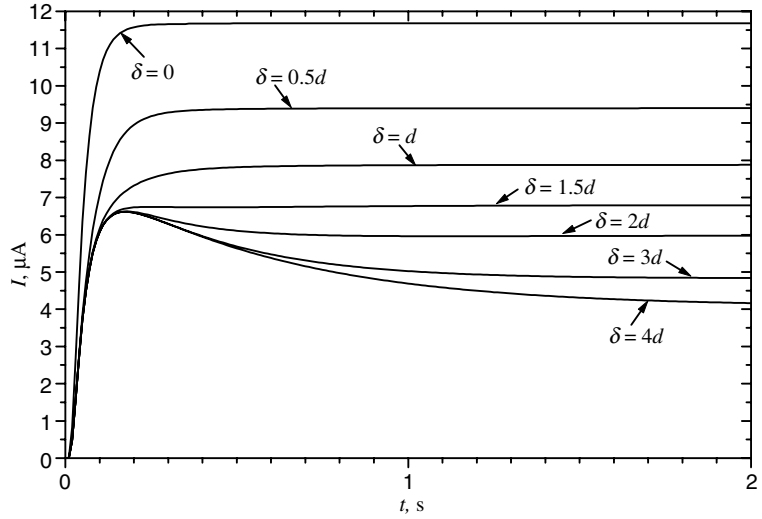

Figure 2. The dynamics of the biosensor current I at the membrane thickness $d = 0.001$ cm and different thickness δ of the diffusion layer, $V_{max} = 10^{-8}$ mol/cm^{3}s.

membrane ($d = 0.01$ cm). Figure 2 shows that the biosensor current is a non-monotonous function of time when the thickness of the diffusion layer exceeds greatly the thickness of the enzyme membrane ($\delta > 1.5d$). In the beginning of the biosensor action, the current increases, while later it starts to decrease. We notice that the biosensor current was distinctly a monotonous function of time in the previous case when the biosensor response is under diffusion control (figure 1). The non-monotonicity of the biosensor current has not been observed also in cases of amperometric biosensors without substrate cyclic conversion at similar conditions [22].

One can see in figure 2 that in the cases when $\delta > 1.5d$, the steady-state current is notably less (about 43%) than in the case when the diffusion layer is neglected, $\delta = 0$. No notable difference is observed between the steady-state currents in all cases $\delta > 1.5d$. Similar effect was also observed in the case of amperometric biosensors acting without substrate cyclic conversion [22].

4.2. The effect of the thickness of diffusion layer on the biosensor response

We investigate the dependence of the steady-state biosensor current on the relative thickness of the diffusion layer. We consider a dimensionless ratio k of the thickness δ of the diffusion layer to the thickness d of the enzyme layer, $k = \delta/d$, $k \geqslant 0$, as the relative thickness of the diffusion layer.

The steady-state current I_∞ is very sensitive to the thickness of the enzyme layer. I_∞ varies even in orders of magnitude [25]. Because of this we normalise

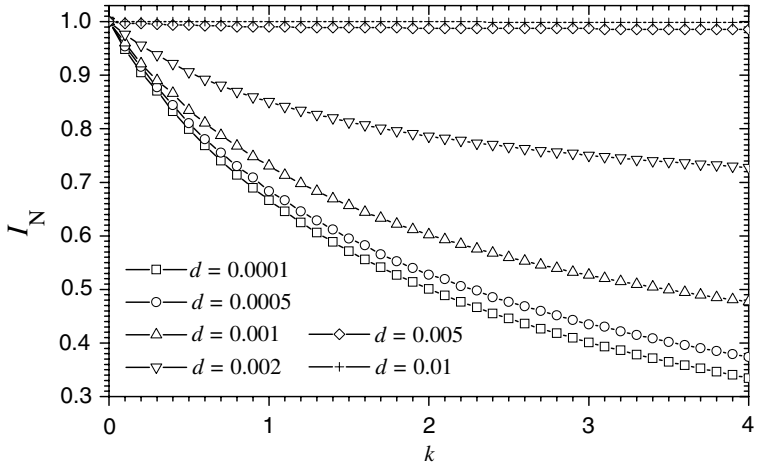

Figure 3. The normalised steady-state biosensor current I_N versus ratio $k = \delta/d$ at $V_{\max} = 10^{-7}$ mol/cm^{3}s and different values of the membrane thickness d (cm).

the steady-state biosensor current to evaluate the effect of the ratio k on the biosensor response. The normalised steady-state biosensor current I_N is expressed by the steady-state current at the thickness δ of the diffusion layer divided by the steady-state current assuming the zero thickness of the diffusion layer

$$I_N(d, \delta) = \frac{I_\infty(d, \delta)}{I_\infty(d, 0)}, \quad d > 0, \ \delta \geqslant 0, \tag{21}$$

where $I_\infty(d, \delta)$ is the steady-state current (see equations (8) and (9)) calculated at given thickness d of the membrane and thickness δ of the diffusion layer.

The biosensor response versus the dimensionless ratio $k = \delta/d$ was investigated at different steady-state enzymatic rates $V_{\max}$ and the membrane thickness d. Results obtained at $V_{\max} = 10^{-7}$ mol/cm^{3}s are depicted in figure 3.

One can see in figure 3 that the steady-state biosensor current notably decreases with increase of the ratio k in cases when the enzyme membrane thickness d is equal to 0.002 cm or less. That decrease is not linear. In cases of relatively thick enzyme membranes ($d \geqslant 0.005$ cm), the influence of the thickness δ of the diffusion layer on the biosensor response is not great. In the case of $d = 0.005$ cm the steady-state current decreases less than 1.5% only, while in the case of $d = 0.0005$ cm it decreases even more than 2.5 times, $I_N \approx 0.37$, when k changes from 0 to 4. Consequently, the mass transport by the diffusion outside the enzyme membrane may be neglected only in the cases of relatively thick membranes.

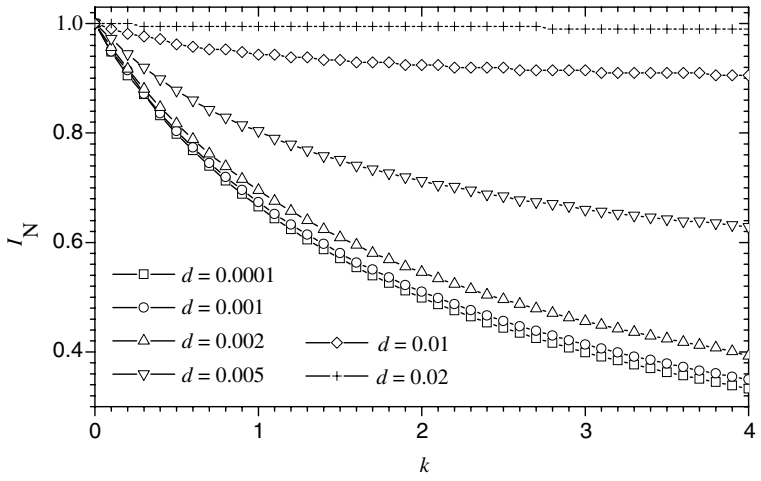

Figure 4. The normalised steady-state biosensor current I_N versus ratio $k = \delta/d$ at $V_{\max} = 10^{-8}$ mol/cm^{3}s and different values of the membrane thickness d (cm).

D_{S_e} and K_M are constant in all our numerical experiments as defined in (19). We express the membrane thickness d_1 through $V_{\max}$ at $\sigma = 1$

$$d_1(V_{\max}) = \sigma \times \sqrt{\frac{D_{S_e} K_M}{V_{\max}}} = \sqrt{\frac{3 \times 10^{-13}}{V_{\max}}}. \tag{22}$$

Comparing the membrane thickness d of 0.002 cm with $d_1(10^{-7}) \approx 0.0017$ cm at which the diffusion modulus σ equals unity for $V_{\max} = 10^{-7}$ mol/cm^{3}s, we notice that the behaviour of the steady-state current favourably depends on that either the enzyme kinetics or the diffusion controls the response.

Figure 3 shows that in cases when the enzyme kinetics distinctly controls the biosensor response ($d \ll d_1$, $\sigma^2 \ll 1$), the steady-state current significantly decreases with increase of the relative thickness of the diffusion layer, i.e. with increase of the ratio k. In cases when the response is distinctly under diffusion control ($d \gg d_1$, $\sigma^2 \gg 1$), variation of the ratio k practically does not effect the steady-state current. Consequently, in cases of relative thick biosensors, the response practically does not depend on the intensity of stirring of the buffer solution. To be sure that these properties are valid at wide range of the steady-state enzymatic rate $V_{\max}$, we calculate the biosensor response also at $V_{\max} = 10^{-8}$ mol/cm^{3}s and the same values of the ratio k as well as membrane thickness d. The results of the calculations are depicted in figure 4.

Since $d_1(10^{-8}) \approx 0.0055$ cm is considerably greater than $d_1(10^{-7})$, the minimal membrane thickness, at which the thickness of the diffusion layer starts to make no notable effect on the biosensor response, is greater at $V_{\max} = 10^{-8}$ mol/cm^{3}s (figure 4) rather than at $V_{\max} = 10^{-7}$ mol/cm^{3}s (figure 3). At the membrane thickness d of 0.005 cm the steady-state current decreases about 1.5 times

($I_N \approx 0.63$) at $V_{max} = 10^{-8}$ mol/cm^{3}s when k changes from 0 to 4. The corresponding decrease is less than 1.5% in the case of $V_{max} = 10^{-7}$ (figure 3). In a case of thicker membrane $d = 0.02$ cm ($\sigma^2 \approx 1$) (figure 4), the steady-state current decreases only about 1.0% when k changes from 0 to 4 and $V_{max} = 10^{-8}$ mol/cm^{3}s. Consequently, in cases of relative thick biosensors ($d \gg d_1$, $\sigma^2 \gg 1$), the biosensor response practically does not depend on the intensity of stirring of the buffer solution at a wide range of the steady-state enzymatic rate V_{max}.

4.3. The importance of the Nernst diffusion layer when solution is well stirred

The thickness δ of the diffusion layer depends upon the nature and stirring of the buffer solution. Usually, the more intensive stirring corresponds to the thinner diffusion layer. That diffusion layer is known as the Nernst diffusion layer [26]. The thickness of the Nernst diffusion layer practically does not depend upon the membrane thickness. In practice, the zero thickness of the Nernst layer can not be achieved. In a case, when the solution to be analysed is stirred by rotation of the enzyme electrode, the thickness of the Nernst diffusion layer may be minimized up to $\delta = 0.0002$ cm by increasing the rotation speed [27]. However, in an another frequently used case, when the solution is stirred in a magnetic stirrer, it is difficult to achieve the thickness δ less than about 0.002 cm.

In the cases when an analyte is well stirred and in powerful motion, the mass transport by diffusion outside the enzyme membrane rather often is neglected [14,21]. We assume that a biosensor model, taking into consideration the Nernst diffusion layer, describes the biosensor action more precisely than an another model where the Nernst diffusion layer is neglected. In addition, we assume that the Nernst diffusion layer of thickness δ may be neglected for a biosensor having membrane thickness d only if the steady-state current calculated considering the Nernst layer is approximately the same as in the case when the Nernst diffusion layer is neglected. Consequently, the Nernst diffusion layer may be neglected if $I_\infty(d, \delta) \approx I_\infty(d, 0)$, i.e. $I_N(d, \delta) \approx 1$.

We investigate the conditions when the Nernst diffusion layer may be neglected to simulate accurately the response of biosensors. To investigate the effect of the Nernst diffusion layer on the biosensor response when the analyte is well stirred and in powerful motion we calculate the normalised steady-state current $I_N(d, \delta)$ at practically minimal thickness of the diffusion layer characteristic for both types of stirring: by electrode rotation and in a magnetic stirrer. Since the effect of the diffusion layer on the biosensor response depends upon the membrane thickness [22], we calculate the normalised current changing the membrane thickness d from 10^{-5} to 4×10^{-2} cm.

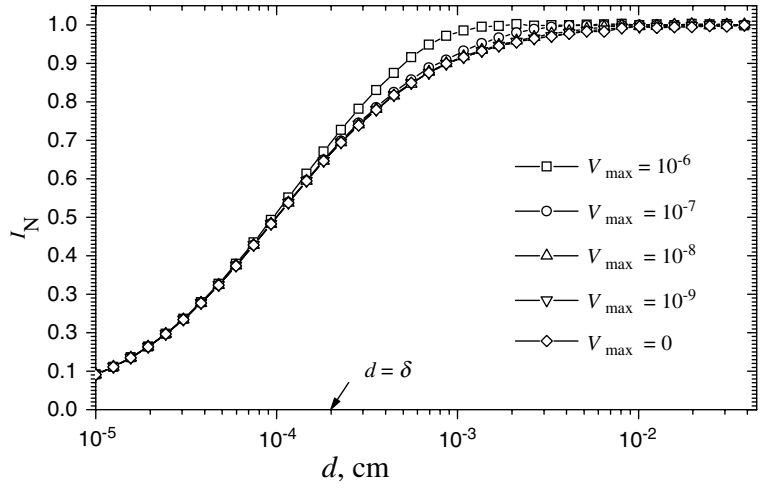

Figure 5. The normalised steady-state biosensor current I_N versus the enzyme membrane thickness d at the thickness $\delta = 0.0002$ cm of the Nernst diffusion layer and different steady-state enzymatic rates V_{max} (mol/cm^{3}s).

Figure 5 shows the results of calculations at the thickness $\delta = 0.0002$ cm while figure 6 shows the results at 10 times thicker ($\delta = 0.002$ cm) than the Nernst diffusion layer.

One can see in figure 5, the effect of the Nernst layer decreases with increase of the membrane thickness d. The Nernst diffusion layer of the thickness of 0.0002 cm should be taken into consideration in all the cases when the enzyme membrane is thinner than about 0.002 cm. Figure 5 shows that the simulated steady state current I_∞ may be even more than 10 times greater ($I_N < 0.1$) than the true current if the Nernst diffusion layer is neglected in cases of thin enzyme membranes, $d \leq 10^{-5}$ cm, when an analyte is well stirred and in powerful motion. The effect of the Nernst diffusion layer becomes slight only in the cases when the membrane is more than 10 times thicker than the diffusion layer, $d > 10\delta = 0.002$ cm. Assuming high speed rotation of the electrode ($\delta = 0.0002$ cm), inactive enzyme ($V_{max} = 0$) and the membrane thickness $d = 10\delta$, the normalised current I_N equals approximately 0.95, i.e. the steady-state current ($I_\infty(0.002, 0.0002)$) in the case when the Nernst diffusion layer is taken into consideration is about 5% less than the steady-state current ($I_\infty(0.002, 0)$) in the case when the Nernst diffusion layer is neglected.

As it is possible to notice in both figures 5 and 6, I_N increases with increase of the membrane thickness d. On the other hand, I_N is less at less steady-state rate V_{max} rather than at higher V_{max}. Consequently, the influence of the Nernst diffusion layer on the biosensor response grows with decrease of the steady-state enzymatic rate. Figures 5 and 6 show, that the effect of the steady-state enzymatic rate V_{max} on the behaviour of I_N as well as I_∞ is very small in cases of

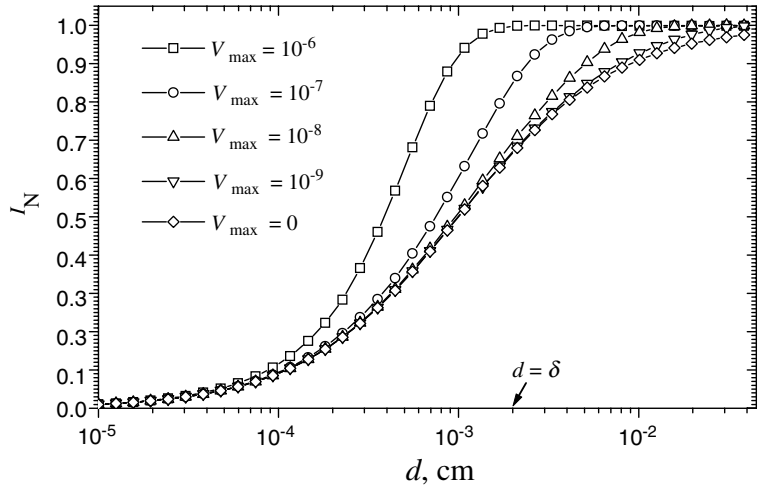

Figure 6. The normalised steady-state biosensor current I_N versus the enzyme membrane thickness d at the thickness $\delta = 0.0002$ cm of the Nernst diffusion layer and different steady-state enzymatic rates V_{max} (mol/cm^{3}s).

thin enzyme membranes, $d < 10^{-4}$ cm. The most significant effect of V_{max} on I_N is notable at the membrane thickness $d \approx 10^{-3}$ for both thickness of the Nernst layer: 0.0002 cm (figure 5) and 0.002 cm (figure 6). However, in the case of the thin Nernst diffusion layer ($\delta = 0.0002$ cm, figure 5) the effect of the steady-state enzymatic rate V_{max} is rather slight in an entire domain of the membrane thickness $d \in [10^{-5}, 4 \times 10^{-2}]$.

Figure 6 shows that the Nernst diffusion layer of the thickness δ of 0.002 cm should be taken into consideration in all the cases when the enzyme membrane is thinner than about 0.02 cm, i.e. $d < 10\delta$.

5. Conclusions

The mathematical model (3)–(6) of operation of amperometric biosensors with substrate cyclic conversion can be used to investigate regularities of the biosensor response in stirred and non-stirred analytes.

In the cases when the thickness δ of the diffusion layer is more than half as great as the thickness d of the enzyme membrane ($\delta > 1.5d$) and the enzyme kinetics distinctly controls the biosensor response (diffusion modulus σ^2 significantly less than unity), the biosensor current is a non-monotonous function of time (figure 2). Otherwise, the biosensor current is a monotonous increasing function of time (figures 1 and 2). In all the cases a steady-state is achieved.

The steady-state current is a monotonous decreasing function of the ratio k of the thickness of the diffusion layer to the thickness of the enzyme membrane

(figures 3 and 4). In particular cases when the biosensor response is distinctly under diffusion control ($\sigma^2 \gg 1$), variation of k practically does not effect the steady-state current. Consequently, in the cases when $\sigma^2 \gg 1$, the biosensor response practically does not depend upon the intensity of stirring of the buffer solution (upon rotation speed of the electrode).

In the cases of low enzymatic activity ($V_{max} \to 0$) the Nernst diffusion layer of the thickness $\delta > 0$ should be taken into consideration if the enzyme membrane is thinner than about 10δ, i.e. $d < 10\delta$ (figures 5 and 6). Increase of V_{max} allows to neglect the Nernst diffusion layer at thinner enzyme membranes than 10δ.

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