

COMPUTATIONAL MODELLING OF MEMBRANE BIOSENSORS ACTING IN STIRRED AND NON-STIRRED SOLUTIONS

Romas Baronas* and Feliksas Ivanauskas^{†,*}

*Vilnius University, Faculty of Mathematics and Informatics
Naugarduko 24, LT-03225 Vilnius, Lithuania
e-mail: romas.baronas@maf.vu.lt
web page: <http://www.maf.vu.lt/~baronas/>
[†]Institute of Mathematics and Informatics
Akademijos 4, LT-08663 Vilnius, Lithuania
e-mail: feliksas.ivanauskas@maf.vu.lt

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Abstract. *This paper presents mathematical models of potentiometric as well as amperometric biosensors, based on an electrode covered with an enzyme membrane. The models involve 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 both the enzyme and the diffusion layers on the biosensor response was investigated. The effect of the diffusion layer on the biosensor response was evaluated for different conditions of the enzymatic reaction and types of mixers. This paper deals also with the conditions when the mass transport in the exterior diffusion region may be neglected to simulate the biosensor response assuming the buffer solution is well-stirred and in powerful motion. The digital simulation was carried out using the finite difference technique.*

1 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. The biosensors signal is proportional to the concentration of measured analyte or a group of analytes. [1, 2, 3].

In potentiometric biosensors, the analytical information is obtained by converting the recognition process into a potential, which is proportional (in a logarithmic fashion) to the concentration of the reaction product [4]. The amperometric biosensors measures the faradic current that arises on the electrode by direct electrochemical oxidation or

reduction of the product. These devices have been widely used in environmental, medical and industrial applications because of their high selectivity, simplicity and low cost [5, 6].

Since it is not generally possible to measure the concentration of substrate inside enzyme domain with analytical devices, starting from seventies various mathematical models of biosensors have been developed and used as an important tool to study and optimise analytical characteristics of actual biosensors [7, 8, 9].

The goal of this investigation is to make models allowing an effective computer simulation of potentiometric and amperometric biosensors acting in stirred as well as non stirred analytes. The developed models are based on the reaction-diffusion equations, containing a non-linear term related to Michaelis-Menten kinetics of the enzymatic reaction [10, 11, 12]. The models 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 [8, 13, 14]. 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 enzyme layer. The behaviour of the potentiometric and amperometric biosensors was compared together. The digital simulation of the biosensor response was carried out using the finite difference technique [15].

2 MATHEMATICAL MODEL

We consider a scheme of catalysed with enzyme (E) substrate (S) conversion to a product (P) [1, 2],



A biosensor may be considered as an electrode, having a layer of enzyme (enzyme membrane) applied onto the electrode surface. Assuming the symmetrical geometry of the electrode and homogeneous distribution of the immobilised enzyme in the enzyme membrane, the biosensor action can be described by the reaction-diffusion system ($t > 0$) [8, 13, 14]:

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

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

where x stands for space, t stands for time, $S_e(x, t)$, $S_b(x, t)$ ($P_e(x, t)$, $P_b(x, t)$) are the substrate (reaction product) concentrations, in the enzyme membrane and buffer 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 maximal enzymatic rate and K_M is the Michaelis constant.

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

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

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

On the boundary between two subregions having different diffusion coefficients we define the matching conditions ($t > 0$),

$$\begin{aligned} D_{S_e} \frac{\partial S_e}{\partial x} \Big|_{x=d} &= D_{S_b} \frac{\partial S_b}{\partial x} \Big|_{x=d}, & S_e(d, t) &= S_b(d, t), \\ D_{P_e} \frac{\partial P_e}{\partial x} \Big|_{x=d} &= D_{P_b} \frac{\partial P_b}{\partial x} \Big|_{x=d}, & P_e(d, t) &= P_b(d, t). \end{aligned} \quad (5)$$

In the bulk solution the concentration of the substrate as well as of the product remains constant ($t > 0$),

$$S_b(d + \delta, t) = S_0, \quad P_b(d + \delta, t) = 0. \quad (6)$$

At the electrode surface ($x = 0$), the boundary conditions depend on the electric activity of the substance. Following the scheme (1) the substrate (S) is electro-inactive,

$$D_{S_e} \frac{\partial S_e}{\partial x} \Big|_{x=0} = 0, \quad t > 0. \quad (7)$$

The reaction product (P) is electro-active substance. The boundary condition for the electro-active substance depends on a type of the electrode. We investigate enzyme electrodes of two types: potentiometric and amperometric.

In the case of potentiometry, the change of the potential is caused by change of the reaction product concentration,

$$D_{P_e} \frac{\partial P_e}{\partial x} \Big|_{x=0} = 0, \quad t > 0. \quad (8)$$

In the case of amperometry, the potential at the electrode is chosen to keep zero concentration of the product,

$$P_e(0, t) = 0, \quad t > 0. \quad (9)$$

The diffusion layer $\{x : x \in (d, d + \delta)\}$ may be treated as a Nernst diffusion layer, which is widely used in modelling of the electrochemical processes [16, 17]. According to the Nernst approach, a layer of thickness δ (the Nernst diffusion layer) remains unchanged with time. Away from it the solution is in motion and uniform in concentration.

3 BIOSENSOR RESPONSE

The measured potential is accepted as a response of potentiometric biosensors. The potential of the biosensor is given by

$$E(t) = E_0 + \frac{R_c T_K}{zF} \ln P_e(0, t), \quad t > 0, \quad (10)$$

where $E(t)$ is the measured potential (in volts) at time t , E_0 is a characteristic constant for the ion-selective electrode, R_c is the universal gas constant, $R_c = 8.314$ J/mol K, T_K is the absolute temperature (K), z is the signed ionic charge, F is the Faraday constant, $F = 9648$ C/mol [18, 19].

We assume, that the system (2)-(8) approaches a steady-state as $t \rightarrow \infty$,

$$E_R = \lim_{t \rightarrow \infty} E(t). \quad (11)$$

E_R is assumed as the steady-state biosensor potential.

In the case of amperometric biosensors, the current depends upon the flux of the electro-active analyte (product) at the electrode surface. A density $I(t)$ of the biosensor current at time t can be obtained explicitly from Faraday's and Fick's laws [8, 20],

$$I(t) = n_e F D_{P_e} \left. \frac{\partial P_e}{\partial x} \right|_{x=0}, \quad (12)$$

where n_e is a number of electrons involved in a charge transfer at the electrode surface, F is Faraday constant.

We assume, that the system (2)-(7),(9) approaches an equilibrium or steady-state when $t \rightarrow \infty$,

$$I_R = \lim_{t \rightarrow \infty} I(t), \quad (13)$$

where I_R is the steady-state biosensor current.

Four parameters: V_{max} , K_M , d and D_{S_e} are among the most important parameters determining the behaviour of the biosensor response [8, 11, 20]. 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 dimensionless 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),

$$\sigma^2 = \frac{V_{max} d^2}{D_{S_e} K_M}. \quad (14)$$

If $\sigma^2 \ll 1$, the enzyme kinetics controls the biosensor response. The response is under diffusion control when $\sigma^2 \gg 1$.

4 SOLUTION OF THE PROBLEM

The problems (2)-(8) and (2)-(7),(9) were solved numerically using the finite difference technique [15, 16]. To simulate the biosensor action for $t \in [0, T]$ we introduced an uniform discrete grid $\omega_h \times \omega_\tau$, where

$$\begin{aligned}\omega_h &= \{x_i : x_i = ih, i = 0, \dots, N_d, \dots, N; hN_d = d, hN = d + \delta\}, \\ \omega_\tau &= \{t_j : t_j = j\tau, j = 0, \dots, M; \tau M = T\}.\end{aligned}\tag{15}$$

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 ($t \geq 0$):

$$\begin{aligned}S(x, t) &= \begin{cases} S_e(x, t), & x \in [0, d], \\ S_b(x, t), & x \in (d, d + \delta], \end{cases} \\ P(x, t) &= \begin{cases} P_e(x, t), & x \in [0, d], \\ P_b(x, t), & x \in (d, d + \delta]. \end{cases}\end{aligned}\tag{16}$$

We assume the following:

$$\begin{aligned}S_i^j &= S(x_i, t_j), \quad P_i^j = P(x_i, t_j), \quad E^j = E(t_j), \quad I^j = I(t_j), \\ i &= 0, \dots, N, \quad j = 0, \dots, M.\end{aligned}\tag{17}$$

We use an implicit difference scheme where the differential equations (2),(3) are replaced with the following difference equations:

$$\begin{aligned}\frac{S_i^{j+1} - S_i^j}{\tau} &= D_{S_e} \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} - \frac{V_{max} S_i^{j+1}}{K_M + S_i^j}, \\ \frac{P_i^{j+1} - P_i^j}{\tau} &= D_{P_e} \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} + \frac{V_{max} S_i^{j+1}}{K_M + S_i^{j+1}},\end{aligned}\tag{18}$$

$$i = 1, \dots, N_d - 1, \quad j = 1, \dots, M,$$

$$\begin{aligned}\frac{S_i^{j+1} - S_i^j}{\tau} &= D_{S_b} \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2}, \\ \frac{P_i^{j+1} - P_i^j}{\tau} &= D_{P_b} \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2},\end{aligned}\tag{19}$$

$$i = N_d + 1, \dots, N - 1, \quad j = 1, \dots, M.$$

The initial conditions (4) are approximated by

$$\begin{aligned}S_i^0 &= 0, \quad i = 0, \dots, N_d - 1, \\ S_i^0 &= S_0, \quad i = N_d, \dots, N, \\ P_i^0 &= 0, \quad i = 0, \dots, N.\end{aligned}\tag{20}$$

The matching and boundary conditions (5)-(7) are approximated as follows:

$$\begin{aligned}D_{S_e}(S_{N_d}^j - S_{N_d-1}^j) &= D_{S_b}(S_{N_d+1}^j - S_{N_d}^j), \\ D_{P_e}(P_{N_d}^j - P_{N_d-1}^j) &= D_{P_b}(P_{N_d+1}^j - P_{N_d}^j), \\ S_N^j &= S_0, \quad P_N^j = 0, \quad S_0^j = S_1^j, \\ j &= 1, \dots, M.\end{aligned}\tag{21}$$

In the case of potentiometric biosensor, the finite difference equations (18)-(21) are followed by the approximation of (8),

$$P_0^j = P_1^j, \quad j = 1, \dots, M. \quad (22)$$

While in the case of amperometry biosensor, the equations (18)-(21) are followed by the approximation of (9),

$$P_0^j = 0, \quad j = 1, \dots, M. \quad (23)$$

The systems of linear algebraic equations were solved efficiently because of the tridiagonality of the matrices of the systems.

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

$$E^j = E_0 + \frac{R_c T_K}{zF} \ln(P_0^j), \quad j = 1, \dots, M. \quad (24)$$

The density of the biosensor current at time $t = t_j$ is calculated as follows:

$$I^j = n_e F D_{S_e} (S_1^j - S_0^j) / h, \quad j = 1, \dots, M. \quad (25)$$

5 NUMERICAL SIMULATION

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

$$\begin{aligned} D_{S_e} = D_{P_e} = 300 \mu\text{m}^2/\text{s}, \quad D_{S_b} = 2D_{S_e}, \quad D_{P_b} = 2D_{P_e}, \\ K_M = 100 \mu\text{M}, \quad E_0 = 0 \text{ V}, \quad z = 1, \quad n_e = 2, \quad T_K = 298 \text{ K}. \end{aligned} \quad (26)$$

The steady-state biosensor response as well as the time moment of occurrence of the steady-state response time were assumed and analysed as ones of the most important characteristics of the biosensors.

In digital simulation, the biosensor response time $T = T_R$ was assumed as the time when the normalised absolute response (potential or current) slope value falls below a given small value ϵ ,

$$T_R = \min_{P_0^j > 0} \left\{ t_j : \left| \frac{E^j - E^{j-1}}{E^j \tau} \right| < \epsilon, \quad j = 1, \dots \right\}, \quad E_R \approx E(T_R), \quad (27)$$

$$T_R = \min_{I^j > 0} \left\{ t_j : \left| \frac{I^j - I^{j-1}}{I^j \tau} \right| < \epsilon, \quad j = 1, \dots \right\}. \quad I_R \approx I(T_R). \quad (28)$$

(27) was used for the potentiometric biosensor while (28) for the amperometric one.

The digital simulator has been programmed in Java language [21].

At zero thickness of the diffusion layer ($\delta = 0$) and low concentrations ($S_0 \ll K_M$) as well as high concentrations ($S_0 \gg K_M$) of the substrate, the steady-state response

can be calculated analytically [7, 19]. The adequacy of the mathematical and numerical models was evaluated using known analytical solutions. The relative difference between the numerical and analytical solutions was less than 1%.

6 RESULTS AND DISCUSSION

Using computer simulation the influence of the thickness of both the enzyme and the diffusion layers on the biosensors response was investigated.

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 stirring is, the thinner diffusion layer is. Furthermore, δ depends upon the type of stirring. No exact analytical expression of δ is available for stirred solutions. δ can be estimated experimentally by measuring the electrode response at given bulk concentration.

6.1 The effect of the thickness of the diffusion layer

We investigate the dependence of the steady-state biosensor response 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, $k = \delta/d$, $k \geq 0$. k is a relative thickness of the diffusion layer.

The steady-state current I_R of an amperometric biosensor as well as the steady-state potential E_R of a potentiometric one are very sensitive to the thickness of the enzyme layer. I_R and E_R vary even in orders of magnitude [20, 22]. Because of this we normalise the biosensor response to evaluate the effect of the ratio k on the response.

The normalised steady-state biosensor potential E_N (current I_N) is expressed by the steady-state potential (current) calculated at the thickness δ of the diffusion layer divided by the steady-state potential (current) assuming the zero thickness of the diffusion layer,

$$E_N(d, \delta) = \frac{E_R(d, \delta)}{E_R(d, 0)}, \quad I_N(d, \delta) = \frac{I_R(d, \delta)}{I_R(d, 0)}, \quad d > 0, \delta \geq 0, \quad (29)$$

where $E_R(d, \delta)$ is the steady-state potential (11) calculated at given thickness d of the membrane and thickness δ of the diffusion layer, $I_R(d, \delta)$ is the corresponding steady-state current (13) of the amperometric biosensor.

The biosensor response versus the dimensionless ratio $k = \delta/d$ was investigated at different values of the maximal enzymatic rate V_{max} and membrane thickness d . Results of the calculation obtained at two values of V_{max} : 10 and 100 $\mu\text{M/s}$ and various values of d are depicted in figure 1 for both types of biosensors: potentiometric and amperometric.

One can see in figure 1a the steady-state biosensor potential notably decreases with increase of the ratio k in the cases when the diffusion modulus σ is less than about 2. In cases of relatively high values of σ ($\sigma > 2$), the influence of the thickness δ of the diffusion layer on the biosensor response is slight only. In the case of $\sigma = 3.65$ ($d = 0.02$ cm,

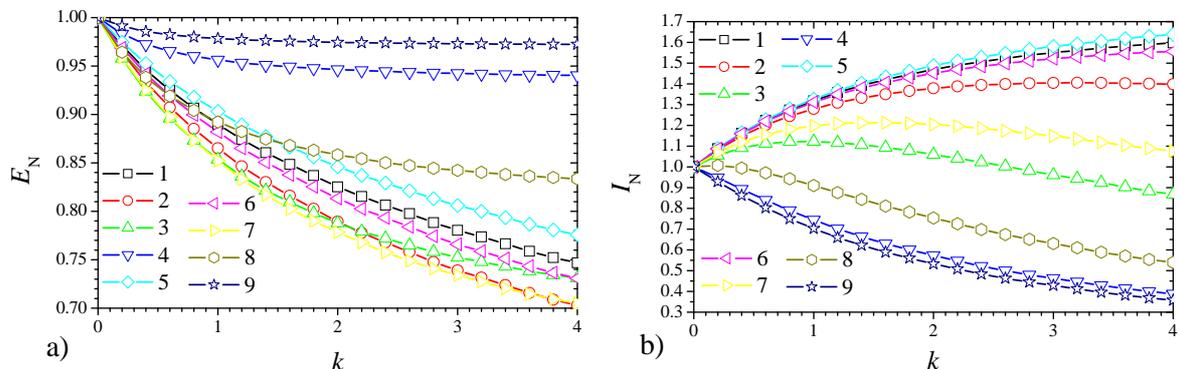


Figure 1: The normalised steady state response of potentiometric (a) and amperometric (b) biosensors versus the ratio $k = \delta/d$ at $V_{max} = 100$ (1-4), $V_{max} = 10 \mu\text{M/s}$ (5-9) and nine diffusion modulus σ : 0.18 (5), 0.29 (1), 0.37 (6), 0.58 (2), 0.91 (7), 1.15 (3), 1.83 (8), 2.89 (4), 3.65 (9).

$V_{max} = 10 \mu\text{M/s}$) the steady-state potential decreases less than 3% only, $E_N \approx 0.972$, while in the case of $\sigma = 0.58$ ($d = 0.001 \text{ cm}$, $V_{max} = 100 \mu\text{M/s}$) it decreases even about 30%, $E_N \approx 0.703$, when k changes from 0 to 4.

Consequently, in the cases when the response of a potentiometric biosensor is considerably under diffusion control ($\sigma > 2$), the mass transport by the diffusion outside the enzyme membrane may be neglected. In those cases the response of potentiometric biosensors practically does not depend on the intensity of stirring of the buffer solution.

In the case of the amperometric biosensor (figure 1b), the behaviour of the normalised steady-state current differs significantly from that of the potentiometric one (figure 1a). I_N is a monotonous decreasing function of the ratio k when the response is under diffusion control ($\sigma > \approx 2$). I_N is a monotonous increasing function of k when the enzyme kinetics controls the biosensor response ($\sigma < \approx 0.5$).

6.2 The effect of the Nernst diffusion layer

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 layer [17]. 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 $\delta = 2 \mu\text{m}$ by increasing the rotation speed [17]. However, in another frequently used case when the solution is stirred in a magnetic stirrer, it is difficult to achieve the thickness δ less than about $20 \mu\text{m}$.

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 [8, 9, 16]. We assume, that a model of the biosensor action, taking into consideration the Nernst diffusion layer, describes the biosensor action more precisely than an another one where the Nernst diffu-

sion 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 response calculated considering the Nernst layer is approximately the same as in the case when the Nernst diffusion layer is neglected.

We introduce the relative error of the biosensor response,

$$R_P(d, \delta) = \left| \frac{E_R(d, \delta) - E_R(d, 0)}{E(d, \delta)} \right|, \quad R_A(d, \delta) = \left| \frac{I_R(d, \delta) - I_R(d, 0)}{I_R(d, \delta)} \right|. \quad (30)$$

$R_P(d, \delta)$ and $R_A(d, \delta)$ may be called the relative errors of the use of the model where the diffusion layer of thickness δ is neglected. Those functions may also be regarded as levels of a reliability of the mathematical model where the Nernst diffusion layer is not taken into account.

We investigate the conditions when the Nernst diffusion layer may be neglected to simulate the response of biosensors accurately. 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 relative errors R_P and R_A at practically minimal thickness of the diffusion layer for both types of stirring: by electrode rotation and in magnetic stirrer. Since the effect of the diffusion layer on the biosensor response significantly depends upon the modulus of diffusion, we calculate the normalised response changing in wide range both: the maximal enzymatic rate V_{max} and the membrane thickness d .

Figure 2 shows the results of calculation at the thickness $\delta = 2 \mu\text{m}$ while figure 3 shows the results at 10 times thicker ($\delta = 20 \mu\text{m}$) the Nernst diffusion layer.

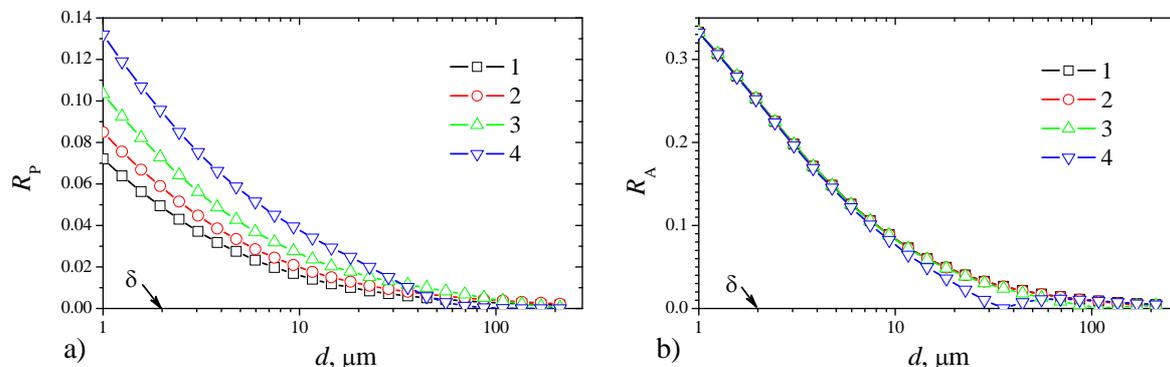


Figure 2: The relative errors R_P and R_A versus the enzyme membrane thickness d at the thickness $\delta = 2 \mu\text{m}$ of the Nernst diffusion layer and four values of V_{max} : 0.1 (1), 1 (2), 10 (3) and 100 (4) $\mu\text{M/s}$.

One can see in figure 2, the effect of the Nernst layer decreases with increase of the membrane thickness d . Figure 2 shows, that the Nernst diffusion layer of the thickness of $2 \mu\text{m}$ should be taken into consideration in all the cases when the enzyme membrane is thinner than about $20 \mu\text{m}$. The simulated steady-state biosensor current I_R may differ even more than 30% ($R_A > 0.3$) from the true current if the Nernst diffusion layer is

neglected in cases of thin enzyme membranes, $d \leq 1 \mu\text{m}$, when the buffer solution is well-stirred and in powerful motion. The potentiometric biosensors are less sensitive to the intensity of stirring. The relative error R_P is more than 2 times less than R_A at the same conditions.

The effect of the Nernst diffusion layer becomes slight only in the cases when the enzyme membrane is more than about 10 times thicker than the diffusion layer, $d > 10\delta = 20 \mu\text{m}$. Assuming the high speed rotation of the electrode ($\delta = 2 \mu\text{m}$) and the membrane thickness $d = 10\delta$, the error R_P varies from 0.01 to 0.03 changing V_{max} between 0.1 and $100 \mu\text{M/s}$, i.e. in the case when the Nernst diffusion layer is taken into consideration, the steady-state potential ($E_R(20, 2)$) of the potentiometric biosensor differs in about 1–3% from the steady-state potential when the Nernst diffusion layer is neglected ($E_R(20, 0)$).

Figure 3 shows very similar effect of the Nernst diffusion layer on the biosensors response also at 10 times thicker ($\delta = 20 \mu\text{m}$) layer. The Nernst diffusion layer of the thickness δ of $20 \mu\text{m}$ should be taken into consideration in all the cases when the enzyme membrane is thinner than about $200 \mu\text{m}$, i.e. $d < 10\delta$.

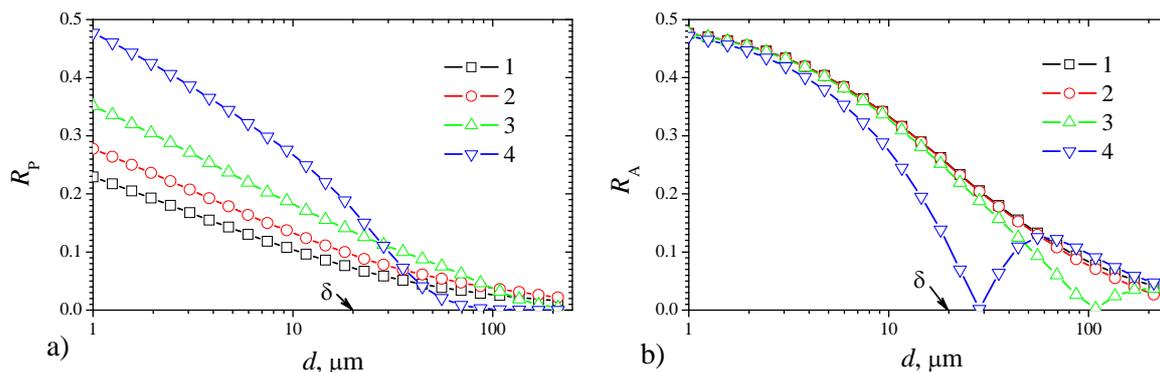


Figure 3: The relative errors R_P and R_A versus the enzyme membrane thickness d at the thickness $\delta = 20 \mu\text{m}$ of the Nernst diffusion layer and four values of V_{max} : 0.1 (1), 1 (2), 10 (3) and 100 (4) $\mu\text{M/s}$.

One can see in figure 3, that the sensitivity to the intensity of stirring is rather similar for both types of electrode: potentiometric and amperometric. The relative error R_P is approximately the same as R_A at the same conditions, while those errors notably differs in the case on thin membrane (figure 2).

As it is possible to notice in figures 2 and 3, the relative error R_P is notable less sensitive to changes of maximal enzymatic rate V_{max} than R_A . Potentiometric biosensors of less enzymatic activity are less sensitive rather than of the higher enzymatic activity.

7 CONCLUSIONS

- The mathematical model (2)-(7), (9) of the operation of the potentiometric biosensors can be used to investigate regularities of the biosensor response in stirred and non-stirred analytes. The model (2)-(8) defines the action of the corresponding

amperometric biosensors.

- The steady-state potential of the potentiometric biosensors is a monotonous decreasing function of the ratio k of the thickness of the diffusion layer to the thickness of the enzyme membrane (figure 1a). In particular cases when the biosensor response is distinctly under diffusion control ($\sigma > 2$), variation of k practically does not effect the steady-state potential. Consequently, in the cases when $\sigma > 2$ the response of potentiometric biosensors practically does not depend upon the intensity of stirring of the buffer solution (upon rotation speed of the electrode).
- 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 2 and 3).

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REFERENCES

- [1] A.P.F. Turner, I. Karube and G.S. Wilson. *Biosensors: Fundamentals and Applications*, Oxford University Press, Oxford, (1987).
- [2] F. Scheller and F. Schubert. *Biosensors*, Elsevier, Amsterdam, Vol. 7, (1988).
- [3] R. Pallás-Areny and J.G. Webster. *Sensors and Signal Conditioning*, John Wiley & Sons, New York, 2nd ed., (2000).
- [4] A. Chaubey and B.D. Malhotra. Mediated biosensors. *Biosens. Bioelectron.*, **17**, 441–456, (2002).
- [5] K.R. Rogers. Biosensors for environmental applications. *Biosens. Bioelectron.*, **10**, 533–541, (1995).
- [6] U. Wollenberger, F. Lisdat and F.W. Scheller. *Frontiers in Biosensorics 2, Practical Applications*, Birkhauser Verlag, Basel, (1997).
- [7] J. Kulys. The development of new analytical systems based on biocatalysts. *Anal. Lett.*, **14**, 377–397, (1981).
- [8] T. Schulmeister. Mathematical modeling of the dynamic behaviour of amperometric enzyme electrodes. *Selective Electrode Rev.*, **12**, 203–260, (1990).

- [9] P.N. Bartlett and K.F.E. Pratt. Modelling of processes in enzyme electrodes. *Biosens. Bioelectron.*, **8**, 451–462, (1993).
- [10] J. Crank. *The Mathematics of Diffusion*. Clarendon Press, Oxford, 2nd ed., (1975).
- [11] R. Aris. *The Mathematical Theory of Diffusion and Reaction in Permeable Catalysts. The Theory of the Steady State*. Clarendon Press, Oxford, (1975).
- [12] L.K. Bieniasz and D. Britz. Recent developments in digital simulation of electroanalytical experiments. *Polish J. Chem.*, **78**, 1195–1219, (2004).
- [13] R. Baronas, F. Ivanauskas and J. Kulys. Computer simulation of the response of amperometric biosensors in stirred and non stirred solution. *Nonlinear Analysis: Modelling and Control*, **8**, 3–13, (2003).
- [14] R. Baronas, F. Ivanauskas and J. Kulys. The effect of diffusion limitations on the response of amperometric biosensors with substrate cyclic conversion. *J. Math. Chem.*, **35**, 199–213, (2004).
- [15] A.A. Samarskii. *The Theory of Difference Schemes*. Marcel Dekker, New York-Basel, (2001).
- [16] D. Britz. *Digital Simulation in Electrochemistry*. Springer-Verlag, Berlin, 2nd ed., (1988).
- [17] J. Wang. *Analytical Electrochemistry*. John Wiley & Sons, New-York, 2nd ed., (2000).
- [18] W.J. Blaedel, T.R. Kissel and R.C. Boguslaski. Kinetic behavior of enzymes immobilized in artificial membranes. *Anal. Chem.*, **44**, 2030–2037, (1972).
- [19] P.W. Carr. Fourier analysis of the transient response of potentiometric enzyme electrode. *Anal. Chem.*, **49**, 799–802, (1977).
- [20] J.J. Kulys. *Analytical Systems Based on Immobilized Enzymes*. Mokslas, Vilnius, (1981) (in Russian).
- [21] J.E. Moreira, S.P. Midkiff, M. Gupta, P.V. Artigas, M. Snir and R.D. Lawrence. Java programming for high performance numerical computing. *IBM Systems J.*, **39**, 21–56, (2000).
- [22] R. Baronas, F. Ivanauskas and J. Kulys. The influence of the enzyme membrane thickness on the response of amperometric biosensors. *Sensors*, **3**, 248–262, (2003).