Journal of Mathematical Chemistry, Vol. 42, No. 3, October 2007 (© 2006)
DOI: 10.1007/s10910-006-9102-0

Computational modelling of the behaviour of potentiometric membrane biosensors

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Received 22 February 2006; revised 15 March 2006

This paper presents a mathematical model of a potentiometric biosensor based on a potentiometric electrode covered with an enzyme membrane. The model is based on the reaction–diffusion equations containing a non-linear term related to the Michaelis–Menten kinetics of the enzymatic reaction. Using computer simulation the influence of the thickness of the enzyme membrane on the biosensor response was investigated. The digital simulation was performed using the finite difference technique. Results of the numerical simulation were compared with known analytical solutions.

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

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

1. Introduction

Biosensors are analytical devices that transform a biological recognition into an electrical signal. The reaction occurs in the membrane where the substrate of interest is converted to a product that causes an electrical response. This response is measured by the transducer and then amplified, processed and displayed [1–4].

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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. These devices have been widely used in environmental, medical and industrial applications because of their high selectivity, simplicity and low cost [5–7].

Since it is not generally possible to measure the concentration of substrate inside enzyme domain with analytical devices, starting from 1970s various mathematical models of biosensors have been developed and used as an important tool to study and optimise analytical characteristics of actual biosensors [8–11]. The goal of this investigation is to make a model allowing an effective computer simulation of potentiometric membrane biosensors acting at wide range of catalytic parameters.

The developed model is based on reaction–diffusion equations [12,13], containing a non-linear term related to the Michaelis–Menten kinetics of the enzymatic reaction. Using computer simulation the influence of the thickness of the enzyme membrane on the biosensor response was investigated. The computer simulation was carried out using the finite difference technique [14].

2. Mathematical model

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

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

A biosensor may be considered as an electrode, having a layer of enzyme applied onto the surface of the probe. Let us assume the symmetrical geometry of the electrode and homogeneous distribution of the 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 of the reaction–diffusion type ($t > 0$):

$$\begin{aligned} \frac{\partial S}{\partial t} &= D\frac{\partial^2 S}{\partial x^2} - \frac{V_{\max} S}{K_{\mathrm{M}} + S}, \\ \frac{\partial P}{\partial t} &= D\frac{\partial^2 P}{\partial x^2} + \frac{V_{\max} S}{K_{\mathrm{M}} + S}, \quad 0 < x < d, \end{aligned} \tag{2}$$

where x stands for space, t stands for time, $S(x,t)$ is the concentration of the substrate, $P(x,t)$ is the concentration of the reaction product, d is the thickness of the enzyme membrane, D is the diffusion coefficient, $V_{\max}$ is the maximal enzymatic rate and K_{M} is the Michaelis constant.

Let $x = 0$ represents the electrode surface, while $x = d$ represents the bulk solution/membrane interface. The biosensor operation starts when the substrate

appears over the surface of the enzyme region. This is used in the initial conditions ($t = 0$)

$$S(x, 0) = 0, \quad 0 \leqslant x < d, \tag{3}$$

$$S(d, 0) = S_0, \tag{4}$$

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

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

If bulk solution is well-stirred and in powerful motion, then the diffusion layer ($0 < x < d$) remains at a constant thickness [9,15,16]. Consequently, the concentration of substrate as well as product over the enzyme surface (bulk solution/membrane interface) remains constant during the biosensor operation. At the electrode surface, we define non-leakage boundary conditions ($t > 0$) [17,18]

$$\left.\frac{\partial S}{\partial x}\right|_{x=0} = \left.\frac{\partial P}{\partial x}\right|_{x=0} = 0, \tag{6}$$

$$S(d, t) = S_0, \tag{7}$$

$$P(d, t) = 0. \tag{8}$$

Typically, the change of potential caused by the reaction product concentration change is measured. The potential is given by

$$E(t) = E_0 + \frac{R_c T_K}{zF} \ln P(0, t), \tag{9}$$

where E is the measured potential (in volts), E_0 is a characteristic constant for the ion-selective electrode. R_c is the universal gas constant, $R_c = 8.314\,\text{J/mol/K}$, T_K is the absolute temperature (K), z is the signed ionic charge, F is the Faraday constant, $F = 9648\,\text{C/mol}$ [17,18]. Typically, the change of E caused by the P concentration change is measuring.

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

$$E_\infty = \lim_{t \to \infty} E(t). \tag{10}$$

E_∞ is assumed as the steady-state biosensor potential.

3. Solution of the problem

Closed mathematical solutions are not usually possible when analytically solving non-linear partial differential equations with complex boundary conditions. Therefore, the problem (2)–(8) was solved numerically using the finite

difference technique [14]. To find a numerical solution of the problem in the domain $[0, d] \times [0, T]$ we introduced an uniform discrete grid $\omega_h \times \omega_\tau$, where

$$\begin{aligned} \omega_h &= \{x_i : x_i = ih,\ i = 0, 1, \ldots, N,\ hN = d\}, \\ \omega_\tau &= \{t_j : t_j = j\tau,\ j = 0, 1, \ldots, M;\ \tau M = T\}. \end{aligned} \tag{11}$$

We assume the following:

$$\begin{aligned} &S_i^j = S(x_i, t_j), \qquad P_i^j = P(x_i, t_j), \qquad E^j = E(t_j), \\ &i = 0, \ldots, N, \quad j = 0, \ldots, M. \end{aligned} \tag{12}$$

An implicit linear finite difference scheme has been built as a result of the difference approximation. The initial conditions (5) we approximated as follows:

$$S_i^0 = 0, \quad i = 0, \ldots, N-1, \tag{13}$$

$$S_N^0 = S_0, \tag{14}$$

$$P_i^0 = 0, \quad i = 0, \ldots, N. \tag{15}$$

Differential equations (2) were approximated by the scheme

$$\begin{aligned} &\frac{S_i^{j+1} - S_i^j}{\tau} = D\frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} - \frac{V_{\max} S_i^{j+1}}{K_{\mathrm{M}} + S_i^j}, \\ &i = 1, \ldots, N-1, \quad j = 0, \ldots, M-1, \end{aligned} \tag{16}$$

$$\begin{aligned} &\frac{P_i^{j+1} - P_i^j}{\tau} = D\frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} + \frac{V_{\max} S_i^{j+1}}{K_{\mathrm{M}} + S_i^{j+1}}, \\ &i = 1, \ldots, N-1, \quad j = 0, \ldots, M-1. \end{aligned} \tag{17}$$

The boundary conditions (6)–(8) were approximated as follows:

$$S_0^j = S_1^j, \qquad S_N^j = S_0, \quad j = 1, \ldots, M, \tag{18}$$

$$P_0^j = P_1^j, \qquad P_N^j = 0, \quad j = 1, \ldots, M. \tag{19}$$

Equations (13)–(15) allow to calculate a solution of the problem on the layer $t = t_0 = 0$. When a solution on a layer t_j ($j = 0, 1, \ldots, M-1$) has been calculated, a solution on the next layer $t = t_{j+1}$ can be calculated in two steps [19]:

(1) calculate values of S_i^{j+1}, $i = 0, \ldots, N$, solving the system of linear finite difference equations (16) and (18);

(2) calculate values of P_i^{j+1}, $i = 0, \ldots, N$, solving the system of linear finite difference equations (17) and (19) using values of S_i^{j+1} calculated in step 1.

The systems of linear algebraic equations were solved efficiently in both steps 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

$$E^j = E_0 + \frac{R_c T_K}{zF} \ln\left(P_0^j\right), \quad j = 1, \ldots, M. \tag{20}$$

4. Computer 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:

$$\begin{aligned} &D = 3.0 \times 10^{-10}\,\mathrm{m^2/s}, \\ &K_M = 0.1\,\mathrm{mol/m^3} = 100\,\mu\mathrm{M}, \\ &E_0 = 0\,\mathrm{V}, \qquad z = 1, \qquad T_K = 298\,\mathrm{K}. \end{aligned} \tag{21}$$

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

In digital simulation, the biosensor response time was assumed as the time when the absolute potential slope value falls below a given small value normalised with the potential value. In other words, the time

$$T_R = \min_{P(0,t)>0} \left\{ t : \left| \frac{1}{E(t)} \frac{\mathrm{d}E(t)}{\mathrm{d}t} \right| < \varepsilon \right\} \tag{22}$$

needed to achieve a given dimensionless decay rate ε was used.

Consequently, the potential $E_R = E(T_R)$ at the biosensor response time T_R was assumed as the steady-state biosensor potential E_∞, $E_R \approx E_\infty$. In calculations, we used $\varepsilon = 10^{-5}$.

The adequacy of the mathematical and numerical models was evaluated using known analytical solutions for potentiometric biosensors. At relatively low concentrations of the substrate, $S_0 \ll K_M$, the steady-state potential can be calculated as follows [8]:

$$E_\infty = E_0 + \frac{R_c T_K}{zF} \ln\left(S_0 \left(1 - \frac{1}{\cosh(\sigma)}\right)\right), \tag{23}$$

$$\sigma^2 = \frac{V_{\max} d^2}{K_M D}. \tag{24}$$

The dimensionless factor σ^2 is known as the diffusion modulus (Damköhler number) [8,13]. The diffusion modulus σ^2 essentially compares the rate of enzyme reaction ($V_{\max}/K_M$) with the diffusion through the enzyme layer (D/d^2). The biosensor response is controlled by the diffusion when $\sigma^2 \gg 1$. If $\sigma^2 \ll 1$, then the enzyme kinetics determines the response.

In the case of very high-substrate concentration, $S_0 \gg K_M$, the stationary potential is expressed as follows [8]:

$$E_\infty = E_0 + \frac{R_c T_K}{zF} \ln\left(\frac{V_{\max} d^2}{2D}\right) = E_0 + \frac{R_c T_K}{zF} \ln\left(\frac{\sigma^2 K_M}{2}\right). \tag{25}$$

The numerical solution of the model (2)–(8) was compared with the analytical ones (23) and (25) at five values of the maximal enzymatic rate $V_{\max}$: 0.1, 1, 10, 100, 1000 μM/s and two values of S_0: 10^{-7} and 10^{-1} M. In all the cases, the relative difference between the numerical and analytical solutions was less than 1%.

Figure 1 shows both: the substrate and the product concentrations at steady-state conditions ($T_R = 116$ s) and at the following intermediate time values: 1, 5, 10, and 20 s, accepting $d = 100\,\mu$m, $V_{\max} = 100\,\mu$M/s, $S_0 = 100\,\mu$M. Since the profiles of the substrate concentration S at $t \geqslant 5$ s are practically identical to the profile S_{116}, they are not marked individually in figure 1.

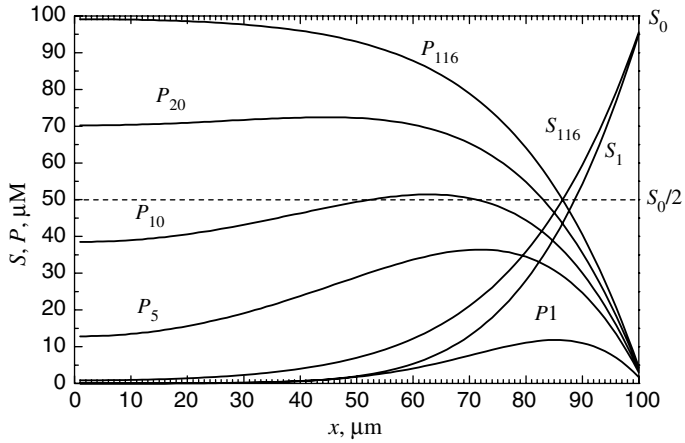

Figure 1. The profile of the substrate (S) and product (P) concentrations at $t = 1$, 5, 10, 20, and 116 s (inside the plot, the subscripts to S and P refer to the time values), $T_R = 116$ s, $d = 100\,\mu$m, $V_{\max} = 100\,\mu$M/s, $S_0 = 100\,\mu$M.

At the steady-state conditions ($\partial S/\partial t = \partial P/\partial t = 0$), the governing equation (2) and the boundary conditions (6)–(8) lead to

$$P(x,t) + S(x,t) = S_0, \quad t \to \infty, \quad x \in [0,d]. \tag{26}$$

Because of this, the symmetry with respect to the axis $S = P = 0.5S_0$ can be noticed at $t = T_R$ in figure 1.

The response time T_R as an approximate steady-state time is very sensitive to the decay rate ε, i.e. $T_R \to \infty$, when $\varepsilon \to 0$. To investigate the behaviour of the response time we employed the time $T_{0.001}$ of the potential differing from the steady-state potential by 0.001 V,

$$T_{0.001} = \min\{t : E(t) > E_R - 0.001\}, \quad E_R = E(T_R), \qquad E_\infty \approx E_R. \tag{27}$$

The approximate steady-state time $T_{0.001}$ equals approximately 78 s at the conditions defined in (21) and in the caption of figure 1.

5. Results and discussion

Using computer simulation we have investigated the dependence of the steady-state biosensor potential on the thickness of the enzyme membrane. These calculations were performed at the following five values of V_{max}: 0.1, 1, 10, 100 and 1000 μM/s to get results for a wide range of values of the maximal enzymatic rate. Figure 2 shows the steady-state (maximal) potentials E_R while figure 3 presents the approximate steady-state time $T_{0.001}$ versus the thickness d of the enzyme layer.

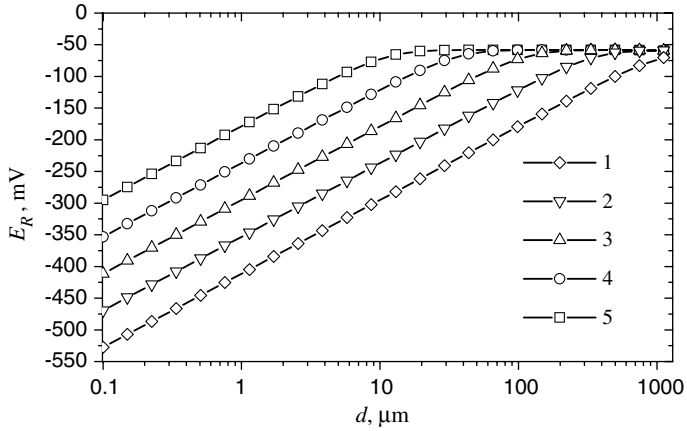

Figure 2. The dependence of the steady-state potential E_R on the thickness d of the enzyme layer at five maximal enzymatic rates V_{max}: 0.1 (1), 1 (2), 10 (3), 100 (4), and 1000 (5) μM/s, $S_0 = K_M = 100\,\mu$M.

One can see in figure 2 that the steady-state biosensor potential E_R is a monotonous increasing function of d at all values of the maximal enzymatic rate V_{max}. However, E_R is practically constant function of d at high values of V_{max} as wells as of d. At a concrete not great thickness d, the higher maximal enzymatic rate V_{max} corresponds to the greater value of E_R.

The stability of the response is one of the most critical characteristics of biosensors [20]. In practice, 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}. Figure 2 shows, that the biosensor steady-state potential can differ 100-fold, when changing V_{max}. The variation is especially notable in cases of relatively thin enzyme membrane. In the cases of relatively thick enzyme membrane, E_R practically does not vary by changing V_{max}. Consequently, a biosensor containing a thicker enzyme layer gives more stable response than a biosensor with thinner layer. However, the thick membrane-based biosensors have very durable response time (figure 3). For example, the response time $T_{0.001}$ is about 48 s when the membrane thickness d equals to 100 μm. The time $T_{0.001}$ is even more durable at thicker enzyme membrane. The time $T_{0.001}$ is approximately an exponentially increasing function of the thickness d. The influence of the maximal enzymatic rate V_{max} on the response time $T_{0.001}$ is slight only. So, the biosensors of relatively thick enzyme membrane are of limited applicability in flow injection systems [21], which are widely used for determination of various compounds.

Thus, a problem of the membrane thickness optimisation arises. The task is to find the thickness of the membrane so small as possible, ensuring the stability of the biosensor response at a range of V_{max} as wide as possible. Let

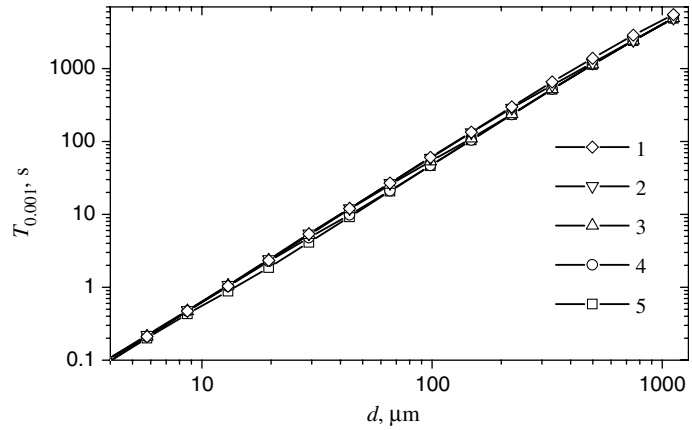

Figure 3. The dependence of the response time $T_{0.001}$ on the thickness d of the enzyme layer. The notation and values of the parameters are the same as in figure 2.

V_1 and V_2 be two values of the maximal enzymatic rate $V_{\max}$ ($V_1 < V_2$) 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 steady-state biosensor potential E_R) 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

$$R(d, V_1, V_2, S_0) = \left| \frac{E_R(d, V_1, S_0) - E_R(d, V_2, S_0)}{E_R(d, V_1, S_0)} \right|, \tag{28}$$

$$d_\delta(V_1, V_2, S_0) = \min_{d>0} \{ d : R(d, V_1, V_2, S_0) < \delta \}, \tag{29}$$

where $E_R(d, V_{\max}, S_0)$ is the biosensor potential calculated at the membrane thickness d, maximal enzymatic rate $V_{\max}$ and substrate concentration S_0.

Let us accept $S_0 = 100\,\mu$M, $V_1 = 100\,\mu$M/s, $V_2 = 1000\,\mu$M/s and $\delta = 0.01$. From the numerical results, presented in figure 2, we found $d_\delta \approx 80\,\mu$m. To evaluate the biosensor stability, we have calculated the responses of a biosensor based on the membrane of thickness $d = d_\delta(V_1, V_2, S_0) = 80\,\mu$m at wide ranges of the substrate concentrations S_0 and of maximal enzymatic rate $V_{\max}$.

Figure 4 shows the potential E_R versus the substrate concentration S_0 at five values of $V_{\max}$: 0.1, 1, 10, 100 and 1000 μM/s. No notable difference (figure 4) is observed between values of E_R, calculated at two largest values of $V_{\max}$: 100 and 1000 μM/s, when the substrate concentration S_0 is less than about 1 mM. Figure 4 expressively shows the stable response of the biosensor, based on the enzyme membrane of thickness $d = 80\,\mu$m, when the maximal enzymatic

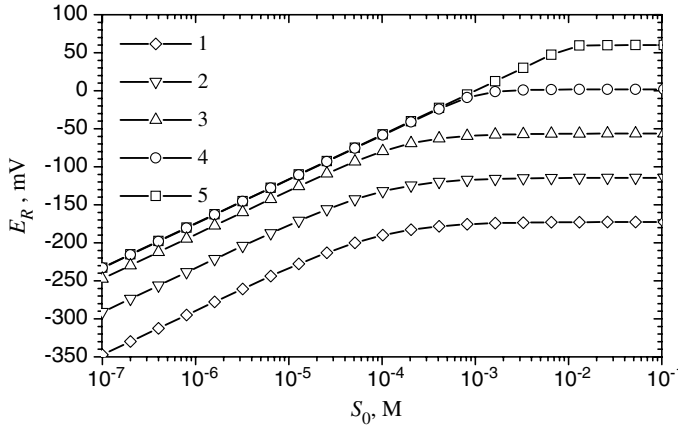

Figure 4. The dependence of the steady-state potential E_R on the substrate concentration S_0 at five maximal enzymatic rates $V_{\max}$: 0.1 (1), 1 (2), 10 (3), 100 (4), and 1000 (5) μM/s, $d = 80\,\mu$m.

rate reduces ten times: from 1000 to 100 μM/s. Although the membrane thickness d_δ was calculated for the substrate concentration $S_0 = 100\,\mu$M, the biosensor response is sufficiently stable also to the substrate of the concentration S_0 being up to about 1 mM. Figure 4 also shows that the response of the biosensor of thickness of 80 μm is approximately constant at the concentration higher than about 10 mM changing $V_{\max}$ from 0.1 to 1000 μM/s. Because of this, such biosensor is practically unuseful to determinate larger substrate concentration.

Figure 5 shows the steady-state potential E_R versus the maximal reaction rate $V_{\max}$ at five values of substrate concentration S_0: 1, 10, 100, 1000, and 10,000 μM and at the same membrane thickness d as above, i.e. $d = 80\,\mu$m. One can see in figure 5, that the response of the biosensor is stable to changes in maximal enzymatic rate $V_{\max}$ from 100 to 1000 μM/s when the concentration S_0 equals or is less than 100 μM. In the case, when $V_{\max} < 100\,\mu$M/s the biosensor response is very sensitive to changes in $V_{\max}$.

Figures 6 and 7 show the influence of the substrate concentration S_0 and of the maximal enzymatic rate $V_{\max}$ on the response time $T_{0.001}$ at the same thickness d of the enzyme membrane, $d = d_\delta = 80\,\mu$m.

Figure 6 presents the effect of substrate concentration S_0 on the response time $T_{0.001}$. One can see in figure 6, that $T_{0.001}$ is a monotonous decreasing function of S_0 at $V_{\max} = 0.1$ and 1 μM/s, and $T_{0.001}$ is a non-monotonic function of S_0 at higher values of $V_{\max}$. The effect of non-monotonous behaviour of the response time versus the substrate concentration has been discussed recently for the cases of amperometric biosensors when the biosensor response is under diffusion control, i.e. $\sigma^2 \gg 1$ [22,23,24]. Let us notice, that in the case of $d = 80\,\mu$m the diffusion modulus σ^2 becomes equal to 1 at $V_{\max} \approx 4.7\,\mu$M/s. As one can see in figure 7, the effect of the maximal enzymatic rate $V_{\max}$ on the response

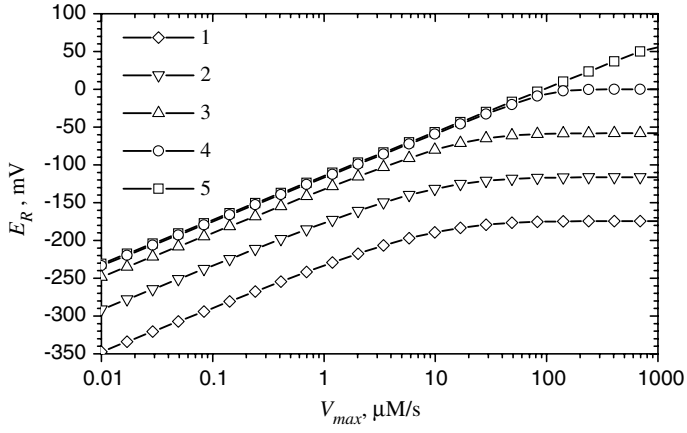

Figure 5. The dependence of the steady-state potential E_R on the maximal enzymatic rate $V_{\max}$ at five substrate concentrations S_0: 1 (1), 10 (2), 100 (3), 1000 (4), and 10000 (5) μM, $d = 80\,\mu$m.

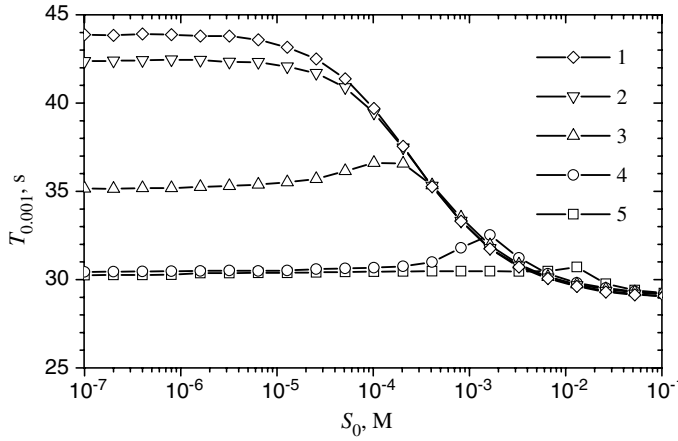

Figure 6. The dependence of the response time $T_{0.001}$ on the substrate concentration S_0. The notation and values of the parameters are the same as in figure 4.

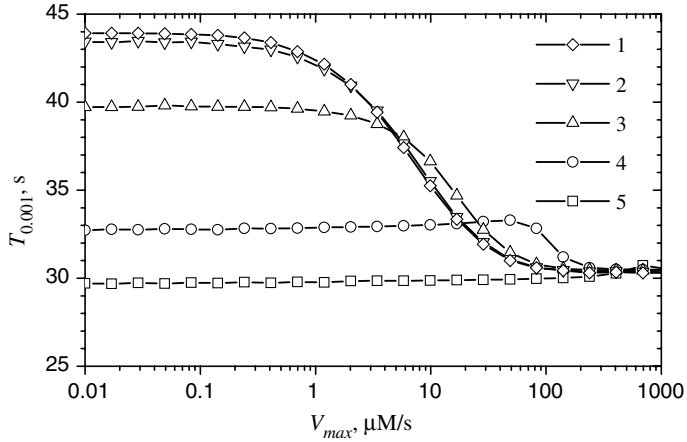

Figure 7. The dependence of the response time $T_{0.001}$ on the maximal enzymatic rate $V_{\max}$. The notation and values of the parameters are the same as in figure 5.

time $T_{0.001}$ is very similar to the effect of substrate concentration S_0. The shape of curves for both types of biosensors: amperometric and potentiometric is very similar (see figures 6 and 7 as well as [23,24]).

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 when the enzymatic rate changes from V_1 to V_2. In this case the minimal thickness d_δ has to be calculated at

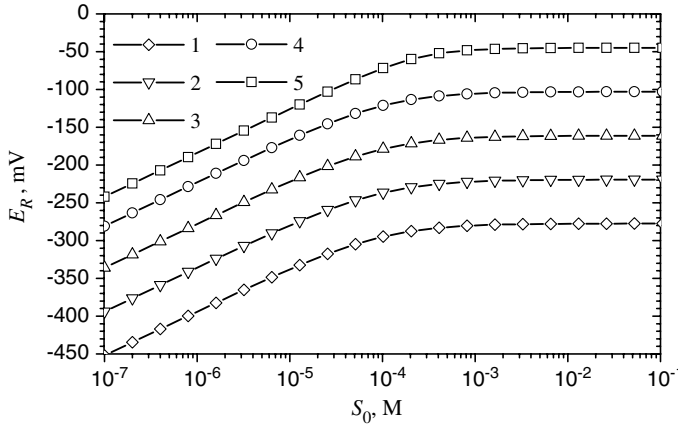

Figure 8. The dependence of the steady-state potential E_R on the substrate concentration S_0, $d = 10\,\mu$m. The notation and values of all other parameters are the same as in figure 4.

the concrete characteristics of biosensor operation: the diffusion coefficient D, ionic charge z, Michaelis constant K_M, temperature T_K and at 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 less sensitive to enzyme inactivation at lower concentrations of the substrate (figure 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 the case when $S_0 \ll K_M$ or $S_0 \gg K_M$, the biosensor potential may be calculated analytically from (23) or (25), otherwise the numerical solution of the models (2)–(8) is preferable for calculation of $E_R(d, V_{max}, S_0)$, used in the framework, expressed by formulas (28) and (29).

To be sure, that the framework, based on definitions (28) and (29), really makes sense 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 enzyme membrane. Figures 8 and 9 show the dependence of the steady state potential E_R on the substrate concentration S_0 and on the enzymatic rate V_{max}, respectively, where the enzyme membrane is eight times thinner, $d = 10\,\mu$m, than in the case presented in figures 4–7.

One can see in figures 8 and 9, the biosensor response is very sensitive to changes of V_{max} at all values of S_0. For example, in a case of $S_0 = 0.1\,\mu$M, the steady-state potential E_R at $V_{max} = V_2 = 1$ mM/s is about 16% (39 mV) higher than E_R at $V_{max} = V_1 = 0.1$ mM/s (figure 8), while the corresponding values of E_R are approximately the same in the case when the membrane is of thickness $d = d_\delta(V_1, V_2, S_0) = 80\,\mu$m at the same values of V_1, V_2, S_0 (figure 4).

Figure 2 shows the significant influence of the membrane thickness on the biosensor response. However, the significance of the influence is different at the

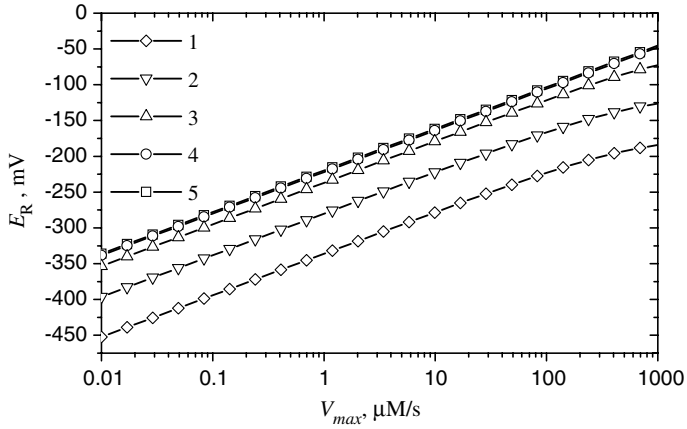

Figure 9. The dependence of the steady-state potential E_R on the maximal enzymatic rates V_{max} at $d = 10\,\mu$m. The notation and values of other parameters are the same as in figure 5.

different membrane thickness. We introduced a resistance B_R of the membrane-based biosensors to changes of membrane thickness [24]. The resistance B_R of a biosensor was expressed as a gradient of the steady-state biosensor potential E_∞ with respect to the membrane thickness d

$$B_R = \frac{dE_\infty}{dd} \approx \frac{dE_R}{dd}. \tag{30}$$

Figure 10 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 figure 2. One can see in figure 10, that the effect of the maximal enzymatic rate V_{max} on B_R is very slight only, while figure 2 shows the significant effect of V_{max} on the steady state potential E_R.

When comparing the behaviour of potentiometric biosensors with the behaviour of the amperometric ones [24], one can see the notable difference in the influence of the enzymatic rate V_{max} on the resistance B_R. In the case of amperometric biosensors having relatively thin enzyme membranes, the enzymatic reaction rate V_{max} effects significantly the resistance B_R.

We have discussed the influence of the membrane thickness d and maximal enzymatic rate V_{max} on the biosensor response. The dimensionless diffusion modulus σ^2 combines directly both these parameters of the mathematical models (2)–(8). The diffusion modules σ^2 increases with increase of both parameters: V_{max} and d.

The numerical experiments presented in figure 2 were repeated with two additional substrate concentrations S_0:10^{-7}, 10^{-4}, 10^{-1} M and plotted as a function E_R of the diffusion modulus σ^2. Figure 11 presents the results of calculations. One can see in this figure, that all the values of E_R calculated at concrete

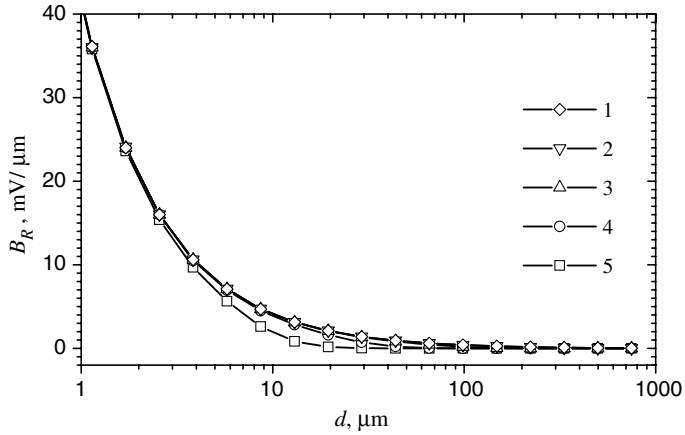

Figure 10. The biosensor resistance B_R versus the thickness d of the enzyme layer. The notation and values of the parameters are the same as in figure 2.

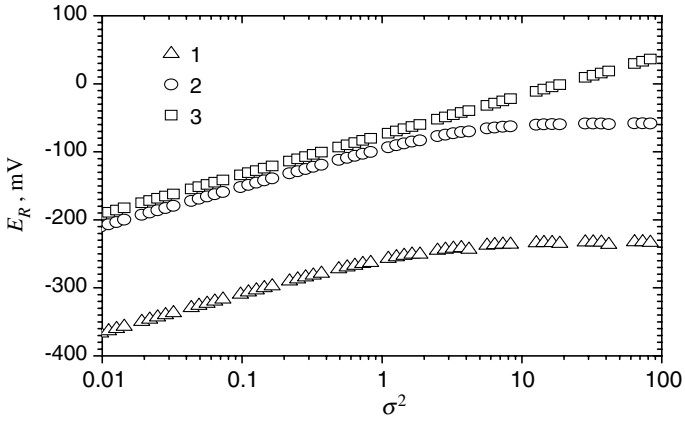

Figure 11. The dependence of the steady-state potential E_R on the dimensionless diffusion modulus σ^2 at three substrate concentrations S_0: 10^{-7} (1), 10^{-4} (2), 10^{-1} (3) M, and five maximal enzymatic rates V_{max}: 0.1, 1, 10, 100, and 1000 μM/s, changing the membrane thickness d.

enzymatic rate V_{max} form one continuous curve. In both extreme cases of S_0: $S_0 \ll K_M$ and $S_0 \gg K_M$ steady state potential is expressed through σ with no additional entries of V_{max} and d (see formulas (23) and (25)). Figure 11 shows that this property is valid also for intermediate values of S_0: $S_0 \approx K_M$. So, the decrease in steady-state potential appeared due to the decrease of V_{max} can really be compensated by increase of the membrane thickness d. This is employed in our framework (28) and (29).

6. Conclusions

The mathematical model (2)–(8) can be successfully used to investigate regularities of the response of potentiometric membrane-based biosensors.

The steady-state biosensor potential E_R is a monotonous increasing function of the enzyme layer thickness d, of the substrate concentration S_0 as well as of the maximal enzymatic rate V_{max} (figures 2, 4, 5, 8, and 9). E_R is a monotonous increasing function also of the diffusion modulus σ^2 (figure 11).

In the cases, when the biosensor response is significantly under diffusion control ($\sigma^2 \gg 1$), the steady-state time $T_{0.001}$ is a non-monotonous function of the substrate concentration S_0 as well as of the maximal enzymatic rate V_{max} (figures 6 and 7). $T_{0.001}$ is a monotonous decreasing function of S_0 as well as of V_{max} when the enzyme kinetics predominates in the biosensor response ($\sigma^2 \ll 1$).

The mathematical models (2)–(8) together with the definitions (28) and (29) describe a way for selection of the membrane thickness d, ensuring a stable biosensor response. In cases when $S_0 \ll K_M$ or $S_0 \gg K_M$, the steady-state potential E_R to be used in (28), may be calculated analytically from (23) and (25), respectively, otherwise the computer simulation based on the models (2)–(8) is preferable for the calculations.

Acknowledgments

This work was partially supported by Lithuanian State Science and Studies Foundation, project No. C-03048.

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