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Calculation of the apparent Michaelis constant for biosensors using reaction-diffusion equations

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Abstract. The relationship between the apparent Michaelis constant and the diffusion module for one and two-layer biosensors is studied using a mathematical model describing action of the amperometric biosensors.

Keywords: biosensor; apparent Michaelis constant; diffusion module.

Introduction

Biosensors are widely used in testing substance concentrations in solutions. Their performance is based on reactions catalysed by enzymes and diffusion of substances. When a biosensor is immersed into a solution, the substance tested (substrate) binds to an enzyme due to diffusion. In an enzyme-catalysed reaction the substrate converts into a product. The resulting product binds to an electrode and acts in an electrochemical reaction, thus causing an electrical current. In amperometric biosensors an electrical current is proportional to the concentration of the substance being tested [1, 2, 5]. The mathematical model of the action of biosensors is based on the substrate and product diffusion equations with a non-linear term describing an enzymatic reaction. A description of enzyme-catalysed reactions in bioamperometric systems is complex, as a kinetic model needs to take account of not only the kinetics of an enzyme-catalysed reaction, but also the influence of the diffusion of substrates, products, intermediate complexes and the environment. While investigating simple systems, kinetic characteristics of enzymes are described using the Michaelis–Menten kinetics model. The rate of an enzyme-catalysed reaction is expressed by non-linear dependence on substrate concentration. This relation is characterized by two constants. One of them is $v_{\max}$, a constant of the maximal reaction rate, which is reached when the concentration of a substrate approaches infinity. The Michaelis constant k_M defines the substrate concentration at which the reaction rate is half its maximal value. The value of k_M is highly significant for developing enzymatic analytical systems, as it determines the linear operating range of such systems. The greater the value of k_M, the wider the linear operating range is. In practice, the apparent Michaelis constant $k_{M,\mathrm{tar}}$ is used, which is the substrate concentration at which the value of the current generated equals half of the maximum steady-state current value. The $k_{M,\mathrm{tar}}$ constant is usually greater in value than k_M. The linear operating range of a biosensor depends on $k_{M,\mathrm{tar}}$. The dependence of this constant on the properties of a biosensor was investigated in [4, 6]. The aim of this research

is to study how the apparent Michaelis constant of a biosensor which has only an enzyme membrane, or an enzyme-containing layer and a diffusion membrane, depends on membrane thickness, diffusion coefficients and the diffusion module.

1 Mathematical model

The substrate and product concentrations $S(t,x)$ and $P(t,x)$ are functions of two variables, the distance to the biosensor electrode x and time t. The values $0 < x < a_e$ correspond to the enzyme-containing layer, and the values $a_e < x < a_e + a_m$ correspond to the points inside the outer membrane. For $0 < x < a_e + a_m$ and $t > 0$ the dynamics of substrate and product concentrations are described by the following nonlinear reaction-diffusion equations [2, 3, 6]:

$$\frac{\partial S}{\partial t} = \frac{\partial}{\partial x}\left(D_S(x)\frac{\partial S}{\partial x}\right) - \alpha(x)\frac{V_{\max} S}{k_M + S}, \tag{1}$$

$$\frac{\partial P}{\partial t} = \frac{\partial}{\partial x}\left(D_P(x)\frac{\partial P}{\partial x}\right) + \alpha(x)\frac{V_{\max} S}{k_M + S}, \tag{2}$$

where the functions $D_S(x)$, $D_P(x)$ and $\alpha(x)$ are defined as follows:

$$D_S(x) = \begin{cases} D_{S_e}, & 0 < x \leqslant a_e, \\ D_{S_m}, & a_e < x \leqslant a_e + a_m, \end{cases} \tag{3}$$

$$D_P(x) = \begin{cases} D_{P_e}, & 0 < x \leqslant a_e, \\ D_{P_m}, & a_e < x \leqslant a_e + a_m, \end{cases} \tag{4}$$

$$\alpha(x) = \begin{cases} 1, & 0 < x \leqslant a_e, \\ 0, & a_e < x \leqslant a_e + a_m. \end{cases} \tag{5}$$

D_{S_e}, D_{P_e} are the substrate and product diffusion coefficients in the enzyme-containing membrane and D_{S_m}, D_{P_m} in the diffusion layer. Enzymatic process takes place only inside the enzyme-containing membrane, therefore $\alpha(x) = 0$ and Eqs. (1), (2) become linear for $a_e < x < a_e + a_m$.

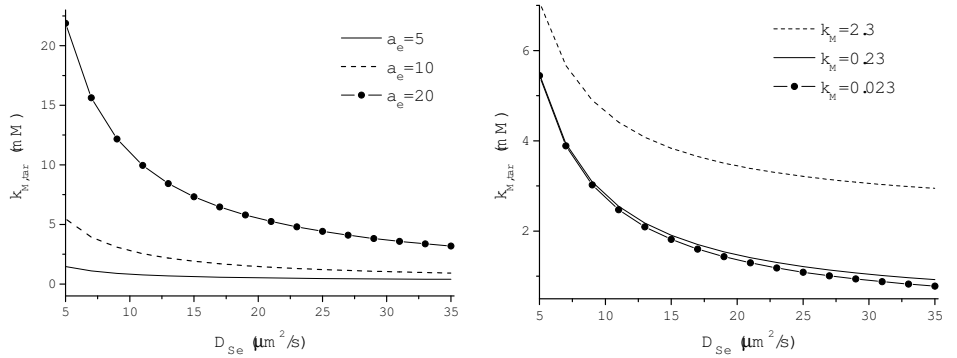
Assume that the concentration of the substrate in the solution, S_0, remains constant during all process time. At the beginning of process ($t = 0$), there is neither substrate nor product inside the enzyme-containing layer and outer membrane:

$$S(0,x) = \begin{cases} 0, & 0 \leqslant x < a_e + a_m, \\ S_0, & x = a_e + a_m, \end{cases}$$

$$P(0,x) = 0, \quad 0 \leqslant x \leqslant a_e + a_m.$$

We suppose that the substrate is an electrochemically inactive substance, hence on the biosensor electrode ($x = 0$) the following boundary conditions are satisfied:

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



(a) (b)

Fig. 1. Dependence of the apparent Michaelis constant of one-layer biosensor on the substrate diffusivity. (a) $k_M = 0.23$ mM, $v_{\max} = 1.1$ mM/s; (b) $a_e = 10$ μm, $v_{\max} = 1.1$ mM/s.

The product can diffuse out through the inner and outer membranes. At the biosensor border with solution ($x = a_e + a_m$) the conditions for substrate and product concentrations are:

$$S(t, a_e + a_m) = S_0, \qquad P(t, a_e + a_m) = 0, \quad t > 0. \tag{7}$$

As a response of electrochemical biosensor the steady-state current density (I) is used:

$$I = \lim_{t\to\infty} i(t), \qquad i(t) = n_e F D_{P_e} \left.\frac{\partial P}{\partial x}\right|_{x=0}, \tag{8}$$

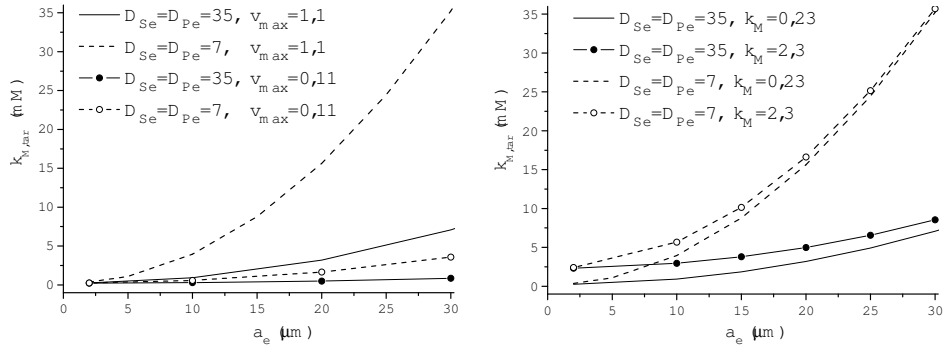
where n_e is the number of electrons involved in the charge transfer at the electrode surface, and F is the Faraday constant. The numerical value of I is calculated by using the formula: $I \approx i(T)$, $T = \min_{i(t)>0}\{t\colon \frac{1}{i(t)}|\frac{di(t)}{dt}| < \delta\}$ with $\delta = 10^{-3}$. The diffusion module allows to compare the rate of the enzyme reaction, $v_{\max}/k_M$, with the mass transport through the enzyme-containing layer, D_{S_e}/a_e^2, [2]:

$$\sigma^2 = \frac{v_{\max} a_e^2}{k_M D_{S_e}}. \tag{9}$$

2 Results

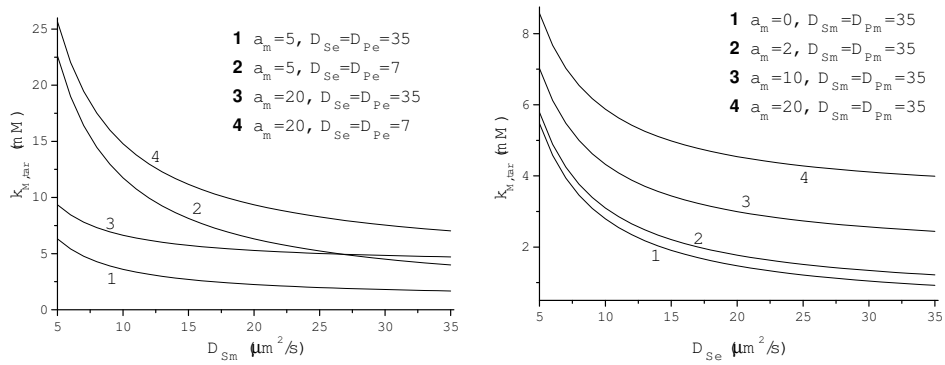
The nonlinear problem (1)–(8) was solved numerically by applying an implicit finite difference scheme which was built on a uniform discrete grid. The following values of the parameters were used in the calculations [4, 5]: $v_{\max} \in [0.1; 1.1]$ mM/s, $k_M \in [0.02; 2.5]$ mM, $a_e \in [2; 20]$ μm, $a_m \in [1; 80]$ μm, $D_{S_e}, D_{P_e}, D_{S_m}, D_{P_m} \in [3; 35]$ μm^2/s.

The results of the calculations for a one-layer biosensor are presented in Figs. 1 and 2 and for a two-layer biosensor they are presented in Fig. 3. Figure 1(a) demonstrates the dependence of the apparent Michaelis constant $k_{M,\text{tar}}$ on the substrate diffusivity D_{S_e}, given that the coefficient of the product diffusion D_{P_e} equals D_{S_e}, and the maximum reaction velocity is $v_{\max} = 1.1$ mM/s. The calculations were performed with the enzymatic layer being 5, 10 and 20 microns thick, respectively. In all three cases, as the diffusion increases, $k_{M,\text{tar}}$ monotonically decreases. The greatest



(a) (b)

Fig. 2. Dependence of the apparent Michaelis constant of one-layer biosensor on the thickness of the enzyme membrane. (a) $k_M = 0.23$ mM; (b) $v_{\max} = 1.1$ mM/s.



(a) (b)

Fig. 3. Dependence of the apparent Michaelis constant of two-layer biosensor on the diffusivity in diffusion layer $D_{S_m} = D_{P_m}$ (a) and in enzyme membrane $D_{S_e} = D_{P_e}$ (b) at $a_e = 10$ μm, $k_M = 0.23$ mM and $v_{\max} = 1.1$ mM/s.

value of $k_{M,\text{tar}}$ is obtained when diffusion is low and the enzymatic layer is thicker ($a_e = 20$ μm, $D_{S_e} = 5$ μm^2/s). As diffusion increases, $k_{M,\text{tar}}$ varies the least when the enzymatic layer is 5 μm thick (solid line), and varies the most when it is 20 μm thick (dotted line). That is also reflected by the change of values of the diffusion module σ^2. For the values of the parameters a_e and D_{S_e} within the range of $[5; 20]$ μm and $[5; 35]$ μm^2/s, respectively, the smallest value is 3.42 and therefore the response of a biosensor is controlled by diffusion. As the diffusion coefficient increases from 5 to 35 μm^2/s, the diffusion module decreases from 23.9 to 3.42 when $a_e = 5$ μm (solid line), and from 382.6 to 54.7 when the thickness is 20 μm (dotted line). If $v_{\max}$ is ten times lower and the other parameters remain the same, the result is the same, as can be seen in Fig. 1, except for the fact that $k_{M,\text{tar}}$ values are ten times smaller. The diffusion module σ^2 decreases by the same number of times. Its smallest value is 0.342 and therefore for some values of a_e and D_{S_e} the response current is controlled by the reaction velocity. In comparison to the curves in Fig. 1, the transition from the kinetic mode to the diffusion mode has not brought about a change in the behaviour

of the curves. The constant was also calculated with different substrate and product diffusion coefficients. If $D_{P_e} = 0.5D_{S_e}$ and $D_{P_e} = 0.1D_{S_e}$ and the other parameters remain the same, we get the same curves as in Fig. 1. A change of the value of the product diffusion coefficient D_{P_e} does not affect the diffusion module σ^2.

The curves in Fig. 1(b) show how $k_{M,\mathrm{tar}}$ values change for different initial Michaelis constant k_M values when $v_{\max} = 1.1$ mM/s. If $k_M = 2.3$ mM, the response is controlled by the reaction velocity, as with the diffusion coefficients from the range studied the diffusion module is less than one. As the Michaelis constant decreases by ten times, the diffusion module increases by the same number of times. The response is therefore limited by diffusion when $k_M = 0.23$ and 0.023 mM. When the diffusion coefficient value is the same, the diffusion modules corresponding to three k_M values differ by ten times. The response being controlled by diffusion results in a small difference between $k_{M,\mathrm{tar}}$ values (solid and dotted lines). However, the difference is substantial when the type of response control changes (solid and dashed lines). If the response is controlled by diffusion, in order to obtain half the current value, the highest substrate concentration is needed when the diffusion module is high and the diffusion is low. If the response depends on the reaction velocity, the biggest $k_{M,\mathrm{tar}}$ value is when the diffusion module is close to one and the diffusion coefficient is small.

The curves in Fig. 2(a) demonstrate how the apparent Michaelis constant changes with the increase in the enzymatic layer thickness and with different maximum reaction velocities. Within the studied range of the varying parameters a_e and D_{S_e}, the diffusion module is greater than one. As before, the greatest $k_{M,\mathrm{tar}}$ value corresponds to the greatest diffusion module and low diffusion (dashed line). As the enzyme layer thickens, $k_{M,\mathrm{tar}}$ increases monotonically. The curves in Fig. 2(b) confirm that the diffusion coefficient has a significant influence on the apparent Michaelis constant. The two solid lines and the two dashed lines do not differ much from each other when the Michaelis constant values are ten times different from one another. As the enzyme layer thickens, $k_{M,\mathrm{tar}}$ values increase significantly when diffusion is low (dashed lines).

Figure 3(a) illustrates how $k_{M,\mathrm{tar}}$ depends on the diffusion coefficients $D_{S_m} = D_{P_m}$. For all the D_{S_m} values curves (1) and (2) correspond to $\sigma^2 = 13.7$, and curves (3) and (4) correspond to $\sigma^2 = 68.3$. The dependence of the apparent Michaelis constant on the diffusion module is now not monotonic. Fig. 3(b) compares the $k_{M,\mathrm{tar}}$ values which were calculated by changing outer membrane thickness. In this case σ^2 does not change as the outer membrane thickness increases.

3 Conclusions

The research into the dependence of the apparent Michaelis constant for one and two-layer biosensors on the parameters characterizing biosensors aimed to determine the relationship between this constant and the diffusion module and establish which parameters have the biggest influence. The results lead to the following conclusions:

1. With knowing solely the diffusion module values, it is impossible to determine exactly how the apparent Michaelis constant value will change.
2. For parameter values within the ranges chosen the greatest apparent Michaelis constant value is obtained when the diffusion module value is the greatest and the diffusion value is the lowest.

3. For parameter values within the ranges chosen the apparent Michaelis constant practically does not change as the product diffusion coefficient increases or decreases when the other parameters are fixed.

References

[1] W.J. Albery and P.N. Bartlet. Amperometric enzyme electrode. Part I. Theory. *J. Electroanal. Chem.*, **194**:211–222, 1985.

[2] R. Baronas, F. Ivanauskas and J. Kulys. *Mathematical Modeling of Biosensors*. Springer Ser. Chem. Sens. Bios. Springer, 2010.

[3] F. Ivanauskas, P. Katauskis and V. Laurinavičius. Mathematical modeling of biosensor action in the region between diffusion and kinetic modes. *J. Math. Chem.*, **52**:689–702, 2014.

[4] F. Ivanauskas, I. Kaunietis, V. Laurinavičius, J. Razumienė and R. Šimkus. Apparent Michaelis constant of the enzyme modified porous electrode. *J. Math. Chem.*, **57**:1516–1526, 2011.

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

[6] O. Štikonienė, F. Ivanauskas and V. Laurinavicius. The influence of external factors on the operational stability of the biosensor response. *Talanta*, **81**:1245–1249, 2010.

REZIUMĖ

Biojutiklių signalo pusėjimo konstantos skaičiavimas, naudojant reakcijos-difuzijos lygtis

P. Katauskis, F. Ivanauskas, S. Laukevičius

Naudojant matematinį modelį, aprašantį amperometrinių biojutiklių veikimą, tiriamas vieno ir dviejų sluoksnių biojutiklių tariamos Michaelio konstantos ir difuzijos modulio ryšys.

Raktiniai žodžiai: biojutiklis, tariamoji Michaelio konstanta, difuzijos modulis.