Apparent Parameters of Enzymatic Plate-Gap Electrode[*]

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Abstract. It was suggested that reaction-diffusion conditions in pores of bulk enzymatic electrode resemble particular conditions in thin enzyme filled gap between parallel conducting plates. The plate-gap model of porous enzymatic electrode is based on the diffusion equations containing a nonlinear term related to the Michaelis-Menten kinetics of the enzymatic reaction inside the gap. Steady state current was calculated for the wide range of given values of substrate diffusion coefficient, depth of the gap and substrate concentrations. Simple approximate relationships between "apparent" parameters of amperometric biosensor (maximal currents and apparent Michaelis constants) and given values of diffusion characterising parameters were derived. Association of these dependences with previously reported relationships led to derive approximate formulae that bind apparent parameters with the complete set of given parameters of the plate-gap enzymatic electrode. The limit case of slow diffusion into deep gap was also characterised. In this specific case, the highest numerical values of the apparent parameters were obtained. However, this gain is achievable at the expense of biosensor response time.

Keywords: reaction-diffusion, modelling, amperometric biosensors, porous electrode.

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1 Introduction

Mathematical modelling is often a useful tool in optimizing the performance of amperometric biosensors [1–4]. Analysis of data by appropriate model can enable us to find out how the response of a particular system can be improved. Amperometric biosensor measures the faradaic current that arises on a working electrode due to electrochemical oxidation or reduction of the products of the biochemical reaction [1–4]. A working electrode is considered to be a complex device consisting of the conducting electrode (metal, carbon, or carbon paste) coated with a biochemical film [1]. Such definitions suggest planar structure of working electrode that is considered in most known to date mathematical models of amperometric biosensors [1–4]. These approaches omit a specific but also widely used in practice class of "non-planar" biosensors that are based on bulk modification of entire electrode material e.g., enzyme modified porous carbon electrodes [5]. Recently, the plate-gap model of porous electrode was suggested [6]. It was shown that reaction-diffusion conditions in pores of bulk electrode resemble particular conditions in the thin gap between parallel conducting plates. The calculations [6] were carried out just for constant values of diffusion coefficients of substrate and product of the enzymatic reaction. The aim of the present paper is computer simulation of the steady state currents at theoretic plate-gap electrode under various diffusion conditions, which are characterized by substrate/product diffusivity D_f (the diffusion coefficients of substrate and product molecules can be assumed to be equal to each other [4,6]) and the depth d_x of the gap. The dependences of calculated steady state currents I on concentrations of the substrate of the enzymatic reaction S can be fitted by the hypherbolic function [6]:

$$I = I_{max}^{app} S/(K_M^{app} + S), \tag{1}$$

where I_{max}^{app} (apparent maximal current) and K_M^{app} (apparent Michaelis constant) are the best fit parameters characterizing an action of the enzymatic biosensors [7]. Therefore, the aim of the current work can be specified as a disclosure of the relationships between commonly used "apparent" parameters and reaction-diffusion parameters of the theoretical plate-gap enzymatic electrode.

2 Plate-gap model of enzyme doped porous electrode

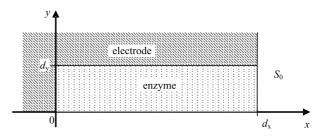During an enzyme-catalyzed reaction

$$S \xrightarrow{E} P \qquad (2)$$

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

$$\nu = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{V_{max}S}{K_M + S}, \qquad (3)$$

where $\nu = \nu(S)$ is the rate of the enzymatic reaction, V_{max} is the maximum enzymatic rate attainable with that amount of enzyme, when the enzyme is fully saturated with substrate, K_M is the Michaelis constant, and t is time.

Let us try to model the reaction-diffusion processes in porous electrode. One can suggest two key features of enzyme doped porous electrode. Firstly, enzyme activity is gradually dispersed in the volume of porous electrode. Secondly, the distances between the enzymatic reaction sites and conducting walls of porous electrode are as short as an average radius of pores. Moreover, pores overlap forming a continuous 3-dimentional network. Considering these features of enzyme modified porous electrode one can suggest the plate-gap model for this reaction-diffusion system. According to this physical model, the enzyme activity is uniformly dispersed in the gap between two parallel conducting plates. The modelled physical system, in general, mimics the main features of the porous electrode. Firstly, the uniform dispersion of the enzyme activity is affirmed according to the definition of the modelled physical system. Secondly, the gap-width dependent characteristic distances between the enzymatic reaction sites and conducting plates of modelled system can be admitted to be similar to the average radius of pores in the porous electrode. In addition, the substrate or product molecules in the modelled plate-gap electrode may diffuse distantly in the directions, which are parallel to the surface of electrode, i.e., as it is in the 3-dimentional network of porous electrode.

To formulate the corresponding mathematical model we assume the symmetrical geometry of the electrode and uniform distribution of the immobilized en-

zyme. This allows formulating the model in two spatial dimensions (Fig. 1). The

Fig. 1. The profile of the biosensor electrode.

dynamics of the considered biosensor system can be described by the reaction-diffusion system

$$\frac{\partial S}{\partial t} = D_f \left(\frac{\partial^2 S}{\partial x^2} + \frac{\partial^2 S}{\partial y^2} \right) - \frac{V_{max} S}{K_M + S},$$

$$0 < x < d_x, \quad 0 < y < d_y, \quad 0 < t \leq T,$$

$$(4)$$

$$\frac{\partial P}{\partial t} = D_f \left(\frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial y^2} \right) + \frac{V_{max} S}{K_M + S},$$

$$0 < x < d_x, \quad 0 < y < d_x, \quad 0 < y \leq T,$$

$$(5)$$

where $S = S(x, y, t)$ is the substrate concentration, $P = P(x, y, t)$ is concentration of the reaction product, D_f is the diffusion coefficient which was assumed to equal for the substrate and the reaction product, d_x is depth of gap; $2d_y$ is gap width; T is full time of biosensor operation to be analyzed.

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

$$S(d_x, y, 0) = S_0, \quad 0 \leq y \leq d_y, \tag{6}$$

$$S(x, y, 0) = 0, \qquad 0 \leq x < d_x, \quad 0 \leq y \leq d_y, \tag{7}$$

$$P(x, y, 0) = 0, \qquad 0 \leq x \leq d_x, \quad 0 \leq y \leq d_y, \tag{8}$$

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

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

$$S(d_x, y, t) = S_0, \quad 0 \leq y \leq d_y, \tag{9}$$

$$P(d_x, y, t) = 0, \quad 0 \leq y \leq d_y, \tag{10}$$

$$P(0, y, t) = 0, \quad 0 \leq y \leq d_y, \tag{11}$$

$$P(x, d_y, t) = 0, \quad 0 \leq x \leq d_x, \tag{12}$$

$$\left.\frac{\partial S(t, x, y)}{\partial x}\right|_{x=0} = 0, \quad 0 \leq y \leq d_y, \tag{13}$$

$$\left.\frac{\partial S(t, x, y)}{\partial y}\right|_{y=d_y} = 0, \quad 0 \leq x \leq d_x, \tag{14}$$

$$\left.\frac{\partial S(t, x, y)}{\partial y}\right|_{y=0} = 0, \quad 0 \leq x \leq d_x, \tag{15}$$

$$\left.\frac{\partial P(t, x, y)}{\partial y}\right|_{y=0} = 0, \quad 0 \leq x \leq d_x. \tag{16}$$

The current is measured as a response of biosensor in a physical experiment. The current depends upon the flux of reaction product at the electrode surface ($x = 0$, $0 \leq y \leq d_y$ and $y = d_y$, $0 \leq x \leq d_x$). Consequently, density $I(t)$ of the current at time t is proportional to the concentration gradient of the product at the surface of the electrode as described by Faraday's law:

$$I(t) = n_e F D_f \left(\int_0^{d_y} \left.\frac{\partial P}{\partial x}\right|_{x=0} dy + \int_0^{d_x} \left.\frac{\partial P}{\partial y}\right|_{y=d_y} dx \right), \tag{17}$$

where n_e is the number of electrons involved is a charge transfer at the electrode surface, and F is Faraday constant, $F \approx 9.65 \cdot 10^5$ C/mol. The steady state current I of the plate-gap electrode is defined as:

$$I = \lim_{t \to \infty} I(t). \tag{18}$$

3 Digital simulation

The following values of the parameters were constant in the numerical simulation of all the experiments

$$\begin{aligned} &K_M = 10^{-6} \text{ mol/cm}^3, \quad V_{max} = 10^{-7} \text{ mol/cm}^3\text{s}, \\ &2d_y = 10^{-4} \text{ cm}, \quad n_e = 2. \end{aligned} \tag{19}$$

The numerical solution of the model was evaluated for different values of the diffusivity D_f (ranged from 10^{-6} to $4 \cdot 10^{-8}$ cm^2/s) and the depth d_x of the gap

(ranged from 0.0004 to 0.01 cm). The steady state currents (18) were calculated for the modelled biosensors responding to the substrate concentrations S_0 (ranged from $3 \cdot 10^{-8}$ to $8 \cdot 10^{-6}$ mol/cm^3). The values of all selected parameters are of the orders that are typical for the amperometric biosensors.

In Figs. 2–4 the numerical values of the steady state currents are plotted against concentration of the substrate for different values of parameters D_f and d_x. The steady state current is practically independent from the diffusion coeffici-
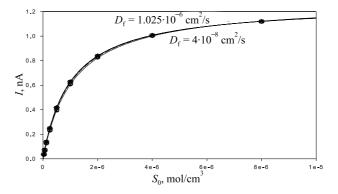

Fig. 2. Theoretical (points) and approximated by hyperbolas responses of the plate-gap electrode for different values of D_f: $4 \cdot 10^{-8}$ (●), $6 \cdot 10^{-8}$ (○), $9 \cdot 10^{-8}$ (▼), $1.35 \cdot 10^{-7}$ (▽), $2 \cdot 10^{-7}$ (■), $3 \cdot 10^{-7}$ (□), $4.5 \cdot 10^{-7}$ (♦), $6.8 \cdot 10^{-7}$ (◇), $1 \cdot 10^{-6}$ (▲) cm^2/s. Given parameter $d_x = 0.0004$ cm.
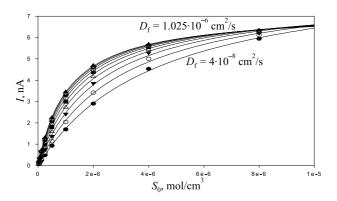

Fig. 3. Theoretical (points) and approximated by hyperbolas responses of the plate-gap electrode for different values of D_f (the numerical values and corresponding symbols as in Fig. 2); $d_x \approx 0.002$ cm.
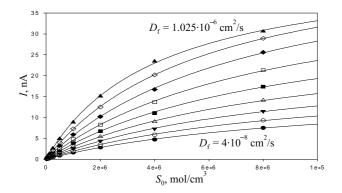
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Fig. 4. Theoretical (points) and approximated by hyperbolas responses of the plate-gap electrode for different values of D_f (the numerical values and corresponding symbols as in Fig. 2); $d_x \approx 0.01$ cm.

ent when the gap is very shallow (Fig. 2). On the other hand, the current is strongly dependent on the diffusion coefficient when the gap is deep (Fig. 4). Seemingly, the difference between these two regimes is whether the response is controlled by enzyme kinetics or by diffusion [1, 4]. As can be seen in Figs. 2–4, each calibration curve can be fairly well approximated by hyperbolas. As was mentioned above, the functions like (1) characterize an action of the enzymatic biosensors [1, 7]. Therefore, we were able: (i) to calculate parameters I_{max}^{app}, K_M^{app}; (ii) and to plot the relationships between these "apparent" parameters and given parameters D_f, d_x.

In Fig. 5 the numerical values of apparent maximal currents of biosensors I_{max}^{app} are plotted against parameter d_x for different values of D_f. It can be seen in this figure that I_{max}^{app} is approximately linearly dependent on d_x and is practically independent on D_f until an appropriate saturation regime is reached. It was estimated that the saturation is started when the characteristic diffusion time parameter d_x^2/D_f is ~ 240 s. When the characteristic time parameter exceeds the appropriate critical value the apparent maximal currents of biosensors are independent on the depth of the gap. Let us define these specific conditions $(d_x^2/D_f > 240$ s) as "saturation conditions". Under saturation conditions the substrate does not penetrate into the deepest zone of the gap without participation in the enzymatic reaction. Consequently, just an upper layer of the gap is working or is "effective" what results in the depth-independent maximal current of the
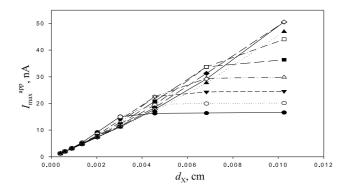
biosensor (Fig. 5).

Fig. 5. Dependences of apparent maximal currents on parameter d_x for different values of D_f (the numerical values and corresponding symbols as in Fig. 2)

Fig. 6 shows the curves of dimensionless parameter K_M^{app}/K_M as a function of $1/D_f$ for different values of d_x. Here the saturation regime is also observed
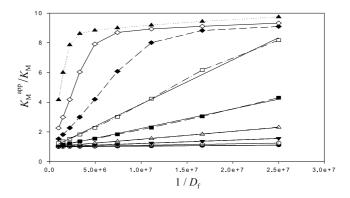

Fig. 6. Dependences of dimensionless parameter K_M^{app}/K_M on parameter $1/D_f$ for different values of d_x: 0.0004 (●), 0.0006 (○), 0.0009 (▼), 0.00135 (▽), 0.002 (■), 0.003 (□), 0.0045 (◆), 0.0068 (◇), 0.01 (▲) cm.

when the substrate diffuses slowly into a relatively deep gap. For the set of given parameters the dimensionless parameter K_M^{app}/K_M saturates at the values close to 10. If one takes into account just moderate parameters D_f and d_x which result in $d_x^2/D_f < 240\,\mathrm{s}$ the simple relationships between apparent and given parameters

can be derived. In this case all curves (Fig. 6) can be approximated by the straight lines ($y = a + bx$) with parameter a approximately equal to 1. The corresponding slope of these straight lines b is approximately proportional to the square of the parameter d_x (Fig. 7). All these observations can be summarized in a simple set
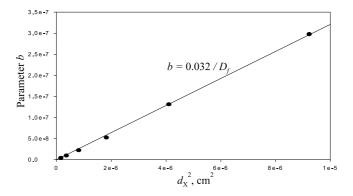

Fig. 7. Slope of straight lines shown in Fig. 6 as a function of d_x^2.

of approximate equations describing the relationships between parameters of the plate-gap enzymatic electrode operating under unsaturated conditions:

$$I_{max}^{app} \approx A_1 d_x, \tag{20}$$

$$K_M^{app}/K_M \approx 1 + A_2 d_x^2/D_f, \tag{21}$$

where A_1 and A_2 are constants ($A_1 \approx 3992.89$, $A_2 \approx 0.032$) representing a set of given parameters K_M and V_{max}. The dependences of I_{max}^{app} and K_M^{app}/K_M on V_{max} and K_M were derived in our previous work on plate-gap electrode [6]:

$$I_{max}^{app} \approx B_1 V_{max}, \tag{22}$$

$$K_M^{app}/K_M \approx 1 + B_2 V_{max}/K_M, \tag{23}$$

where B_1 and B_2 are constants representing a set of given parameters d_x and D_f. The combination of these approximate formulae (20)–(23) result in the final relationship between apparent parameters and the complete set of the reaction-diffusion parameters of the plate-gap electrode operating under unsaturated con-

ditions ($d_x^2/D_f < 240\,\text{s}$):

$$I_{max}^{app} \approx C_1 V_{max} d_x, \tag{24}$$

$$K_M^{app}/K_M \approx 1 + C_2 V_{max} d_x^2 / K_M D_f, \tag{25}$$

where C_1 and C_2 are constants ($C_2 \approx A_2 K_M/V_{max} \approx 0.32$). In practice, I_{max}^{app} is a measure of the sensitivity, and K_M^{app} is a measure of the linearity of the amperometric biosensor [1, 7]. Therefore, it is preferable to design biosensors exhibiting high values of I_{max}^{app} and K_M^{app}. As was shown above, the highest numerical values of these apparent parameters are observable when biosensors are operating under saturated conditions. However, in this case, the high values of the apparent parameters are achieved at the expense of biosensor response time (which is of order $\sim d_x^2/D_f$). Obviously, the saturated regime cannot be regarded as preferable when fast response times (< 4 min) are required. Fast responses are potentially obtainable at electrodes operating under unsaturated conditions. The optimisation of these electrodes can be carried out using simple equations (24) and (25).

4   Conclusions

The reaction diffusion conditions in porous electrodes have been modelled. Two-dimensional plate-gap model of enzyme doped electrode (4)–(18) allows to calculate the steady state currents for the wide range of given parameters (Figs. 2–4). Theoretical calibration curves of modelled biosensors can be readily approximated by hyperbolas (Figs. 2–4) in order to obtain apparent parameters which are legitimated in practical experimentation. Relationships between apparent and given parameters are rather simple when unsaturated conditions are taken into account (Figs. 5–7). Simple set of approximate relationships between apparent and given parameters (24), (25) has been derived for the case of unsaturated conditions. The saturated conditions of the enzymatic electrode result in the high values of apparent parameters. However, this gain is achievable at the expense of biosensor response time, which inevitably must exceed several minutes.

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