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Computer simulation of the steady state currents at enzyme doped carbon paste electrodes

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The plate-gap model of porous enzyme doped electrode has been proposed and analyzed. It was suggested that reaction diffusion conditions in pores of bulk electrode resemble particular conditions in thin gap between parallel conducting plates. The model is based on the diffusion equations containing a nonlinear term related to the Michaelis–Menten kinetic of the enzymatic reaction inside gap. Steady state current was calculated for the wide range of given parameters and substrate concentrations. All dependences of current on substrate concentration were approximated by hyperbolas in order to obtain "apparent" parameters (maximal currents and apparent Michaelis constants) of modelled biosensors. Simple approximate relationships between given and apparent parameters were derived. The applicability of theoretical plate-gap model was tested for the case of carbon paste electrodes which were doped with PQQ – dependent glucose dehydrogenase. It was found, that soluble glucose dehydrogenase based biosensors exhibit characteristic features of the theoretical plate-gap biosensors.

KEY WORDS: reaction–diffusion, modelling, amperometric biosensors, carbon, PQQ-dependent dehydrogenase

1. Introduction

The most widely used type of biosensors are amperometric biosensors [1]. Mathematical modelling is often a useful tool in optimizing the performance of these biosensors [2–7]. 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

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by electrochemical oxidation or reduction of the products of the biochemical reaction [1–8]. 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 which is considered in most known to date mathematical models of amperometric biosensors [2–7]. It should be noted, that 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 [8]. The dimensionality of such electrodes is rarely taken into account. As an exceptional case, the simulations of carbon fibre microelectrodes could be mentioned [9].

The aim of present work was computer simulation of steady state currents at enzyme modified screen printed porous electrode under specific to this system reaction–diffusion conditions. Dependences of the catalytic current on concentration of substrates of enzymatic reaction represent theoretical calibration curves of the modelled amperometric biosensor. Sets of theoretical calibration curves enable to obtain specific to porous electrode "apparent parameters" (Michaelis constants and maximal currents) characterizing amperometric biosensor as an analytical device [10]. The final goal of this work was verification of the proposed model by comparison of theoretical and experimental responses for biosensors based on PQQ-dependent glucose dehydrogenases.

2. Materials and methods

Carbon black (CB) was synthesized from carbon monoxide during the Boudouard's reaction on the Fe catalyst [11]:

$$2CO_{(g)} - (Fe_{(k)}) \rightarrow C_{(gr)} + CO_{2(g)}$$

followed by subsequent purification with HCl [12].

The protocol of CBs: C1 and C2 modification consisted of nine technological operations. Objectives of using these operations can be divided into three blocks:

(a) delamination of graphite structure carried out using H_2SO_4 (C1), KOH (C1, C2), and H_3BO_3 (C1, C2) [13];

(b) tailoring of functional groups carried out in the presence of HNO_3 (C2), Br_2 + Fe (C1), H_2O_2 (C1)and $N_2H_4{\cdot}2HCl$ (C2) [14];

(c) modification of particle shape carried out by melting with Zn (C1) and S (C2) [15].

The CB samples were tested microscopically, using an optical microscope LEITZ, and an atomic force microscope Digital Instruments NanoScope III. The average diameter of carbon particles was ~20 nm (C1) and ~100 nm (C2).

The screen-printed carbon electrodes (CE) were designed as described previously [16]. Aiming to prepare CE with different roughness, carbon samples C1 and C2 were mixed in following ratios: 100:0; 75:25; 50:50; 25:75 and 0:100. Further all of these mixed carbon examples were used for CE preparation in similar way. Two types of PQQ-glucose dehydrogenases: intracellular soluble glucose dehydrogenase (s-GDH) L.M.D. 79.41 from *Acinetobacter calcoacetics* purified according to the known protocol [17] (specific activity 1568 U/mg) and the membrane-bound enzyme (m-GDH) (EC1.1.99.17) purified from *Erwinia* sp. 34-1 [18] (specific activity 12 U/mg) were immobilized on the electrode (working area 0.125 cm^2) by adsorption of 3 μl of enzyme solution for 30 min. The layer of an immobilized enzyme was covered with semi-permeable terrylene film.

All electrochemical measurements were performed using a conventional three-electrode system containing screen-printed carbon electrode as the working electrode, a platinum wire as the counter and an Ag/AgCl in saturated KCl as reference electrodes. Steady state currents of biosensors were recorded on the polarographic analyzer PGZ 402 (Radiometer Analytical, France).

About 0.05 M acetate buffer (pH 6.0) containing 1 mM of Ca^{2+} was used as a default buffer. All inorganic reagents were of analytical reagent grade and organic reagents were suitable for electron microscopy. Bidistilled and ultra-filtered water was obtained using a "Purator Bi" (Glas Keramic, Berlin, Germany).

Dependences of experimentally measured steady state currents (I) on concentrations of substrates of enzymatic reactions (S) were fitted by hypherbolic functions:

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

where $I_{\max}^{\text{app}}$ and $K_{\text{M}}^{\text{app}}$ are parameters characterizing action of enzymatic biosensors [10].

3. Plate-gap model of enzyme doped porous electrode

During an enzyme-catalyzed reaction

$$S \xrightarrow{E} P \tag{2}$$

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:

$$\nu = \frac{\mathrm{d}P}{\mathrm{d}t} = -\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{V_{\max} S}{K_M + S}, \tag{3}$$

where $v = v(S)$ is the rate enzymatic reaction, $V_{\max}$ is the maximal 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's try to model the reaction–diffusion processes in porous electrode. One can suggest two key features of enzyme doped porous electrode. First, enzyme activity is gradually dispersed in the volume of porous electrode. Second, the distances between enzymatic reaction sites and conducting walls of porous electrode are as short as an average radius of pores. Taking into account these evident features of enzyme modified porous electrode one can suggest the simple plate-gap model for this reaction–diffusion system. In this physical model enzyme activity is uniformly dispersed in the gap between two parallel conducting plates.

To formulate the corresponding mathematical model we assume the symmetrical geometry of the electrode and uniform distribution of the immobilized enzyme. This allows formulating the model in two spatial dimensions (figure. 1). The dynamics of the considered biosensor system can be described by the reaction-diffusion system

$$\frac{\partial S}{\partial t} = D_f \left(\frac{\partial^2 S}{\partial x^2} + \frac{\partial^2 S}{\partial y^2} \right) - \frac{V_{\max} S}{K_M + S}, \quad 0 < x < d_x, \quad 0 < y < d_y, \quad 0 < t \leqslant T, \quad (4)$$

$$\frac{\partial P}{\partial t} = D_f \left(\frac{\partial^2 P}{\partial x^2} + \frac{\partial^2 P}{\partial y^2} \right) + \frac{V_{\max} S}{K_M + S}, \quad 0 < x < d_x, \quad 0 < y < d_y, \quad 0 < t \leqslant T, \quad (5)$$

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 of substrate in the gap, 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 ($t = 0$)

$$S(d_x, y, 0) = S_0, \quad 0 \leqslant y \leqslant d_y, \quad (6)$$

$$S(x, y, 0) = 0, \quad 0 \leqslant x < d_x, \quad 0 \leqslant y \leqslant d_y, \quad (7)$$

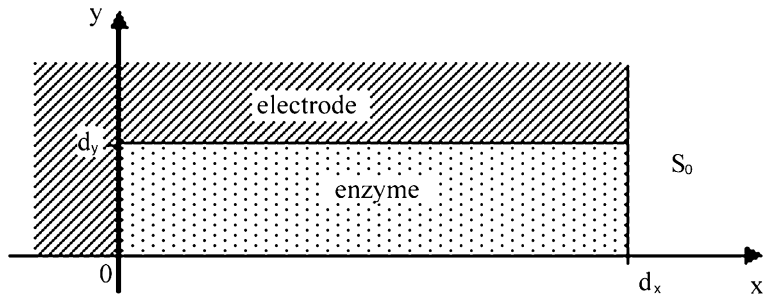

Figure 1. The profile of the biosensor electrode.

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

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

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

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

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

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

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

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

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

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

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

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 ($x = 0$, $0 \leqslant y \leqslant d_y$ and $y = d_y$, $0 \leqslant x \leqslant d_x$). Consequently, a 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:

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

where n_e is number of electrons involved is a charge transfer at the electrode surface, and F is Faraday constant, $F \approx 9.65 \times 10^5$ C/mol.

4. Digital simulation

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

$$D_f = 10^{-6}\mathrm{cm}^2/s, \quad d_x = 5 \cdot 10^{-3}\mathrm{cm}, \quad \mathrm{n}_e = 2. \tag{18}$$

Numerical solution of the model was evaluated for different values of the parameters $V_{\max}(10^{-8}, 2.5{\cdot}10^{-8}, 5{\cdot}10^{-8}, 7.5{\cdot}10^{-8}, 10^{-7}$ mol/cm^{3}s), $K_{\mathrm{M}}(10^{-7}, 2.5{\cdot}10^{-7},$

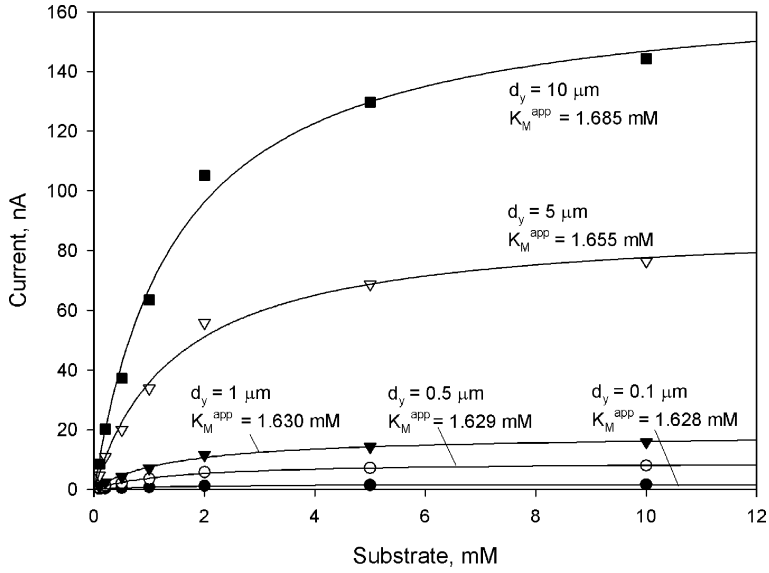

Figure 2. Theoretical dependences of steady state currens on concentration of substrate for different values of gap width (points). Given parameters: $K_M = 1$ mM, $V_{max} = 0.1$ mM/s. Corresponding approximations by hyperbolas as well as numerical values of apparent parameter K_M^{app} are presented.

$5{\cdot}10^{-7}$, $7.5{\cdot}10^{-7}$, 10^{-6}, $2.5{\cdot}10^{-6}$, $5{\cdot}10^{-6}$, $7.5{\cdot}10^{-6}$, 10^{-5} mol/cm^3), $2d_y$ (10^{-5}, $5{\cdot}10^{-5}$, 10^{-4}, $5{\cdot}10^{-4}$, 10^{-3} cm). Steady state currents were calculated for modelled biosensors responding to various substrate concentrations S_0 (10^{-7}, $2.5{\cdot}10^{-7}$, $5{\cdot}10^{-7}$, 10^{-6}, $2.5{\cdot}10^{-6}$, $5{\cdot}10^{-6}$, 10^{-5} mol/cm^3). Values of all selected parameters are of the orders that are typical for real amperometric biosensors. Data of calculations are presented in convenient for biochemical research dimensions: millimoles per litre (mM) for substrate concentrations and mM/s for maximal rates.

Theoretical response-time curves of biosensors for different values of gap width are exemplified in figure 2. According to expectations, higher volume of enzyme filled gap resulted in higher currents. Each curve was approximated by hyperbolas and apparent K_M^{app} of modelled amperometric biosensor were calculated (figure 2). It could be noticed, that, first, apparent Michaelis constants are higher than given parameter K_M, and, second, these apparent constants are independent on gap width, when gap is thin. These features are characteristic to all sets of parameters V_{max} and K_M (data not shown). Therefore, in further numerical simulations we assumed constant gap width ($2d_y = 10^{-4}$ cm).

In figures 3–5 numerical values of steady state currents are plotted against concentration of substrate for different values of parameters K_M and V_{max}. As can be seen on these figures, each calibration curve fairly well can be approximated by hyperbolas. Therefore we were able to plot relationships between "apparent" and given parameters for the families of calibration curves.

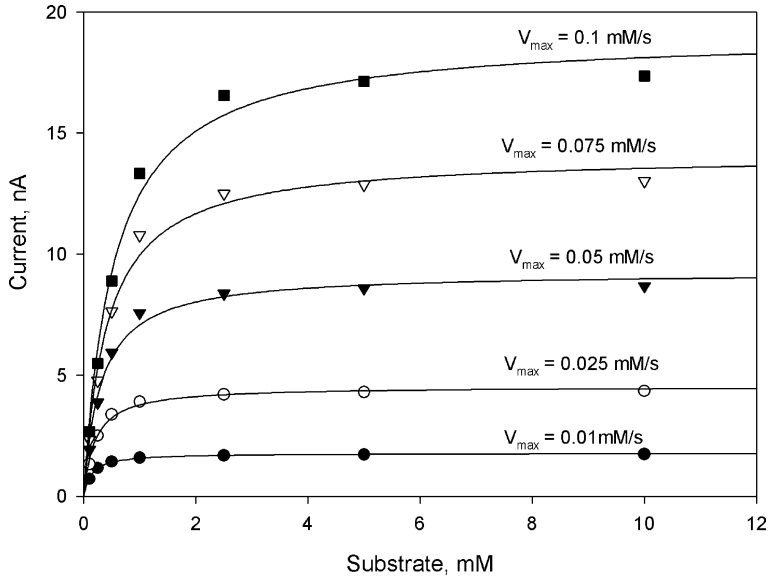

Figure 3. Theoretical (points) and approximated by hyperbolas responses of plate-gap electrode for different values of $V_{\max}$. Given parameters: $K_{\mathrm{M}} = 0.1\,\mathrm{mM}$, $d_y = 10^{-4}\mathrm{cm}$.

In figure 6 numerical values of apparent maximal current of biosensors ($I_{\max}^{\mathrm{app}}$) are plotted against parameter $V_{\max}$ for different parameters K_{M}. It can be seen on this figure that $I_{\max}^{\mathrm{app}}$ is linearly dependent on $V_{\max}$ and is practically independent on K_{M}. Therefore, apparent maximal current can be regarded as a measure of maximal rate of enzymatic reaction inside gap.

Figure 7 shows curves of dimensionless parameter $K_M^{\mathrm{app}}/K_{\mathrm{M}}$ as a function of $V_{\max}$ for different parameters K_{M}. All these curves were approximated by straight lines ($y = a + bx$) with parameter a approximately equal to 1.0. Corresponding slope of these straight lines b is approximately proportional to inverse of given parameter K_{M} (figure 8). All these observations can be summarized in simple set of approximate equations, describing relationships between given and "apparent" parameters of plate-gap enzymatic electrode:

$$I_{\max}^{\mathrm{app}} \approx C_1\, V_{\max}, \tag{19}$$

$$K_{\mathrm{M}}^{\mathrm{app}}/K_{\mathrm{M}} \approx 1 + C_2\, V_{\max}/K_{\mathrm{M}}, \tag{20}$$

where C_1 and C_2 are constants.

5. Experimental verification of mathematical model

Theoretically, if plate-gap model of working electrode is valid for a particular type of biosensor, one can expect that apparent Michaelis constants are not

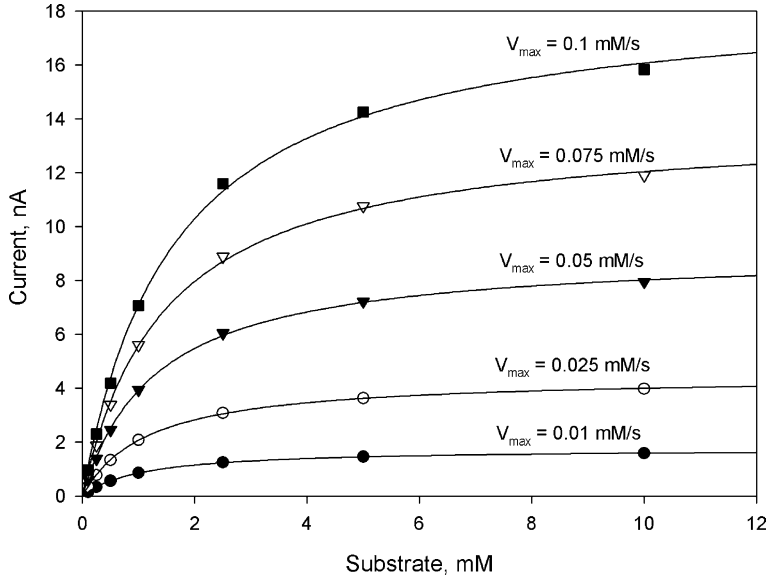

Figure 4. Theoretical (points) and approximated by hyperbolas responses of plate-gap electrode for different values of $V_{\max}$. Given parameters:$K_M = 1\,\text{mM}$, $d_y = 10^{-4}\text{cm}$.

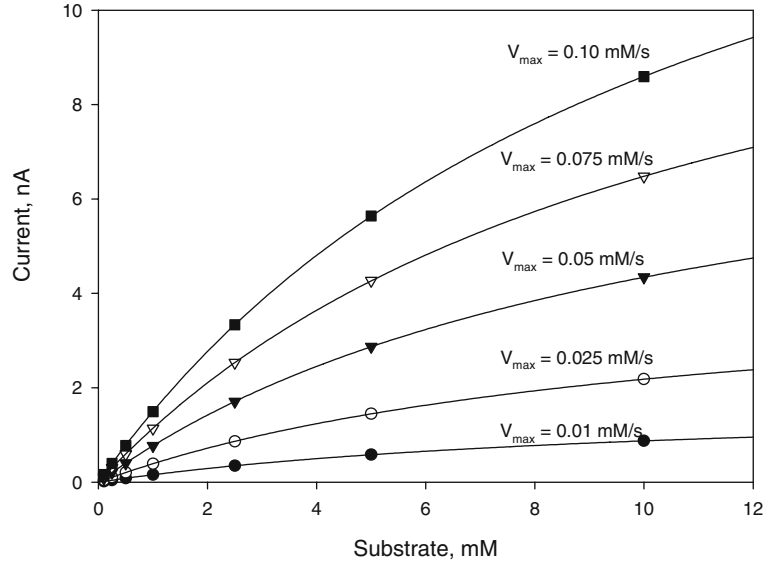

Figure 5. Theoretical (points) and approximated by hyperbolas responses of plate-gap electrode for different values of $V_{\max}$. Given parameters: $K_M = 10\,\text{mM}$, $d_y = 10^{-4}\text{cm}$.

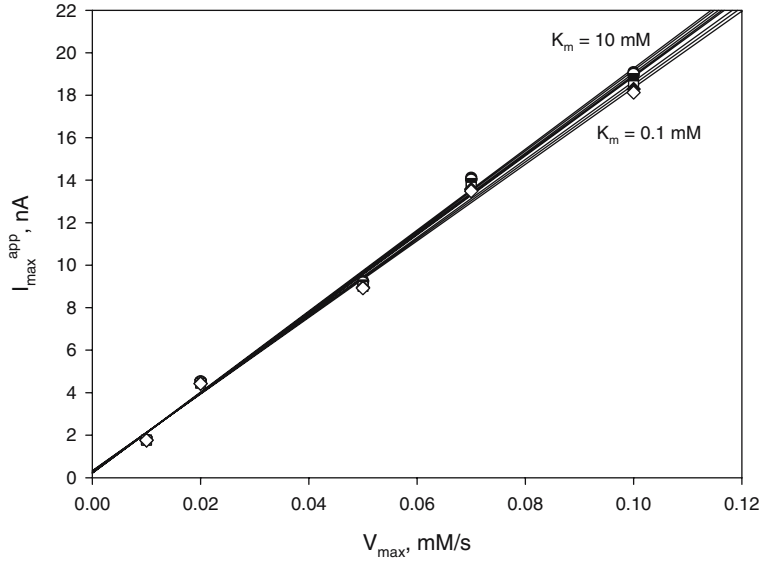

Figure 6. Dependences of apparent maximal currents on given parameters $V_{\max}$ for different parameters K_{M}.

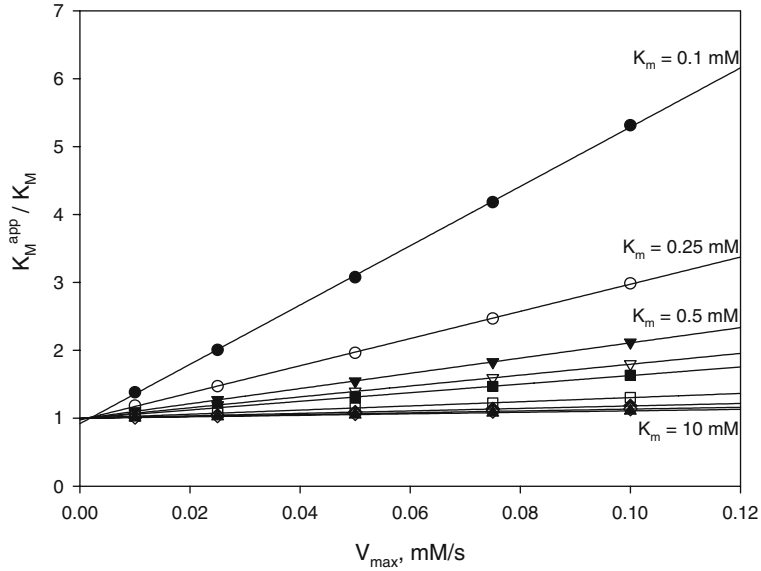

Figure 7. Dependences of dimensionless parameter $K_{\mathrm{M}}^{\mathrm{app}}$ / K_{M} on given parameter $V_{\max}$ for different parameters K_{M}.

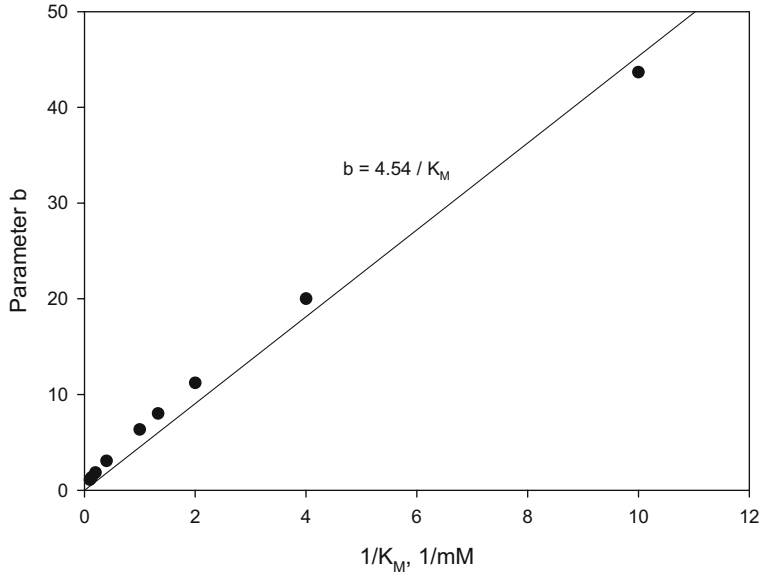

Figure 8. Slope of straight lines shown in figure 7 as a function of $1/K_M$.

dependent on the gap width or diameters of pores in porous electrodes. Experimentally, these diameters were varied by changing proportion of carbons (C1 and C2) in carbon pastes. The dependences of apparent Michaelis constants on composition of carbon paste are shown in figure 9. It can be seen on this figure, that K_M^{app} for biosensors based on soluble GDH is practically independent on the roughness of the paste. Contrary to expectation, apparent Michaelis constant for insoluble GDH based biosensors rise sharply with increasing roughness of the paste. Moreover, biocatalytic currents are not detectable for these electrodes when concentration of rough fraction exceeds 25%. It means, that both enzymes possess different sorption behaviour on carbon paste electrodes as was recently shown [19].

Plate-gap model predict linear relationship between apparent parameters of biosensors (K_M^{app} and I_{max}^{app}). The couples of these parameters were experimentally obtained by changing concentration of soluble GDH and by aging of biosensors. As can be seen in figure 10, there is close to linear correlation between apparent parameters for these biosensors. Presumably, a soluble enzyme is uniformly dispersed in a bulk of electrode and obeys conditions for suggested model.

6. Conclusions

The reaction diffusion conditions in carbon paste electrodes were modelled. Two-dimensional plate-gap model of enzyme doped electrode (4)–(17) allows to

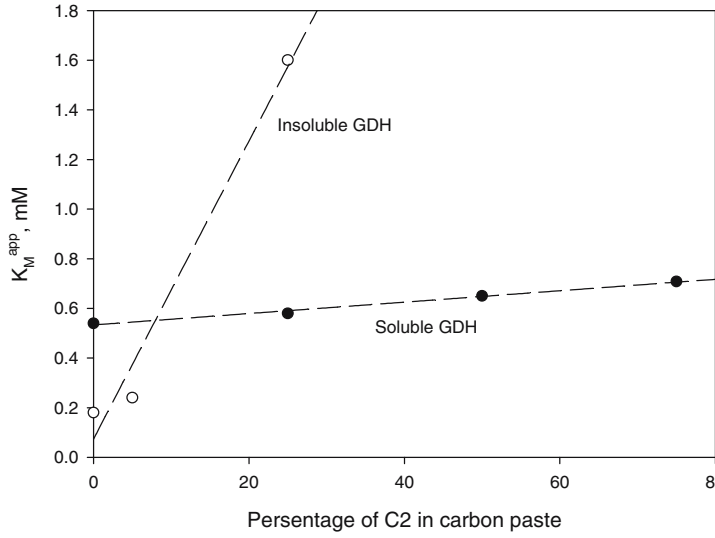

Figure 9. Dependences of apparent Michaelis constants of GDH based biosensors on composition of carbon paste. Concentration of enzyme in doping solution was 10 g/l.

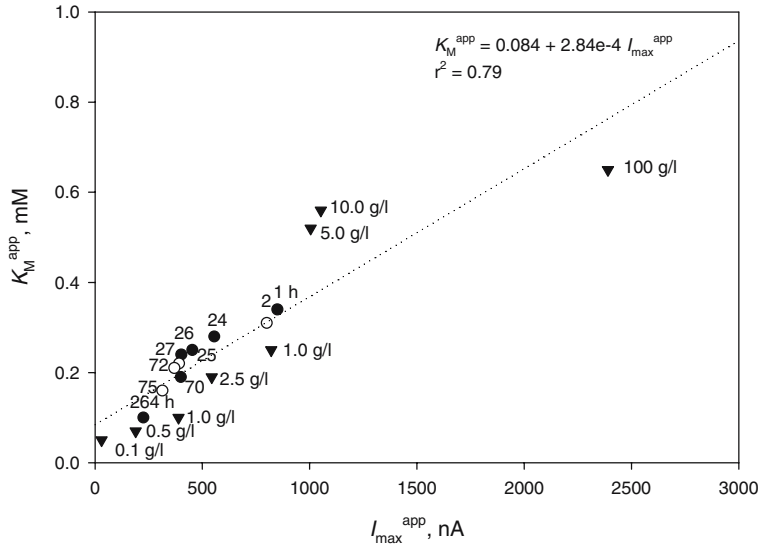

Figure 10. Correlation between parameters of calibration curves for C1 carbon and soluble GDH based biosensor 1 (○), biosensor 2 (●) (for both cases – concentration of enzyme in doping solution was 1.7 g/l) and set of biosensors doped with different amount of enzyme (▼). An age of particular biosensor (in hours) or concentrations of enzyme solutions (g/l) which were used during doping procedure are shown at each point.

calculate steady state currents for the wide range of given parameters (figures. 2–5). Theoretical calibration curves of modelled biosensors can be readily approximated by hyperbolas (figure 2–5) in order to obtain apparent parameters which are legitimated in practical experimentation. Relationships between apparent and given parameters are close to linear (figure 6–8). Simple set of approximate relationships between apparent and given parameters (19–20) indicates that apparent maximal current is a measure of maximal rate of enzymatic reaction and apparent Michaelis constant is a measure of linear combination between given Michaelis constant and given maximal rate. Consequent linear relationship between apparent parameters can be readily tested for real electrodes like was done for the case of PQQ-dependent glucose dehydrogenase modified carbon paste working electrodes (figure 10).

Acknowlegdment

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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] G.G. Guilboult and G. Nagy, Anal. Chem. 45 (1973) 417.
[3] L.D. Mell and J.T. Maloy, Anal. Chem. 47 (1975) 299.
[4] L.D. Mell and J.T. Maloy, Anal. Chem. 48 (1976) 1597.
[5] J. Kulys, V.V. Sorochinski and R.A. Vidziunaite, Biosensors 2 (1986) 135.
[6] T. Schulmeister, J. Rose and F. Scheller, Biosens. Bioelectron. 12 (1997) 1021.
[7] R. Baronas, F. Ivanauskas, J. Kulys and M. Sapagovas, J. Math. Chem. 34 (2003) 227.
[8] L. Gorton, Electroanalysis 7 (1995) 23.
[9] M. Somasundrum and K. Aoki, J. Electroanal. Chem. 530 (2002) 40.
[10] D.R. Thevenot, K. Toth, R.A. Durst and G.S. Wilson, Pure Appl. Chem. 71 (1999) 2333.
[11] G. Brauer (Herausg.), *Hahdbuch der präparativen anorganischen Chemie, B. 2* (Ferdinand Enke Verlag, Stuttgart, 1975).
[12] J. Barkauskas, V. Samanavičiūtė and D. Uždavinienė, J. Therm. Anal. Calorim. 66 (2001) 371.
[13] C.M. Hagg, and M. Skyllas-Kazacos, J. Appl. Electrochem. 32 (2002) 1063.
[14] M.C. Hennion, J. Chromatogr. A 885(2000), 73.
[15] A. Gorbunov, O. Jost, W. Pompe and A. Graff, Carbon 40 (2002) 113.
[16] J. Razumiene, V. Gureviciene, V. Laurinavicius and J.V. Grazulevicius, Sens. & Actuat. B 78 (2001) 243.
[17] P. Dakter, J. Frank, J. Duine., Biochem. J. (part II), 239(1986) 163.
[18] L. Marcinkevičienė, I. Bachmatova, R. Semėnaitė, R. Rudomanskis, G. Bražėnas, R. Meškienė and R. Meškys, R. Biotechnol. Lett. 21(1999) 187.
[19] V. Laurinavičius, J. Razumiene, A. Ramanavicius, A.D. Ryabov. Biosensors & Bioelectron. 20 (2004) 1217.