

# The Numerical Analysis of Enzyme Membrane Thickness on the Response of Amperometric Biosensor

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**Abstract.** This paper presents a one-dimensional-in-space mathematical model of an amperometric biosensor. The model is based on the reaction-diffusion equations containing a non-linear term related to Michaelis-Menten kinetics of the enzymatic reactions. The model is solved numerically by applying the Finite Difference Method (FDM). This model describes the biosensor response to active time of enzymatic reaction in membrane layer. Using numerical solution, the influence of the thickness of enzyme membrane on the biosensor current response was investigated. The numerical results of the biosensor operation showed the monotonous change of the biosensor current response versus the membrane thickness.

**Mathematics Subject Classification:** 65Z05, 65M06

**Keywords:** amperometric biosensor, reaction-diffusion, Michaelis-Menten kinetics, Finite Difference Method, membrane thickness

## 1. Introduction

Biosensors are devices that combine the selectivity and specificity of a biologically active compound with a signal transducer and an electronic amplifier

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[1–4]. The transducer converts the biochemical signal to an electronic signal. The biosensor signal is proportional to the concentration of measured analyte or a group of analytes. The biosensors are classified according to the nature of the physical transducer. Amperometric biosensors measure the current on an indicator electrode due to direct oxidation of the products of the biochemical reaction. In case of the amperometric biosensors the potential at the electrode is held constant while the current flow is measured. The amperometric biosensors are reliable, relatively cheap and highly sensitive for environment, clinical and industrial purposes.

Starting from the publication of Clark and Lyons [1], the amperometric biosensors became one of the popular and perspective trends of biochemistry. The understanding of the kinetic regularities of biosensors is of crucial importance for their design. Mathematical models can explain such regularities. The general features of amperometric response were analyzed in the publications of Mell and Maloy [5, 6]. Some later reports were also devoted to the modeling and investigation of the amperometric biosensor response [7–11].

The developed model is based on non-stationary diffusion equations [12], containing a non-linear term related to Michaelis-Menten kinetic of the enzymatic reaction. The numerical method of the biosensor response was carried out using the Finite Difference Method (FDM) [13, 14]. The software has been programmed in C language. The program built was employed to investigate the influence of the enzyme membrane thickness, substrate concentration as well as the maximal enzymatic rate on biosensor response.

## 2. Mathematical Model

During an enzyme-catalysed reaction



the mixture of substrate ( $S$ ) binds to the enzyme ( $E$ ) to form enzyme-substrate complex. While it is a part of this complex, the substrate ( $S$ ) is converted to the product ( $P$ ). The rate of the reaction is the rate of appearance of the product. This rate is known to depend upon the concentration of substrate.

Let us consider an amperometric biosensor, which can be treated as enzyme electrode, having a layer of enzyme immobilized onto the surface of the probe. Assuming no interaction between analysed substrates (compounds) of the mixture, the symmetrical geometry of the electrode, homogeneous distribution of immobilized enzyme in the enzyme reaction with the diffusion described by Fick's law leads to the following equations:

$$\frac{\partial S}{\partial t} = D_S \frac{\partial^2 S}{\partial x^2} - \frac{V_{\max} S}{K_M + S}, \quad 0 < x < d, 0 < t \leq T, \quad (2)$$

$$\frac{\partial P}{\partial t} = D_P \frac{\partial^2 P}{\partial x^2} + \frac{V_{\max} S}{K_M + S}, \quad 0 < x < d, 0 < t \leq T, \quad (3)$$

where  $V_{\max}$  is the maximal enzymatic rate of biosensor attainable with that amount of enzyme, when the enzyme is fully saturated with substrate ( $S$ ),  $K_M$  is Michaelis constant,  $S$  is the concentration of substrate ( $S$ ),  $P$  is concentration of the reaction product ( $P$ ),  $d$  is thickness of the enzyme layer,  $t$  is time,  $T$  is full time of biosensor operation to be analysed,  $D_S$  and  $D_P$  are diffusion coefficients of the substrate  $S$  and product  $P$ , respectively.

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

$$S(x,0) = \begin{cases} 0, & 0 \leq x < d, \\ S_0 & x = d, \end{cases} \quad (4)$$

$$P(x,0) = 0, \quad 0 \leq x \leq d, \quad (5)$$

where  $S_0$  is the concentration of substrate initial over the biosensor.

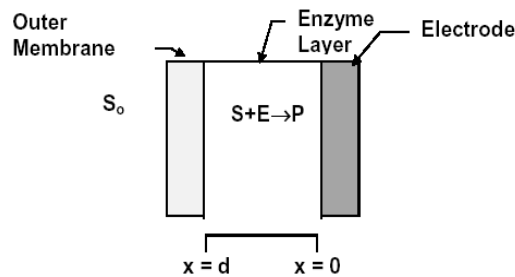


Fig. 1 Schematic representation of enzyme-electrode surface in amperometric biosensor

Because of electrode polarization, the concentration of the reaction product at the electrode surface is being permanently reduced to zero. If the substrate is well-stirred and in powerful motion, then the diffusion layer ( $0 < x < d$ ) will remain at a constant thickness. Consequently, the concentration of substrate as well as product over the enzyme surface (bulk solution/membrane interface) remains constant while the biosensor contact with the substrate. When the analyte disappears, a buffer solution swills the enzyme surface, reducing the substrate concentration at this surface to zero. Because of substrate (analyte) remaining in the enzyme membrane, the mass diffusion as well as the reaction still continues some time even after the disconnected of the biosensor and substrate.

This is used in the boundary conditions ( $0 < t \leq T$ ) given by:

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

$$S(d,t) = \begin{cases} S_0, & t = 0, \\ 0, & t > 0, \end{cases} \quad (7)$$

$$P(0,t) = P(d,t) = 0. \quad (8)$$

The current is measured as a response of a biosensor in a physical experiment. The biosensor current depends upon the flux of reaction product at the electrode surface, i.e., at border  $x = 0$ . Consequently, density  $I(t)$  of the biosensor current, as a results of the reaction of the substrate  $S$  with the product  $P$  at time  $t$ , is proportional to the concentration gradient of the product at the surface of the electrode as described by Faraday's law:

$$I(t) = n_e F D_P \left. \frac{\partial P}{\partial x} \right|_{x=0}, \quad (9)$$

where  $n_e$  is a number of electrons involved in a charge transfer at the electrode surface, and  $F$  is Faraday constant,  $F = 96485$  C/mol.

### 3. Solution of the Mathematical Model

Let us assume the problem (2)-(8) formulation for a substrate  $S$  and reaction product  $P$ . Let  $V_{\max}$  be the maximal enzymatic rate of the modeled biosensor,  $S$  is the concentration of substrate  $S$  and  $P$  is concentration of the reaction product  $P$ . The problem (2)-(8), reformulated for substrate  $S$  and reaction product  $P$ , was solved numerically using FDM. 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:

$$\begin{aligned} \omega_h &= \{x_i : x_i = ih, \quad i = 0, \dots, N_1; hN_1 = d\}, \\ \omega_\tau &= \{t_j : t_j = j\tau, \quad j = 0, \dots, N_A, \dots, N_2; \tau N_A = T_A, \tau N_2 = T\}. \end{aligned} \quad (10)$$

Let us assume the following:

$$S_i^j = S(x_i, t_j), \quad P_i^j = P(x_i, t_j), \quad i = 0, \dots, N_1; \quad j = 0, \dots, N_2. \quad (11)$$

An implicit linear finite difference scheme has been built as a result of the difference approximation. The initial conditions (4) and (5) we approximated as follows:

$$\begin{aligned} S_i^0 &= 0, \quad i = 0, \dots, N_1 - 1, \quad S_{N_1}^0 = S_0, \\ P_1^0 &= 0, \quad i = 0, \dots, N_1. \end{aligned} \quad (12)$$

Differential equations (2) and (3) were approximated by scheme:

$$\frac{S_i^{j+1} - S_i^j}{\tau} = D_S \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} - \frac{V_{\max} S_i^{j+1}}{K_M + S_i^j}, \quad i = 1, \dots, N_1 - 1, \quad (13)$$

$$\frac{P_i^{j+1} - P_i^j}{\tau} = D_P \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} + \frac{V_{\max} S_i^{j+1}}{K_M + S_i^{j+1}}, \quad j = 1, \dots, N_2 - 1. \quad (14)$$

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

$$\begin{aligned} S_0^j &= S_i^j, \quad j=1, \dots, N_2, \\ S_N^j &= S_0, \quad j=1, \dots, N_A, \end{aligned} \quad (15)$$

$$\begin{aligned} S_N^j &= 0, \quad j = N_A + 1, \dots, N_2, \\ P_0^j &= 0, \quad P_{N_1}^j = 0, \quad j=1, \dots, N_2. \end{aligned} \quad (16)$$

Equations (12) allow to calculate a solution of the problem on the layer  $t = t_0 = 0$ . When a solution on a layer  $t_j$  has been calculated, a solution on the next layer  $t = t_{j+1}$  can be calculated in two steps:

- 1) calculate values of  $S_i^{j+1}$ ,  $i = 0, \dots, N_1$ , solving the system of linear equations (13), (15);
- 2) calculate values of  $P_i^{j+1}$ ,  $i = 0, \dots, N_1$ , solving the system of linear equations (14), (16) using values of  $S_i^{j+1}$ , which have been calculated in step 1.

The system of linear algebraic equations can be solved efficiently in both steps above because of the tridiagonality of matrices of the systems.

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

$$I(t_j) = \frac{n_e F D_P (P_1^j - P_0^j)}{h}, \quad j = 0, \dots, N_2. \quad (17)$$

In step 1), only values of the following parameters:  $D_S$ ,  $D_P$ ,  $V_{\max}$ , and  $S_0$  vary when one computer simulation changes the next one.

#### 4. Data Synthesis

The develop computer simulation software was employed to generate data for a calibration of an amperometric biosensor. The mathematical model as well as the numerical solution of the problem was evaluated for different values of the membrane thickness  $d$ . The following values of the parameters were constant in the numerical simulation of all the experiments:

$$\begin{aligned} D_S &= D_P = 3 \times 10^{-6} \text{ cm}^2/\text{s}, \\ K_M &= 1 \times 10^{-7} \text{ mol/cm}^3, \\ n_e &= 2, \\ d &= 0.001, 0.0014, 0.0018, 0.0022, 0.0026 \text{ and } 0.003 \text{ cm}. \end{aligned}$$

The evolution of the biosensor currents were characterized by the following value of the maximal enzymatic rate  $V_{\max}$ :

$$V_{\max} = 1 \times 10^{-8} \text{ mol/cm}^3 \text{ s}.$$

The value of the substrate concentration  $S_0$  of the evolution of the biosensor currents was employed:

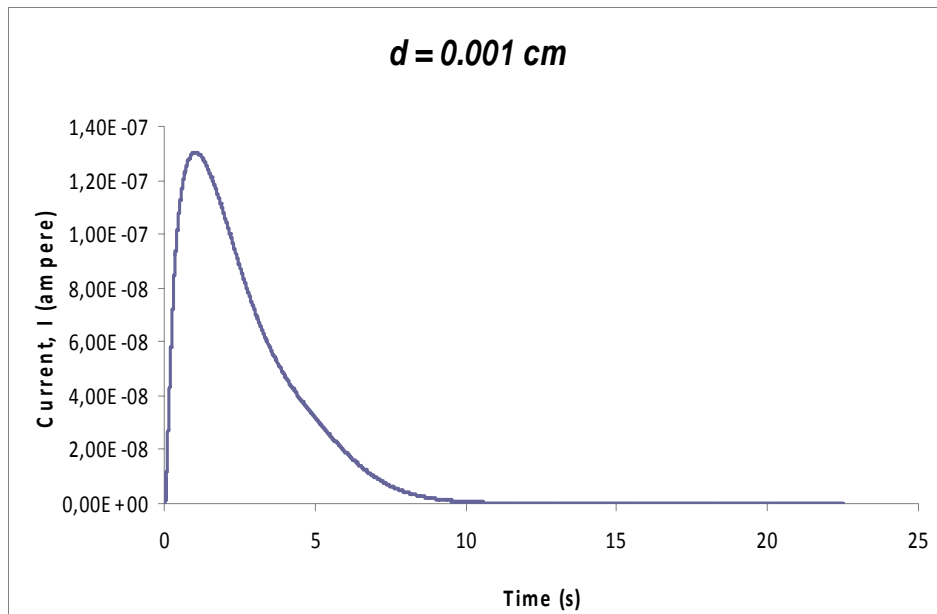
$$S_0 = 2 \times 10^{-8} \text{ mol/cm}^3.$$

The active time  $T_A$  of enzymatic reaction in membrane layer is:

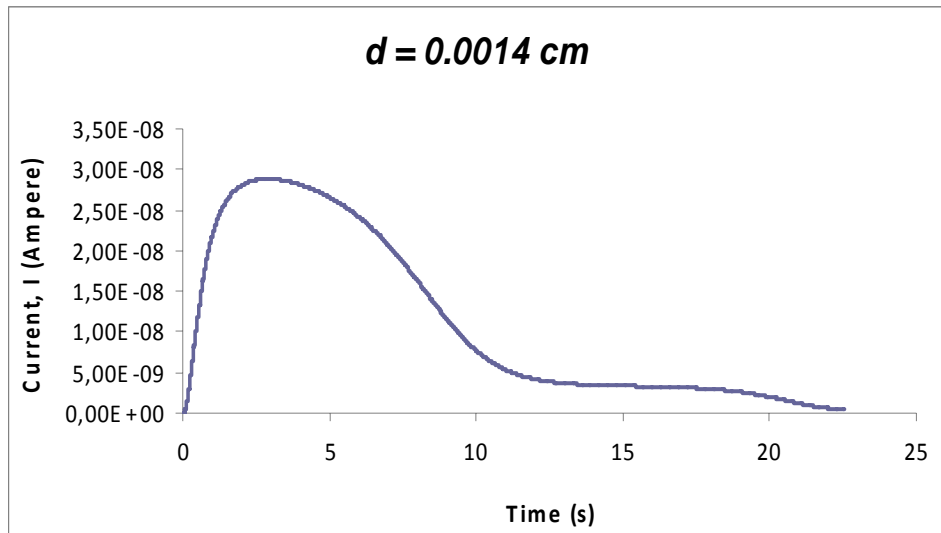
$$T_A = 0.3 \text{ s.}$$

## 5. Results and Discussion

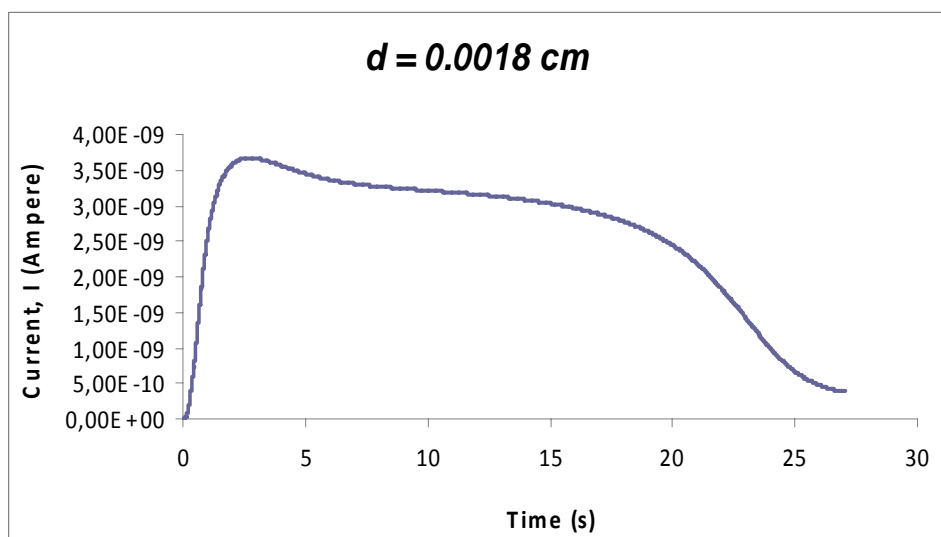
The evolution of the biosensor current at the maximal enzymatic rate  $V_{\max}$  of  $1 \times 10^{-7} \text{ mol/cm}^3\text{s}$  and active time of enzymatic reaction  $T_A = 0.3 \text{ s}$  is presented in Fig. 2. The biosensor response was modeled for biosensors having six different membrane thicknesses  $d$ : 0.001, 0.0014, 0.0018, 0.0022, 0.0026 and 0.003 cm. One can see in Fig.2 the biosensor current appears with some delay at relatively thick enzyme layers. This delay increases with increase of the enzyme membrane thickness. The biosensor response is notable higher at thinner membrane ( $d = 0.001 \text{ cm}$ ) than at thicker five ( $d = 0.0014, 0.0018, 0.0022, 0.0026$  and  $0.003 \text{ cm}$ ).



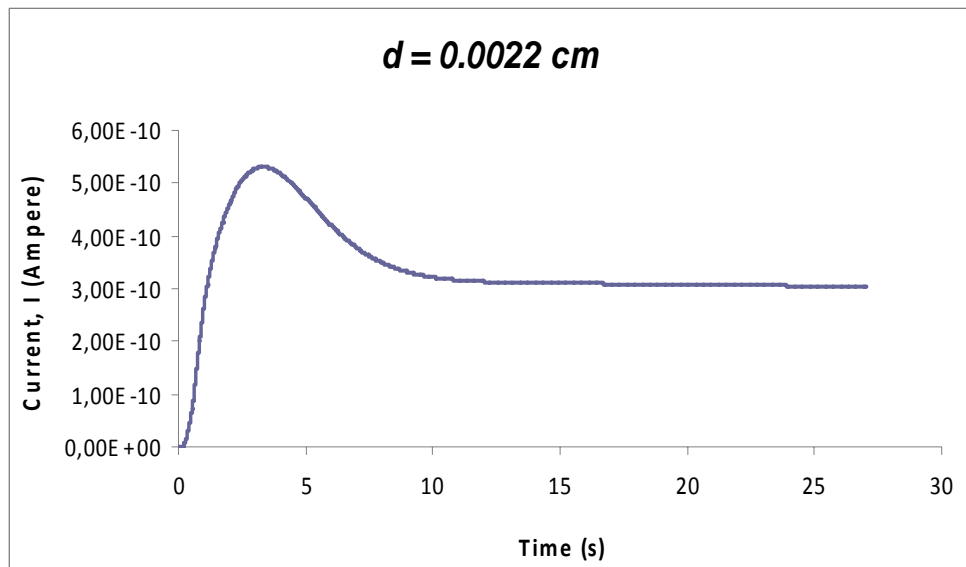
(a)



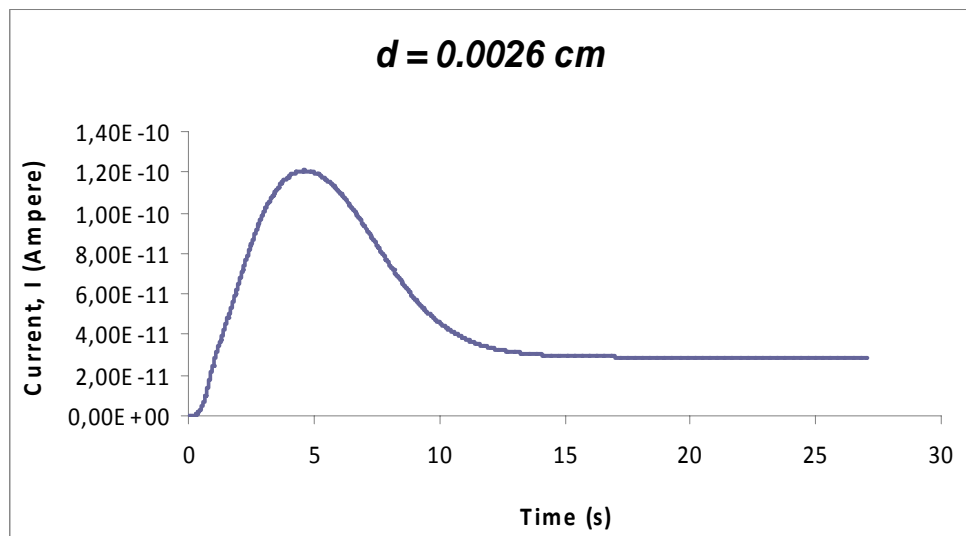
(b)



(c)



(d)



(e)



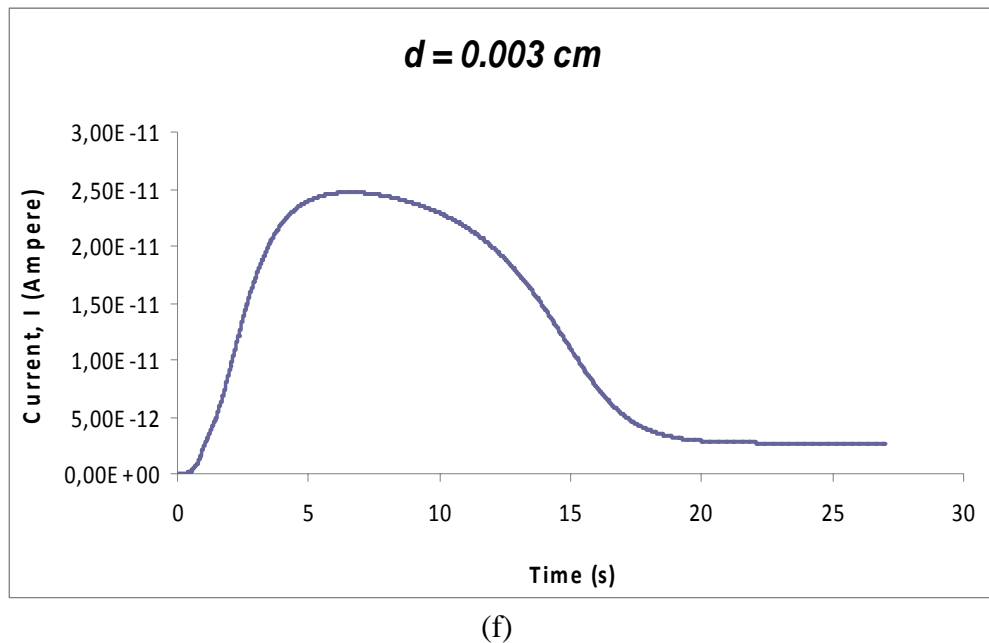


Fig. 2 The dynamics of the biosensor current at the maximal enzymatic rate  $V_{\max} = 1 \times 10^{-7} \text{ mol/cm}^3\text{s}$  and six membrane thickness  $d$ : (a) 0.001, (b) 0.0014, (c) 0.0018, (d) 0.0022, (e) 0.0026 and (f) 0.003 cm,  $S_0 = 2 \times 10^{-8} \text{ mol/cm}^3$ .

## 6. Conclusions

The mathematical model (2)-(9) of amperometric biosensor operation can be successfully used to investigate the kinetic regularities of enzyme membrane-based sensors. The biosensor current is a monotonous function of membrane thickness  $d$  at a value of active time ( $T_A$ ) of enzymatic reaction in membrane layer (Fig. 2). Consequently, the maximal biosensor current increases with decreasing of the membrane thickness  $d$ . The greater the membrane thickness  $d$ , the biosensor response will become minimum and unstable.

## References

- [1] L.C. Clarc and C. Loys, Electrode system for continuous monitoring in cardiovascular surgery, *Ann. N.Y. Acad.Sci.*, **102** (1962), 29–45.
- [2] A.P.F Turner, I. Karube and G.S Wilson, *Biosensors: Fundamentals and Applications*, Oxford University Press, Oxford, 1987.
- [3] F. Scheller and F. Schubert, *Biosensors*, Elsevier (1992), Amsterdam, **7**.
- [4] A. Chaubey and B.D. Malhotra, Mediated biosensors, *Biosens. Bioelectron.*, **17** (2002), 441–456.

- [5] C.D. Mell and J.T. Maloy, A model for the amperometric enzyme electrode obtained through digital simulation and applied to the glucose oxidase system, *Anal. Chem.*, **47** (1975), 299–307.
- [6] C.D. Mell and J.T. Maloy, Amperometric response enhancement of the immobilized glucose oxidase enzyme electrode, *Anal. Chem.*, **48** (1976), 1597.
- [7] J.J. Kulys, V.V. Sorochinski and R.A. Vidziunaite, Transient response of bienzyme electrodes, *Biosensors*, **2** (1986), 135–146.
- [8] T. Schulmeister, Mathematical modeling of the dynamics of amperometric enzyme electrodes, *Selective Electrode Rev.*, **12** (1990), 260–303.
- [9] V.V. Sorochinskii and B.I. Kurganov, Steady-state kinetics of cyclic conversions of substrate in amperometric bienzyme sensors, *Biosens. Bioelectron.*, **11**(3) (1996), 225–238.
- [10] K. Yokoyama and Y. Kayanuma, Cyclic voltammetric simulation for electrochemically mediated enzyme reaction and determination of enzyme kinetic constants, *Anal. Chem.*, **70**(16) (1998), 3368–3376.
- [11] R. Baronas, F. Ivanauskas and J. Kulys, Modelling a biosensor based on the heterogeneous microreactor, *J. Math. Chem.*, **25**(2–3) (1999), 245–252.
- [12] J. Crank, *The mathematics of diffusion*, 2nd ed., Clarendon Press, Oxford, 1975.
- [13] A. Samarskii, *Difference Scheme Theory*, Nauka, Moskow (in Russian), 1983.
- [14] D. Britz, *Digital simulation in electrochemistry*, 2nd ed., Springer-Verlag, Berlin, 1988.

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