Modelling a biosensor based on the heterogeneous microreactor

R. Baronas [a], F. Ivanauskas [a,b] and J. Kulys [c]

[a] *Faculty of Mathematics and Informatics, Vilnius University, Naugarduko 24, 2600 Vilnius, Lithuania*
E-mail: Feliksas.Ivanauskas@maf.vu.lt
[b] *Institute of Mathematics and Informatics, Akademijos 4, 2600 Vilnius, Lithuania*
[c] *Laboratory of Enzyme Chemistry, Institute of Biochemistry, Mokslininku 12, 2600 Vilnius, Lithuania*

Received 16 March 1999

Modelling of the amperometric biosensors based on carbon paste electrodes encrusted with a single heterogeneous microreactor is analyzed. The microreactor was constructed from CPC-silica carrier and was loaded with glucose oxidase. The model is based on non-stationary diffusion–reaction equations containing a non-linear term related to the enzymatic reaction. A homogenization process having an effective algorithm for the digital modelling of the operation of the microreactor is proposed. The influence of the size, geometrical form, and the position of a microreactor on the operation of biosensors are investigated.

1. Introduction

The goal of this research is to propose a model allowing us an effective digital modelling, and also to investigate the influence of the geometry of a microreactor on the operation of biosensors.

Recently, the amperometric biosensors based on carbon paste electrodes (CPEs) encrusted with a single microreactor (MR) have been constructed for the determination of glucose [3,4]. The MRs were prepared from CPC-silica carrier and were loaded with glucose oxidase (GO), mediator and acceptor. A numerical model of the operation of biosensors has been designed. The model is based on non-stationary diffusion equations containing a non-linear term related to the enzymatic reaction. In the simplest case, this term is given by the Michaelis–Menten equation:

$$\frac{\mathrm{d}u}{\mathrm{d}t} = \frac{au}{b+u}, \tag{1}$$

where a represents the maximal enzymatic rate, b the Michaelis constant, and u the substrate concentration.

The problems in the modelling arise because of the possibility to solve analytically such type of equations. In the digital modelling, the heterogeneous nature of MR, the determination of the boundary conditions, and the overload of calculation are the main problems.

2. Definition and homogenization of the model

Let Ω be the area of the container (buffer solution) which was filled with some substrate, and Ω_0 the area of MR ($\Omega_0 \subset \Omega$). Since the microreactor is constructed from CPC-silica carrier (CPC) and is loaded with glucose oxidase (GO), let the whole MR area Ω_0 consist of two areas: Ω_{0C}, the CPC-carrier, and Ω_{0G}, the glucose oxidase ($\Omega_0 = \Omega_{0C} \cup \Omega_{0G}$) (figure 1). Let Γ be the whole surface of the container, and Γ_1 only the base of the container.

The operation of biosensors includes the heterogeneous enzymatic process (reaction) and diffusion. The stimulus of the reaction is MR, but the reaction performs only in the area Ω_{0G} of MR which was filled with glucose oxidase. The model consists of a system of the following non-linear differential equations of the reaction–diffusion type:

$$\frac{\partial u}{\partial t} = d_1 \Delta u - f(u), \tag{2}$$

$$\frac{\partial \nu}{\partial t} = d_1 \Delta \nu + f(u), \tag{3}$$

$$d_1|_{\Omega_{0G}} = d_1|_{\Omega \backslash \Omega_0} = d, \qquad d_1|_{\Omega_{0C}} = 0, \tag{4}$$

$$f|_{\Omega_{0G}} = au/(b+u), \qquad f|_{\Omega_{0C}} = f|_{\Omega \backslash \Omega_0} = 0, \tag{5}$$

where Δ is the Laplace operator, d is the diffusion rate, u is the substrate concentration, ν is the concentration of the reaction product, and t is time. The initial conditions ($t = 0$) are

$$u|_{\Omega_0} = 0, \qquad u|_{\Omega \backslash \Omega_0} = u_0, \qquad \nu|_{\Omega} = 0. \tag{6}$$

The boundary conditions ($t > 0$) are

$$\left.\frac{\partial u}{\partial n}\right|_{\Gamma} = 0, \qquad \left.\frac{\partial \nu}{\partial n}\right|_{\Gamma \backslash \Gamma_1} = 0, \qquad \nu|_{\Gamma_1} = 0, \tag{7}$$

where $(\partial u/\partial n)|_{\Gamma}$ is a derivative of u with respect to the normal direction to the surface Γ and $(\partial \nu/\partial n)|_{\Gamma \backslash \Gamma_1}$ is a derivative of ν in the normal direction to the surface $\Gamma \setminus \Gamma_1$.

Due to the technology of the construction of MR, the number of cells which are filled with glucose oxidase is very large, so an average size of a cell is much less than the size of MR. The number of the cells and the geometrical shape of the cells cannot be precisely defined. For that reason, it is hopeless to solve (2)–(7) analytically and even to design an effective algorithm for the numerical calculations.

The model (2)–(7) was reduced by the homogenization process [1]. Let N be the ratio of the volume of MR to the volume of the glucose oxidase which fills the MR cells

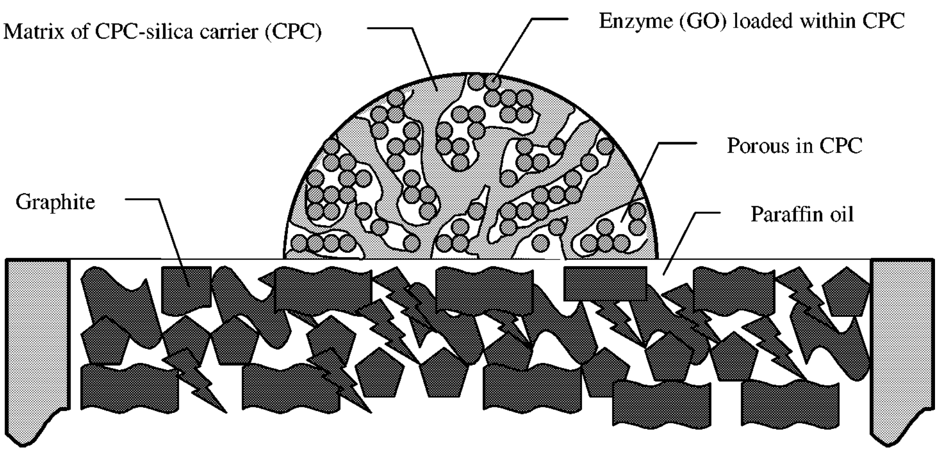

Figure 1. A principal structure of a heterogeneous microreactor was constructed from CPC-silica carrier and was loaded with glucose oxidase. The average size of a cell which is filled with glucose oxidase is much less than the size of MR. The geometrical shapes of cells are not precisely defined.

(it is easy to calculate this ratio experimentally), i.e., $N = \text{volume}(\Omega_0)/\text{volume}(\Omega_{0G})$. By using the homogenization process, the definition of the non-linear term related to the enzymatic reaction was simplified and the model was reduced to

$$\frac{\partial \overline{u}}{\partial t} = d_2 \Delta \overline{u} - \frac{1}{N} f(\overline{u}), \quad (8)$$

$$\frac{\partial \overline{\nu}}{\partial t} = d_2 \Delta \overline{\nu} + \frac{1}{N} f(\overline{u}), \quad (9)$$

$$d_2|_{\Omega \setminus \Omega_0} = d, \qquad d_2|_{\Omega_0} = d_3, \quad (10)$$

$$f|_{\Omega_0} = a\overline{u}/(b + \overline{u}), \qquad f|_{\Omega \setminus \Omega_0} = 0, \quad (11)$$

where $\overline{u} \approx u$, $\overline{\nu} \approx \nu$, and the value d_3 of the diffusion rate in the area of MR depends on the diffusion rate d, the geometry of MR, and the ratio N (see [1] and below).

The initial conditions and the boundary conditions are the same as above ((6) and (7), respectively).

3. Digital modelling of an experiment

The model (8)–(11), (6), (7) was used for digital modelling of the real experiment. The container was modelled as a hemisphere of radius R and MR was modelled as a hemisphere of radius R_0. MR was placed on the center of the base of the container. Approximately, a half of the volume of MR was loaded with the glucose oxidase (GO) and the second half was the CPC-silica carrier (CPC) (i.e., the ratio $N \approx 2$) (figure 1). Due to the ratio $N \approx 2$, the value $d/4$ was accepted as the homogenized diffusion rate

d_3 in the area of MR. Due to symmetry, the model in the spherical coordinates was reduced to the system in two space variables.

The finite-difference technique [6] was used for discretisation of the model. We introduced a non-uniform discrete grid to avoid an overload of calculations due to the condition $R_0 \ll R$. An exponentially increasing step of the grid was used in the direction r, while a constant step was used in v and t directions.

A system of linear equations of implicit finite difference schemes was built as a result of the difference approximation. To decrease the order of the system of linear equations, the variable direction method [6] was used. The resulting system was solved iteratively.

The current of the biosensor is measured in order to understand the dynamics of reaction-diffusion in real experiment. The current was expressed as

$$I = mFd_2 \iint_{\Gamma_1} \frac{\partial \nu}{\partial n}\bigg|_{\Gamma_1} \mathrm{d}\Gamma_1, \tag{12}$$

where $m = 2$ is the number of electrons and $F \approx 9.65 \times 10^4$ C/mol is Faraday's constant. The calculated current of a biosensor was compared with the experimental data.

The model (6)–(11) was used in the numerical experiments with the following values of the parameters:

$$\begin{aligned} &R = 1 \text{ cm}, \qquad R_0 = 0.0424 \text{ cm}, \qquad d = 6.7 \times 10^{-6} \text{ cm}^2/\text{s}, \\ &a = 4.4 \times 10^{-5} \text{ mol/cm}^3\,\text{s}, \qquad b = 8.3 \times 10^{-5} \text{ mol/cm}^3, \\ &u_0 = 10^{-6} \text{ mol/cm}^3. \end{aligned} \tag{13}$$

The model was realized in C/C++ programming language, compiled by the IBM VisualAge C++ for OS/2 compiler and was tested in the environment of the operating system OS/2 Warp 4.0. The program runs about 10 min to simulate a 100 s long reaction on a PC based Intel Pentium II 350 MHz microprocessor.

4. Influence of the size, form, and position of a microreator

The dynamics of a current is considered in a parametrization of the radius of MR, i.e., the dependence on the size of a microreactor is considered in the case where the value of the diffusion rate d and the values of all other parameters are the same as defined above in (13). MR was modelled as a hemisphere. The evolution of the current for the radius of MR equal to $0.25R_0$, $0.5R_0$, R_0, $1.5R_0$, and $2.0R_0$ (here R_0 is the same as in (13)) is presented in figure 2.

One can see that the values of the current (including the maximal current) increase if the radius of the microreactor increases and this growth is non-linear.

Several other geometrical shapes of MR differing from the hemisphere were used to analyze the dependence of the operation of MR on the form of MR.

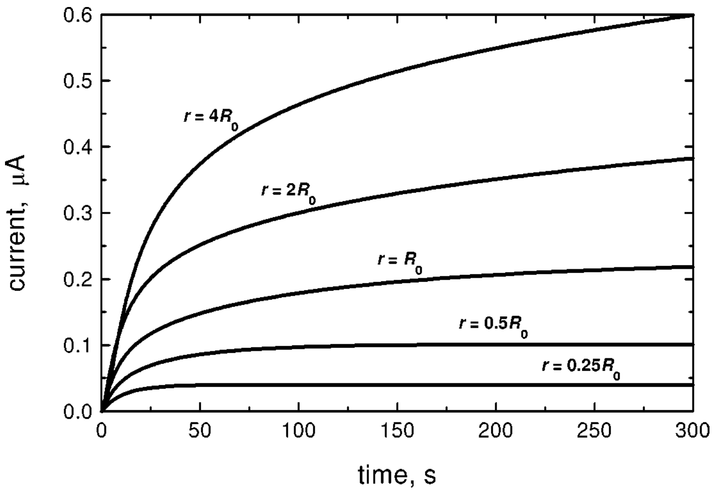

Figure 2. The dependence of the current on the size of a microreactor solid. The microreactor was modelled as one half of a sphere of radius r, where r is from the set $\{0.25R_0, 0.5R_0, R_0, 1.5R_0, 2.0R_0\}$. R_0 and the values of all other parameters are defined in (13).

Firstly, MR was modelled as a hemi-ellipsoid of revoliution. In the Cartesian coordinate system it is given by

$$\frac{x^2}{a^2} + \frac{y^2}{a^2} + \frac{z^2}{c^2} \leqslant 1, \quad z \geqslant 0, \tag{14}$$

where a and c are semi-axes of the ellipsoid. The current was calculated for several different values of a and c keeping the volume of every ellipsoid equal to the volume of a sphere of radius R_0 in order to have the same volume of MR as that used in real experiments and in test calculations. Thus, the volume of MR was kept constant, and only a geometrical form was changed. The following cases were analyzed:

(a) the semi-axis c is equal to the one-fourth of the semi-axis a;

(b) the semi-axis c is equal to the one-half of the semi-axis a;

(c) the semi-axis c is equal to the semi-axis a;

(d) the semi-axis c is twice as long as the semi-axis a;

(e) the semi-axis c is four times the semi-axis a.

The results of calculation are presented in figure 3. The form of MR appears to be important for the current and it is especially important at the initial stage of the reaction. Here the current grows faster as the area of the base of MR increases. Later this importance decreases. The area of the base of MR is important for the maximal current and the time moment of the occurrence of the maximal current. The maximal value of the current increases and the time moment of its occurrence decreases as the area of the base increases even if the volume of MR remains unchanged.

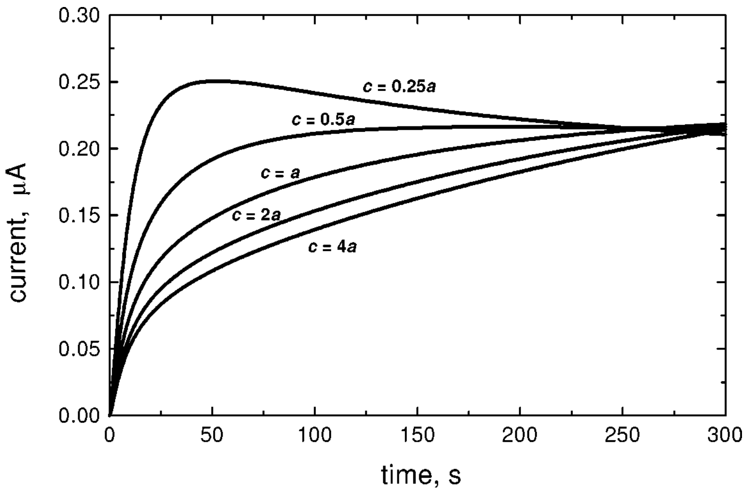

Figure 3. The dynamics of the current in the case where the microreactor is a hemi-ellipsoid of revolution. a and c are, respectively, the semi-axes of the ellipsoid in the x- and z-direction (in the Cartesian coordinate system, see (14)). The volume of every ellipsoid is equal to the volume of a sphere of radius R_0.

A half of a torus was used as a second geometrical shape of MR. Let r be the radius of a circle which draws the torus and R the radius of the leading circle of the torus, i.e., R is the distance between the z-axis and the center of a circle which is rotated around the z-axis. The center of the rotating circle is on the plane $z = 0$. Several different values of both radiuses r and R were used to determine the dependence of the current on this geometrical form of MR. The volume of every torus was equal to the volume of a sphere of radius R_0 (see (13)). Microreactors of the shape of the upper part of a torus ($z \geqslant 0$) were used in calculation. In a special, case where $R = 0$, MR is a half of a sphere. The dynamics of the current in the case where MR was modelled as one-half of a torus is depicted in figure 4.

In all the numerical experiments discussed above, as well as in the physical experiments, MR was placed on the base of a container. We investigated the dynamics of the current when MR was lifted up. Since the current arises only when some concentration of the reaction product is reached on the base of the container, the current emerges with delay if MR is lifted up. The time of delay depends on an altitude. This was the reason why we simulated the reaction for a longer time now compared to the previous numerical experiments. The MR in the form of a sphere was used in the analysis. The radius of MR to be lifted up was derived from R_0 (R_0 is defined in (13)) to have the volume of MR the same as it was in the test experiments, where MR was modelled as a hemisphere. Let h be the altitude of MR; more precisely, h is the distance between the center of MR and the base of a container. The results of numerical experiments for several values of altitude h are shown in figure 5. It appears that the altitude of MR is very important for the dynamics of the current. The delay increases and the current grows much slower if the altitude increases.

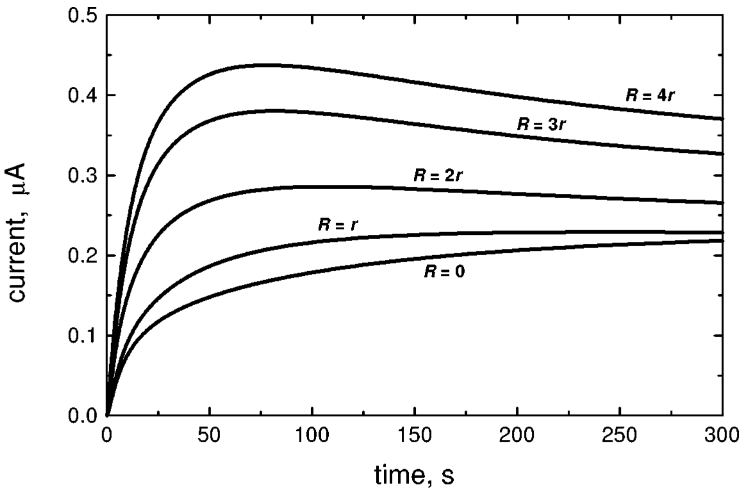

Figure 4. The dynamics of the current in the case where the microreactor is a half of a torus. r is the radius of the leading circle rotated to get the torus and R is the radius of that rotation around the z-axis (in the Cartesian coordinate system). The center of the leading circle is on the plane $z = 0$. The volume of every torus is equal to the volume of a sphere of radius R_0 (see (13)).

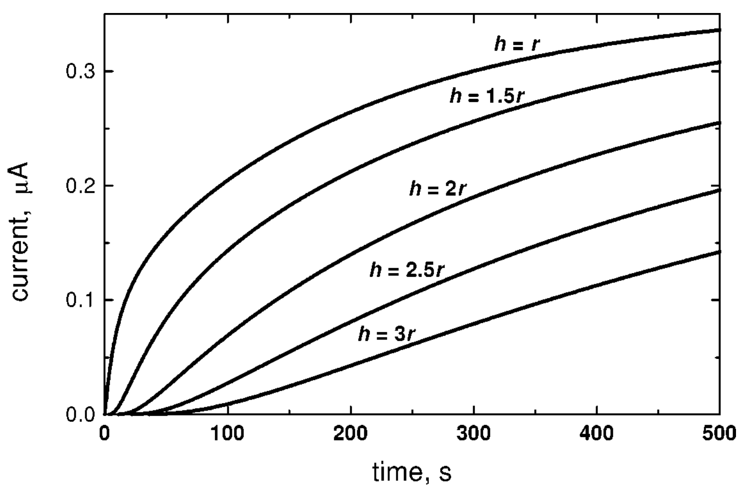

Figure 5. The dynamics of the current in the case where the microreactor is lifted up from the base of the container. MR is a sphere of radius r. The volume of the sphere is equal to the volume of a hemisphere of radius R_0 (see (13)). h is the distance between the center of MR and the base of a container.

References

[1] N.S. Bakhvalov and G.P. Panasenko, *Homogenisation of Processes in Periodic Media* (Nauka, Moscow, 1984) (in Russian).

[2] K. Kotchetkov, A.F. Botchkov, B.A. Dmitriev, A.I. Usov, O.S. Tchizchov and V.N. Schibaev, *Chemistry of Carbohydrates* (Khimia, Moscow, 1967) (in Russian).

[3] J. Kulys, The carbon paste electrode encrusted with a microreactor as glucose biosensor, Biosens. Bioelectr. (1999), submitted.
[4] J. Kulys, A. Palaima and G. Urbelis, Employing heterocyclic dihydropolyazines for amperometric glucose sensing, Anal. Lett. 31(4) (1998) 569.
[5] D.R. Lide, ed., *Handbook of Chemistry and Physics*, 74th ed. (CRC Press, Boca Raton, FL, 1993–1994).
[6] A.A. Samarskij, *The Theory of Difference Schemes* (Nauka, Moscow, 1989) (in Russian).
[7] K. Weibel and H.J. Bright, The glucose oxidase mechanism, J. Biol. Chem. 246 (1971) 2734.