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Numerical simulation of the influence of the fluctuations of the biosensor's parameters on its response

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Abstract. The mathematical model of the electrochemical glucose biosensor based on the enzymatic conversion of the substrate and the diffusion of the substrate was used. The influence of the fluctuations of the membrane thickness, the diffusion coefficients and pH were modelled and their impact was evaluated at different modes of an action of the biosensor.

Keywords: biosensor, reaction-diffusion, modelling, reproducibility.

Introduction

Biosensors as an analytical instrument have found wide application in medicine, environment, and food-quality control. However, the application of the biosensors is limited by a low reliability of the biosensor operation. This is due to a number of different parameters. Enzymes are not stable for a long time and are sensitive to a number of factors. As a model biosensor, a well-known electrochemical glucose biosensor based on glucose oxidase was selected [2]. The goal of this paper is mathematical modelling and evaluation of the influence of two main parameters of the biosensor – pH fluctuations and diffusion fluctuations. We evaluated the weight of these factors on the biosensor response.

1. Mathematical model

Suppose that substrate S conversion to product P was catalysed by the enzyme E

$$S \xrightarrow{E} P.$$

Consider a biosensor as a flat amperometric device with a layer of enzyme and outer membrane. It follows that the model has two regions. In the first region (outer membrane) only mass transport limited by diffusion takes place. In the second region (enzyme layer) enzymatic conversion of glucose to gluconic acid, oxygen to hydrogen peroxide and mass transport are limited by diffusion. A mathematical model of a biosensor is based on the system of the diffusion equations with a nonlinear term

corresponding to the Michaelis–Menten kinetics of the enzymatic reaction (see [1] and references therein). Let us suppose that the symmetry of a biosensor allows to describe changes in the concentration of the substrate and the product in the biosensor by the following system of reaction-diffusion equations. In the porous membrane region only the processes of diffusion take place:

$$\frac{\partial S}{\partial t} = d_{Sm}\frac{\partial^2 S}{\partial x^2}, \quad a_e \leqslant x \leqslant a_e + a_m, \tag{1}$$

$$\frac{\partial P}{\partial t} = d_{Pm}\frac{\partial^2 P}{\partial x^2}, \tag{2}$$

where x and t are space and time, respectively, $S(x,t)$ is the concentration of the substrate (glucose) and $P(x,t)$ is the concentrations of the reaction product (hydrogen peroxide) in the membrane region. a_e is the thickness of the enzyme layer, a_m is the thickness of the diffusion layer (outer membrane). d_{Sm} and d_{Pm} are the diffusion coefficients of the substrate and the reaction product in the membrane. The thickness of the diffusion layer remains constant. The concentration of the substrate as well as the product over the outer membrane surface remains constant while the biosensor keeps in touch with the substrate ($t > 0$). So, the following boundary conditions are satisfied:

$$S(t, a_e + a_m) = S_0, \qquad P(t, a_e + a_m) = 0, \tag{3}$$

where S_0 is the substrate concentration in the bulk solution.

In the second region the diffusion of both the substrate and the product, and the reaction take place:

$$\frac{\partial S}{\partial t} = d_{Se}\frac{\partial^2 S}{\partial x^2} - \frac{V_{\max} S}{K_M + S}, \qquad 0 \leqslant x \leqslant a_e, \tag{4}$$

$$\frac{\partial P}{\partial t} = d_{Pe}\frac{\partial^2 P}{\partial x^2} + \frac{V_{\max} S}{K_M + S}, \tag{5}$$

where K_M is the Michaelis constant for glucose, d_{Se} and d_{Pe} are the diffusion coefficients of the substrate and the reaction product in the enzyme layer. The activity of the enzyme depends on pH of solution. It is described by the following equation:

$$V(pH) = \frac{\tilde{V}_{\max}}{10^{-pH+pK_{a1}} + 10^{-pK_{a1}+pH} + 1}, \quad V_{\max} = \max V(pH), \tag{6}$$

where pK_{a1} and pK_{a2} are the values of the ionogenic groups of the enzyme active centre responsible for the activity, $\tilde{V}_{\max}$ is the maximal enzymatic rate.

Let $x = 0$ represent the surface of the electrode, while $x = a_e + a_m$ is the boundary between the diffusion layer and the buffer solution. The concentration of the reaction product at the surface of electrode is equal to zero due to the fast electrochemical oxidation of the hydrogen peroxide. As the substrate is an electro-inactive substance, the following boundary conditions for $t > 0$ can be applied to the system:

$$\frac{\partial S}{\partial x}(t, 0) = 0, \quad P(t, 0) = 0. \tag{7}$$

The biosensor operation starts when some substrate appears in the bulk solution. This is used in the initial conditions:

$$S(0,x)=0, \quad S(0,a_e+a_m)=S_0, \tag{8}$$

$$P(0,x)=0, \quad P(0,x)=0. \tag{9}$$

On the boundary between two regions with different diffusion coefficients we define the compatibility conditions ($t>0$). These conditions mean that the concentrations and the fluxes of the substrate and the product through the outer membrane are equal to the corresponding concentrations and fluxes entering the surface of the enzyme layer

$$S(t,a_e-0)=S(t,a_e+0), \tag{10}$$

$$P(t,a_e-0)=P(t,a_e+0), \tag{11}$$

$$d_{Se}\frac{\partial S}{\partial x}(t,a_e-0)=d_{Sm}\frac{\partial S}{\partial x}(t,a_e+0), \tag{12}$$

$$d_{Pe}\frac{\partial P}{\partial x}(t,a_e-0)=d_{Pm}\frac{\partial P}{\partial x}(t,a_e+0). \tag{13}$$

Both concentration functions S and P are continuous in the entire domain.

The steady-state biosensor current I (the biosensor response) is one of the most important characteristics of biosensors. The recorded current is a response of a biosensor to the glucose concentration in the bulk. The current depends upon the flux of the reaction product at the electrode surface. The current density $i(t)$ at time t can be obtained explicitly from Faraday's law and Fick's law using the flux of the product concentration at the surface of the electrode

$$i(t)=n_e s F d_{Se}\frac{\partial P}{\partial x}(t,0),$$

where n_e is a number of electrons, involved in the charge transfer at the electrode surface, s is the square of the flat electrode and F is the Faraday constant, $F=96485$ C/mol. The steady state current I is defined as

$$I=\lim_{t\to\infty} i(t). \tag{14}$$

2. Digital simulation

Serious difficulties can arise when one tries to solve analytically a multidimensional nonlinear system of partial differential equations with complex boundary conditions (1)–(5), (7)–(13). Therefore, the problem was solved numerically. All simulations were carried out using MATLAB. We solved the initial-boundary value problems for systems of parabolic partial differential equations. The finite difference technique was applied for discretization of the mathematical model. A uniform discrete grid was introduced in x direction. The second order approximation to the solution is made on the mesh in x direction. The time integration was achieved with dynamically selected time step. Having a numerical solution of the system of partial differential equations, the density of the biosensor current was calculated.

To obtain the concentration profiles in the steady state, the calculations were continued while the concentration profiles stopped changing. Simulation time 5000 s was chosen so that it guarantees an accurate simulation of the steady-state current (14). The steady-state currents were calculated for the modelled biosensor response when the substrate concentration S_0 changes up to 35 mM.

Using computer simulation the influence of the variations of pH as well as the variations of the diffusion coefficient and the thickness of the outer membrane on the biosensor response was investigated. The following values of the parameters were constant in the numerical simulation of all the experiments: the square of the flat electrode $s = 2$ mm^2; the thickness of the enzyme layer $a_e = 4$ μm; the diffusion coefficients of the substrate and the reaction product in the enzyme layer $d_{Pe} = d_{Se} = 70$ μm^2/s; $K_M = 0.23$ mM; $\tilde{V}_{\max} = 1.1$ mM/s, $pK_{a1} = 3.5$, $pK_{a2} = 7.3$. The numerical solution of the model was evaluated for different values of input parameters. In all numerical experiments the diffusion coefficients of the substrate d_{Sm} and the reaction product d_{Pm} in the membrane were changed from 3 μm^2/s to 12 μm^2/s. The thickness of the diffusion layer a_m was changed from 5 μm to 25 μm. In this simulation pH value was calculated at the point of maximal activity of the enzyme. In the case of the given values of ionogenic groups pK_a $pH = 5.4$.

3. Results and discussion

As a sensitive element of the mode of the biosensor action K_M and $V_{\max}$ were selected. The fluctuations of K_M directly reflects the integrated number of impacts of a number of factors affecting the biosensor action. Taking into account that limited acceptable fluctuations of the biosensor response should not exceed 5%, we calculated how K_M can be changed to satisfy this requirement. Data are presented in Fig. 1. Limited decrease of K_M will be symmetric (not shown). As can be seen in the case of a thin membrane and a high diffusion coefficient the biosensor operates in a kinetic mode and the response of the biosensor is very sensitive to any fluctuations of the value of the K_M. Calculations show, that the increase of the membrane thickness 5 times increases the allowed fluctuations of the K_M from 30.8 % to 111.5 %. It means that the biosensor is switching to the diffusion mode of action and the impact of fluctuations of the enzyme structure and the capacity to bind the substrate reduces 3.6 times. Thereby, the impact of the enzyme selectivity on the biosensor selectivity also decreases. The biosensor is sensitive to the fluctuations of the diffusion coefficient. The decrease of the permeability of the membrane 4 times switches the mode of action of the biosensor to the diffusive mode. This allowed the fluctuations of K_M to increase up to 3 times. In a deep diffusive mode, the fluctuations of K_M up to 400 % do not influence significantly the biosensor response.

Another parameter affecting the action of the biosensor is pH. Its dependence of glucose oxidase is determined by two ionogenic groups with pK_a equal to 3.5 and 7.3 (calculated from the data presented in [3]), see Eq. 6. As can be expected, pH affects the ratio of the diffusion/kinetic modes, thereby, it also affects the impact of the enzyme capacity to bound the substrate. The higher the activity of the enzyme, the deeper the diffusion mode of action is, the lower is the impact of the pH dependence. Data are presented in Fig. 2.

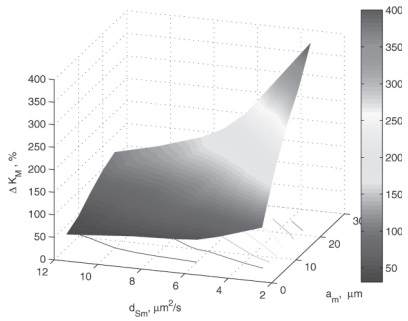

Figure 1. Dependence of the limited increase of K_M on membrane thickness and diffusion coefficient.

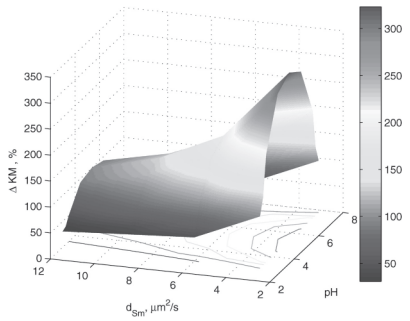

Figure 2. Dependence of the limited increase of K_M on pH and membrane diffusion coefficient.

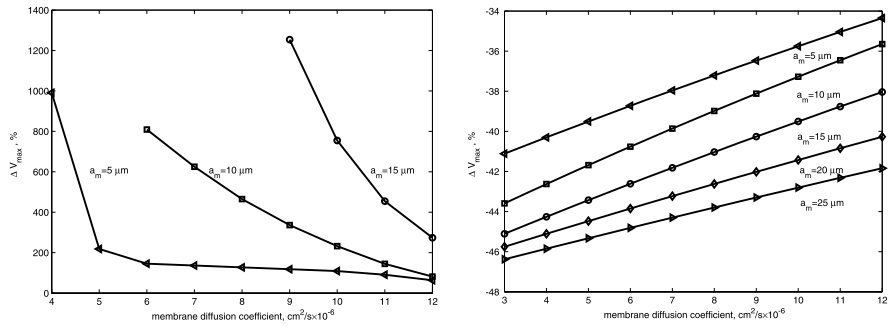

Figure 3. Dependence of the limited increase and decrease of $V_{\max}$ on diffusion coefficient at different membrane thickness.

The activity of the enzyme directly impacts the response of the biosensor, thereby, the allowed fluctuations of the $V_{\max}$ can be applied in a much shorter diapason, than K_M. The fluctuations of the enzyme activity directly affect the ratio of the kinetic/diffusion modes of action, thereby, increasing and decreasing of the enzyme activity. Therefore their impacts will not be symmetric as can be seen in Fig. 3. During the exploitation of the biosensor we can expect inactivation of the enzyme rather than unexpected activation. The increase of the enzyme activity can switch the mode of the biosensor action into the diffusion mode, thereby further increase of the enzyme activity does not influence the response of the biosensor, and the model does not describe the process.

4. Conclusions

In this study we have demonstrated a mathematical model, allowing to predict the reproducibility of the biosensor and evaluate the influence of the membrane thickness, the diffusion coefficient and pH on the metrological parameters of the biosensor. The model shows, that the fluctuations of K_M affects the response of the biosensor much

weaker than the fluctuations of the enzyme activity. The importance of the sensitivity of the enzyme activity on pH has been evaluated in different modes of the action of the biosensor.

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References

1. R. Baronas, J. Kulys, F. Ivanauskas. Modelling amperometric enzyme electrode with substrate cyclic conversion. *Biosensors & Bioelectronics*, 19(8):915–922, 2004.
2. V.A. Laurinavicius, J.J. Kulys, V.V. Gureviciene, K.J. Simonavicius. Flow-through and catheter biosensors with an extended concentration range. *Biomed. Biochim. Acta*, 48(11/12):905–909, 1989.
3. R. Wilson, A.P.F. Turner. Flow-through and catheter biosensors with an extended concentration range. *Biosensors & Bioelectronics*, 7(3):165–185, 1992.

REZIUMĖ

O. Štikonienė, F. Ivanauskas. Parametrų svyravimo įtakos biojutiklio atsakui skaitinis modeliavimas

Darbe pristatomas biojutiklių su fermentiniu sluoksniu, uždengtu difuzine membrana, veikimo mechanizmų matematinis modeliavimas. Buvo padaryta storio, difuzijos koeficientų ir pH įtakos atsako tikslumui kompiuterinė analizė.

Raktiniai žodžiai: biojutiklis, modeliavimas, reakcijos ir difuzijos lygtys.