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The influence of external factors on the operational stability of the biosensor response

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ABSTRACT

The behaviour of the electrochemical glucose biosensor based on the glucose oxidase was examined in the diffusion and the kinetic modes of the action. The sensitivity and linearity of the biosensor can be monitored changing the permeability of the outer membrane of the biosensor. The mathematical model based on the enzymatic conversion of the substrate and the diffusion of the substrate was created. 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. Taking into account that limited acceptable fluctuations of the biosensor response should not exceed 5%, we calculated how $K_{M(app.)}$ and V_{max} can move to satisfy this requirement. In a deep diffusive mode (thick highly acetylated membrane), the fluctuations of $K_{M(app.)}$ up to 400% do not influence significantly the biosensor response. In the diffusion mode of action of the biosensor, the limit of the V_{max} fluctuations is on the level of 34%. The increase of the thickness of the membrane 5 times, increases the limit of fluctuations only to 19%. The reduction of the permeability of the membrane 4 times increases the level of limited fluctuations about 10–16%. The novelty of this work is binding into one system the fluctuations of pH and diffusion parameters and demonstrating the interdependence of them as an integrated factor of the reliability of the biosensor response.

1. Introduction

Biosensors as an analytical instrument have found wide application in medicine, environment, and food-quality control [1,2]. 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. The stability of the biosensor action depends both on the stability of the sensing element of the biosensor, i.e. usually an enzyme or an enzymatic complex, and the stability of the matrix. Enzymes are not stable for a long time and are sensitive to a number of factors. The activity of an enzyme is limited by the pH. A number of compounds can activate and even more often inhibit the activity of the enzyme. The complicated construction of the biosensor, usually consisting of several semi-permeable layers is very often sensitive to the fluctuations of the pressure in the bulk, especially in mixing or flow-through conditions. Fluctuations of the pH and the concentration of salts can change the diffusion parameters of the biosensor membranes, thereby, changing the response of the biosensor.

The main goal of this paper is mathematical modelling and evaluation of the influence of two main parameters of the biosensor–pH fluctuations and diffusion fluctuations. pH fluctuations can occur in the bulk during a number of processes outside the biosensor, such as the microbiological, the action of enzymes, and as a result of different chemical reactions taking place in the bulk. The shift of the pH can be the result of an enzyme reaction inside the biosensor. The shift of the pH can change the activity of the enzyme, because almost all enzymes are pH-dependent. The shift of the pH can also evoke the shrinking of the membranes, thus the diffusion parameters and the diffusion distance can be changed. All these shifts will influence the response of the biosensor. We evaluated the weight of these factors on the biosensor response.

2. Experimental

2.1. Biosensor

As a model biosensor, a well-known electrochemical glucose biosensor based on glucose oxidase was selected [3]. Glucose oxidase (*Asp. niger* sp., K_M = 0.23 mM) in albumin gel layer was

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deposited on the surface of the flat Pt electrode and covered with diffusion membrane made from cellulose or acetylated cellulose. Electrochemical registration of the oxidation current of the produced (hydrogen peroxide) was performed at potential 0.6 V *vs.* Ag/AgCl reference electrode.

2.2. Mathematical model

Suppose that the substrate (S) conversion to product (P) was catalysed by the enzyme:

$$S \xrightarrow{E} P \tag{1}$$

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 non-linear term corresponding to the Michaelis–Menten kinetics of the enzymatic reaction [4–8]. 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:

$$\begin{aligned} \frac{\partial S_m}{\partial t} &= d_{S_m}\frac{\partial^2 S_m}{\partial x^2}, \quad a_e \le x \le a_e + a_m, \\ \frac{\partial P_m}{\partial t} &= d_{P_m}\frac{\partial^2 P_m}{\partial x^2}, \end{aligned} \tag{2}$$

where x and t are space and time respectively, $S_m(x,t)$ is the concentration of the first substrate (glucose) and $P_m(x,t)$ 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_{S_m} and d_{P_m} 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 (bulk solution/membrane interface) remains constant while the biosensor keeps in touch with the substrate ($t > 0$). So, the following boundary conditions are satisfied:

$$S_m(t,\ a_e + a_m) = S_0, \quad P_m(t,\ a_e + a_m) = 0. \tag{3}$$

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:

$$\begin{aligned} \frac{\partial S_e}{\partial t} &= d_{S_e}\frac{\partial^2 S_e}{\partial x^2} - \frac{V_{\max} S_e}{K_M + S_e}, \quad 0 \le x \le a_e, \\ \frac{\partial P_e}{\partial t} &= d_{P_e}\frac{\partial^2 P_e}{\partial x^2} + \frac{V_{\max} S_e}{K_M + S_e}, \end{aligned} \tag{4}$$

where $S_e(x,t)$, $P_e(x,t)$ are the concentrations of the substrate and the reaction product in the enzyme layer, respectively. $V_{\max}$ is the maximal enzymatic rate. K_M is the Michaelis–Menten constant for glucose. d_{S_e} and d_{P_e} are the diffusion coefficients of the substrate and the reaction product in the enzyme layer, respectively. The concentration of the reaction product at the surface of electrode ($x = 0$) 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:

$$\frac{\partial S_e}{\partial t}(t,\ 0) = 0, \quad P_e(t,\ 0) = 0. \tag{5}$$

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 biosensor operation starts when some substrate appears in the bulk solution. This is used in the initial conditions ($t = 0$):

$$\begin{aligned} &S_e(0,\ x) = 0, \quad S_m(0,\ a_e + a_m) = S_0, \\ &P_e(0,\ x) = 0, \quad P_m(0,\ x) = 0. \end{aligned} \tag{6}$$

On the boundary between two regions with different diffusion coefficients we define the compatibility conditions ($t > 0$). These conditions mean that the concentration 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:

$$\begin{aligned} &S_e(t,\ a_e - 0) = S_m(t,\ a_e + 0), \\ &P_e(t,\ a_e - 0) = P_m(t,\ a_e + 0), \\ &d_{S_e}\frac{\partial S_e}{\partial x}(t,\ a_e - 0) = d_{S_m}\frac{\partial S_m}{\partial x}(t,\ a_e + 0), \\ &d_{P_e}\frac{\partial P_e}{\partial x}(t,\ a_e - 0) = d_{P_m}\frac{\partial P_m}{\partial x}(t,\ a_e + 0). \end{aligned} \tag{7}$$

We introduce the concentration S of the substrate and the concentration P of the reaction product in the entire domain as follows ($t > 0$):

$$\begin{aligned} S(t,\ x) &= \begin{cases} S_e(t,\ x), & 0 \le x \le a_e, \\ S_m(t,\ x), & a_e \le x \le a_e + a_m, \end{cases} \\ P(t,\ x) &= \begin{cases} P_e(t,\ x), & 0 \le x \le a_e, \\ P_m(t,\ x), & a_e \le x \le a_e + a_m. \end{cases} \end{aligned} \tag{8}$$

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

Biosensor development and modelling from both chemical and mathematical point of view are presented in a monograph [9].

3. Results and discussion

3.1. Digital simulation

Serious difficulties can arise when one tries to solve analytically a multidimensional non-linear system of partial differential equations with complex boundary conditions. Therefore, the problem was solved numerically [10,11]. 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.

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:

$$i(t) = n_e F d_{Se}\frac{\partial P_e}{\partial x}(t,\ 0), \tag{9}$$

where n_e is a number of electrons, involved in the charge transfer at the electrode surface, and F is the Faraday constant, $F = 96{,}485$ C/mol. The steady-state current I is defined as:

$$I = \lim_{t\to\infty} i(t). \tag{10}$$