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Modelling an amperometric biosensor acting in a flowing liquid

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SUMMARY

This paper presents a mathematical model of the amperometric biosensor based on an electrode covered with an enzyme membrane. The model involves three regions: the enzyme layer where enzymatic reaction as well as mass transport by diffusion takes place, a diffusion-limiting region where only diffusion takes place, and a convective region, where the analyte concentration is maintained constant. Using computer simulation the influence of the biosensor geometry as well as the flow intensity on the biosensor response was investigated. This paper also deals with the conditions when the mass transport in the exterior region may be neglected to simulate the biosensor response assuming that the buffer solution is in intense flow and in powerful motion. The digital simulation was carried out using the finite difference technique.

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KEY WORDS: reaction–diffusion; modelling; biosensor

1. INTRODUCTION

Biosensors are analytical devices made up of a biological entity, usually an enzyme, that recognizes a specific analyte and a transducer that translates the changes in the bio-molecule into an electrical signal [1]. The biosensors yield a signal, which is proportional to the concentration of the measured analyte. The amperometric biosensors measure the faradaic current that arises on the electrode by direct electrochemical oxidation or reduction of the reaction product. These devices have been widely used in environmental, medical and industrial applications because of their high selectivity, simplicity and low cost [2].

In the literature, mathematical models have been widely used as an important tool to study and optimize analytical characteristics of actual biosensors [3]. We consider a system where a membrane biosensor is used for an analysis of a continuously flowing analyte over the membrane surface.

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The goal of this investigation is to make a model involving enzymatic reaction in enzyme membrane and mass transport inside as well as outside the membrane. Although practical biosensors contain a multilayer enzyme membrane [4], the model biosensors containing the exploratory monolayer membrane are widely used to study the biochemical behaviour of biosensors [3, 5].

The developed model is based on the reaction–diffusion equations, containing a non-linear term related to Michaelis–Menten kinetics of the enzymatic reaction [6, 7]. The model involves three regions: the enzyme layer where enzymatic reaction as well as the mass transport by diffusion takes place, a diffusion-limiting region where only a mass transport by diffusion takes place, and a convective region, where the analyte concentration is maintained constant [3, 8].

Using computer simulation the influence of the thickness of the enzyme membrane as well the diffusion layer on the biosensor response was investigated. The conditions when the exterior mass transport may be neglected were investigated. The computer simulation was carried out using the finite difference technique [9, 10].

2. MATHEMATICAL MODEL

A biosensor may be considered as an electrode, having a layer of enzyme (enzyme membrane) applied onto the electrode surface. Assuming the symmetrical geometry of the electrode and homogeneous distribution of the immobilized enzyme in the enzyme membrane, the biosensor action can be described by the reaction–diffusion system ($t>0$) [3, 8]

$$\frac{\partial S_e}{\partial t}=D_{S_e}\frac{\partial^2 S_e}{\partial x^2}-\frac{V_{\max}S_e}{K_M+S_e},\quad \frac{\partial P_e}{\partial t}=D_{P_e}\frac{\partial^2 P_e}{\partial x^2}+\frac{V_{\max}S_e}{K_M+S_e},\quad x\in(0,d) \tag{1}$$

$$\frac{\partial S_b}{\partial t}=D_{S_b}\frac{\partial^2 S_b}{\partial x^2},\quad \frac{\partial P_b}{\partial t}=D_{P_b}\frac{\partial^2 P_b}{\partial x^2},\quad x\in(d,d+\delta) \tag{2}$$

where x stands for space, t stands for time, $S_e(x,t), S_b(x,t)(P_e(x,t), P_b(x,t))$ are the substrate (reaction product) concentrations in the enzyme and in the liquid, respectively, d is the thickness of the enzyme membrane, δ is the thickness of the diffusion layer, D_{S_e}, D_{S_b}, D_{P_e}, D_{P_b} are the diffusion coefficients, $V_{\max}$ is the maximal enzymatic rate and K_M is the Michaelis constant.

Let $x=0$ represent the electrode surface, while $x=d$ represent the boundary layer between the liquid and the enzyme membrane. The biosensor operation starts when some substrate appears in the liquid ($t=0$)

$$\begin{aligned} S_e(x,0)&=0,\quad P_e(x,0)=0,\quad x\in[0,d]\\ S_b(x,0)&=0,\quad P_b(x,0)=0,\quad x\in[d,d+\delta)\\ S_b(d+\delta,0)&=S_0,\quad P_b(d+\delta,0)=0 \end{aligned} \tag{3}$$

where S_0 is the concentration of the substrate to be analysed.

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On the boundary between two subregions we define the matching conditions ($t>0$)

$$\begin{aligned} D_{S_e}\frac{\partial S_e}{\partial x}\bigg|_{x=d} &= D_{S_b}\frac{\partial S_b}{\partial x}\bigg|_{x=d}, \quad S_e(d,t)=S_b(d,t) \\ D_{P_e}\frac{\partial P_e}{\partial x}\bigg|_{x=d} &= D_{P_b}\frac{\partial P_b}{\partial x}\bigg|_{x=d}, \quad P_e(d,t)=P_b(d,t) \end{aligned} \tag{4}$$

In the bulk solution the concentration of the substrate as well as the product remains constant ($t>0$)

$$S_b(d+\delta,t)=S_0, \quad P_b(d+\delta,t)=0 \tag{5}$$

At the electrode surface ($x=0$) the potential is chosen to maintain zero concentration of the reaction product

$$P_e(0,t)=0, \quad D_{S_e}\frac{\partial S_e}{\partial x}\bigg|_{x=0}=0, \ \ t>0 \tag{6}$$

We assume that system (1)–(6) approaches a steady state as $t\to\infty$. A density $i(t)$ of the biosensor current at time t can be obtained explicitly from Faraday's and Fick's laws [3]

$$i(t)=n_e F D_{P_e}\frac{\partial P_e}{\partial x}\bigg|_{x=0}, \quad I=\lim_{t\to\infty} i(t) \tag{7}$$

where n_e is the number of electrons involved in a charge transfer at the electrode surface, F is the Faraday constant and I is the steady-state biosensor current.

The biosensor response is known to be under mass transport control if the enzymatic reaction in the enzyme layer is faster than the mass transport [3, 6]. The dimensionless diffusion modulus (Damköhler number) σ^2 essentially compares the rate of enzymatic reaction (V_{max}/K_M) with the diffusion through the enzyme layer (D_{S_e}/d^2)

$$\sigma^2=\frac{V_{max}d^2}{D_{S_e}K_M} \tag{8}$$

If $\sigma^2\ll 1$ then the enzyme kinetics controls the biosensor response. The response is under diffusion control when $\sigma^2\gg 1$.

3. SOLUTION OF THE PROBLEM

Problem (1)–(6) was solved numerically using the finite difference technique [9, 10]. We introduced a non-uniform discrete grid in both directions: x and t. An implicit finite difference scheme has been built as a result of the difference approximation of the model. The resulting systems of linear algebraic equations were solved efficiently because of the tridiagonality of their matrices. The digital simulator has been programmed in Java language [11].

The mathematical model as well as the numerical solution of the model was evaluated for different values of the maximal enzymatic rate V_{max}, substrate concentration S_0, the thickness d of

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the enzyme layer and the thickness δ of the external layer. The following values of the parameters were constant in all the numerical experiments:

$$\begin{aligned} &D_{S_e} = D_{P_e} = 300\,\mu m^2/s, \quad D_{S_b} = D_{P_b} = 2D_{P_e} \\ &K_M = 100\,\mu M, \quad n_e = 2 \end{aligned} \tag{9}$$

At low concentrations ($S_0 \ll K_M$) as well as high concentrations ($S_0 \gg K_M$) of the substrate, the steady-state response can be calculated analytically [3]. The adequacy of the mathematical and numerical models was evaluated using known analytical solutions. The relative difference between the numerical and analytical solutions was less than 1%.

4. RESULTS AND DISCUSSION

Using computer simulation the influence of the thickness of both the enzyme and the diffusion layers on the biosensors response was investigated.

The thickness d of the enzyme membrane of a biosensor can usually be measured physically rather precisely. The thickness δ of the diffusion layer depends upon the flowing of the buffer solution. The thickness δ is inversely proportional to the intensity of the flowing. δ can be estimated experimentally [12].

4.1. The effect of the thickness of the diffusion layer

We investigate the dependence of the steady-state biosensor response on the relative thickness of the diffusion layer. We consider a dimensionless Biot number Bi to express the ratio of the internal mass transfer resistance to the external one [6]. Because of high sensitivity of the maximal biosensor current to the thickness of the enzyme layer we normalize the steady-state current [5]

$$I_N(Bi) = \frac{I(Bi)}{I(\infty)}, \quad Bi = \frac{d/D_{S_e}}{\delta/D_{S_b}} = \frac{D_{S_b} d}{D_{S_e} \delta} \tag{10}$$

where $I(Bi)$ is the steady-state current (7) calculated at given Biot number Bi. $I(\infty)$ corresponds to the biosensor response for zero thickness of the external diffusion layer, $\delta = 0$.

The biosensor response *versus* the Biot number Bi was investigated at different values of the maximal enzymatic rate V_{max}, the substrate concentration S_0 and the membrane thickness d. Results of the calculation obtained at two values of V_{max}: 10 and 100 μM/s and various values of the thickness d are depicted in Figure 1.

One can see in Figure 1 that the steady-state biosensor current is a monotonous increasing function of the Biot number Bi when the response is under diffusion control ($\sigma > \approx 1.5$) [5]. I_N is a non-monotonous function of Bi when the enzyme kinetics controls the biosensor response ($\sigma < \approx 1.5$). In the cases when $\sigma \approx 1$ the steady-state biosensor current varies slightly at $Bi > \approx 1$. At all values of the diffusion modulus σ, the steady-state current varies slightly at $Bi > 10$.

4.2. The effect of the Nernst diffusion layer

The thickness δ of the external diffusion layer depends upon the nature and intensity of flowing of the buffer solution. Usually, the more intense flow corresponds to the thinner diffusion layer [12]. That diffusion layer is known as the Nernst layer. The thickness of the Nernst diffusion

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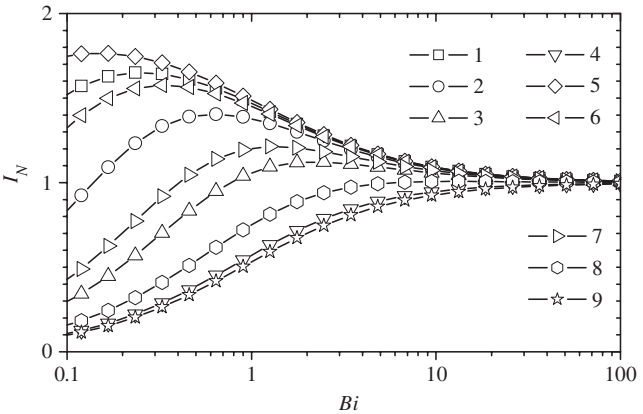

Figure 1. The normalized steady-state current I_N *versus* the Biot number Bi at $V_{max}=100$ (1–4), $V_{max}=10\,\mu M/s$ (5–9), $S_0=K_M$, and nine diffusion modulus σ: 0.18 (5), 0.29 (1), 0.37 (6), 0.58 (2), 0.91 (7), 1.15 (3), 1.83 (8), 2.89 (4), 3.65 (9).

layer practically does not depend upon the membrane thickness. In practice, the zero thickness of the Nernst layer cannot be achieved [12]. The thickness of the Nernst diffusion layer may be minimized up to $\delta=2\,\mu m$ by increasing the flowing speed [12].

In the cases when an analyte is in powerful motion, the mass transport by diffusion outside the enzyme membrane is neglected rather often [3, 10]. We assume that a model of the biosensor action taking into consideration the Nernst diffusion layer describes the biosensor action more precisely than another one where the Nernst diffusion layer is neglected. In addition, we assume that the Nernst diffusion layer of thickness δ may be neglected for a biosensor having membrane thickness d only if the steady-state response calculated considering the Nernst layer is approximately the same as in the case when the Nernst diffusion layer is neglected.

We introduce the relative error of the biosensor response

$$R(Bi)=\frac{|I(Bi)-I(\infty)|}{I(Bi)} \tag{11}$$

$R(Bi)$ may be called the relative error of the use of the model where the diffusion layer of thickness δ is neglected at the Biot number Bi. This function may also be regarded as a level of a reliability of the mathematical model where the Nernst diffusion layer is not taken into account.

We investigate the conditions when the Nernst diffusion layer may be neglected to simulate the response of biosensors accurately. To investigate the effect of the Nernst diffusion layer on the biosensor response when the analyte is in intense flow we calculated the relative error R at practically minimal thickness of the diffusion layer. Since the effect of the diffusion layer on the biosensor response significantly depends upon the diffusion modulus, we calculate the normalized response changing in a wide range both the maximal enzymatic rate V_{max} and the membrane thickness d. Figure 2 shows the results of calculation at the thickness $\delta=2\,\mu m$ of the Nernst diffusion layer.

One can see in Figure 2 that the effect of the Nernst layer decreases with an increase in the Biot number Bi as well as in the membrane thickness d. Figure 2 shows that the Nernst diffusion layer of the thickness of $2\,\mu m$ should be taken into consideration in all the cases when the enzyme

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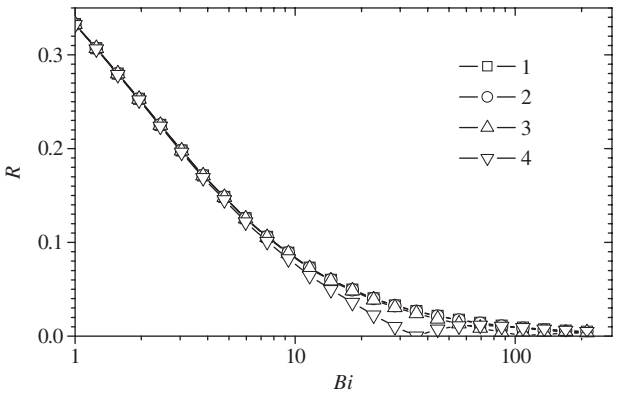

Figure 2. The relative error R *versus* the Biot number Bi at the thickness $\delta = 2\,\mu$m of the Nernst diffusion layer and four values of V_{max}: 0.1 (1), 1 (2), 10 (3) and 100 (4) μM/s, $S_0 = K_M$.

membrane is thinner than about 50 μm ($Bi = 50$). The simulated steady-state biosensor current I may differ even more than 30% ($R > 0.3$) from the true current if the Nernst diffusion layer is neglected in the cases of thin enzyme membranes, $Bi \leqslant 1$, when the buffer solution is in intense flow. The effect of the Nernst diffusion layer becomes slight only in the cases when the Biot number is greater than about 50.

5. CONCLUSIONS

The mathematical model (1)–(6) can be used to investigate regularities of the biosensor response in flowing and non-flowing analytes.

If the biosensor response is distinctly under diffusion control then the steady-state biosensor current I is a monotonous increasing function of the Biot number Bi. In the cases when the enzyme kinetics controls the biosensor response, I is a non-monotonous function of Bi (Figure 1).

The Nernst diffusion layer should be taken into consideration when an analytical system based on an amperometric biosensor acts under conditions when the Biot number is less than about 50 (Figure 2).

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