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Nonlinear effects of diffusion limitations on the response and sensitivity of amperometric biosensors

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ABSTRACT

The dependencies of the internal and external diffusion limitations on the response and sensitivity of amperometric biosensors are investigated computationally using a two-compartment model, and a framework for evaluating and selecting the biosensor configuration is proposed. Enzyme-based biosensors are mathematically modelled by a system of reaction-diffusion equations containing a nonlinear term related to Michaelis-Menten kinetics. The biosensor response, sensitivity and stability are numerically analysed at the transition and steady state conditions in a wide range of model parameters, and attractive biosensor configurations are derived. The performed calculations show nonlinear effects of internal and external diffusion limitations on the biosensor current, response time and sensitivity as well as on the linear range of the calibration curve. The phenomenon of the non-monotonicity of the biosensor response is also investigated.

1. Introduction

Catalytic biosensors are analytical devices based on the enzyme-catalyzed conversion of analytes of interest into redox active products [1,2]. Amperometric biosensors produce the output anodic or cathodic current which in most cases is proportional to the concentration of the substrate. These instruments have found wide applications ranging from clinical through to environmental and industrial [2–4].

Despite numerous advantages of biosensors, biological materials suffer from several drawbacks. A limited stability of the sensing element, a high price of purified enzymes, a relatively short linear range of the calibration curve and a high background current are among the main drawbacks restricting wider use of biosensors [4,5].

Due to the drawbacks of free-diffusing redox compounds, especially with respect to continuous monitoring of the analyte, an application of different semi-permeable membranes permit to build reliable and highly sensitive bioelectrocatalytic systems [4,6]. Commercial biosensors often contain a thin layer of polyvinyl alcohol, polyurethane, cellulose, terylene or other material, which prevents the enzyme from dissolution [3]. An opportunity to increase the biosensor sensitivity as well as to prolong the linear range of the calibration curve by increasing the diffusion barrier to the substrate is an important feature of enzyme-based electrochemical biosensors, especially due to the possibility to increase the sensitivity at different limitations of the biosensor action [1,2,5].

The prediction of both, geometric and catalytic parameters, is of crucial importance for solving analytical problems and development of novel biosensors. Using mathematical and computational modelling to characterize the biosensor response in a wide range of input parameters can guide the experimental work, thus reducing development time and costs [6,7].

When modelling biosensors with selective or dialysis membranes, multi-layer models are required to achieve a sufficient accuracy of the model [7–9]. Nevertheless, even mono layer models, in which the external mass transport by diffusion is ignored, are still used in different applications due to the model simplicity [10–12]. Starting from 70ths till now, two-compartment models as a particular case of the multi-layer models have been extensively used in the modelling of biosensors [7,13–17].

In a two-compartment model, a biosensor is modelled as a relatively thin layer of an enzyme applied onto the sensing surface and an outer diffusion layer [5,7]. Various attractive features of the biosensors have been identified or approved by applying two compartment models [15,17–19]. Particularly, it was found that by applying a thick highly acetylated membrane the apparent Michaelis constant for a electrochemical glucose oxidase biosensor can be increased up to 400% keeping a stable biosensor response [20].

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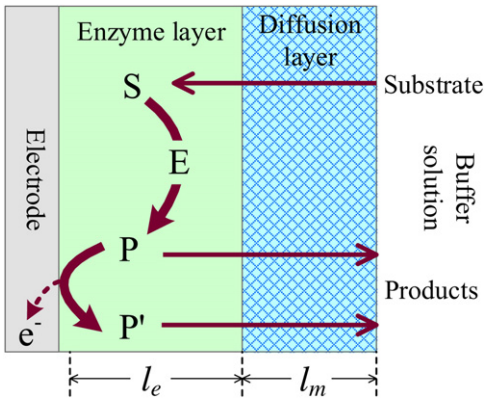

Fig. 1. Schematic representation of the amperometric biosensor.

The goal of this work was to investigate in detail the dependencies of the internal and external diffusion limitations on the response and sensitivity of amperometric biosensors modelled by a two-compartment model, based on reaction-diffusion equations containing a nonlinear term related to Michaelis-Menten kinetics [7,21]. The biosensor current, sensitivity, response time and stability were numerically analysed at transition and steady state conditions in a wide range of model parameters, and attractive configurations of biosensors have been determined. The computational simulation was carried out using the finite difference technique [22]. The performed calculations showed nonlinear effects of the internal and external diffusion limitations on the biosensor current, response time and sensitivity as well as on a linear range of the calibration curve. The phenomenon of the non-monotonicity of the biosensor response was also investigated.

2. Mathematical modelling

2.1. Biosensor principal structure

An amperometric biosensor to be modelled is considered as an electrode and a relatively thin layer of an enzyme (enzyme membrane) applied onto the electrode surface followed by outer semi-permeable membrane [2,4,5,23]. The schematic view of the modelled biosensor is presented in Fig. 1, where l_e and l_m stand for thicknesses of the enzyme layer and outer membrane, respectively.

In the enzyme layer we consider the enzyme-catalyzed reaction

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}, \tag{1}$$

where the substrate (S) combines reversibly with an enzyme (E) to form a complex (ES). The complex then dissociates into the product (P) and the enzyme is regenerated [23,24].

Assuming the quasi steady state approximation, the concentration of the intermediate complex (ES) does not change and may be neglected when modelling the biochemical behaviour of biosensors [2,23,25]. In the resulting scheme, the substrate (S) is enzymatically converted to the product (P),

$$\mathrm{S} \xrightarrow{\mathrm{E}} \mathrm{P} \tag{2}$$

At the electrode surface, the electro-active product P is converted into a species P′ having no influence on the function of the biosensor,

$$\mathrm{P} \rightarrow \mathrm{P}' \pm e^- \tag{3}$$

2.2. Mathematical model

The biosensor model involves three regions: the enzyme layer (enzyme membrane) where the enzymatic reaction (2) as well as the mass transport by diffusion of both compounds (the substrate S and the product P) take place, a semi-permeable membrane where only the mass transport by diffusion takes place, and a convective region where the analyte concentration is maintained constant.

2.2.1. Governing equations

Assuming the symmetrical geometry of both membranes and the homogeneous distribution of the immobilized enzyme in the enzyme layer leads to a two compartment model [3,7,21]. The dynamics of the concentrations of the substrate S as well as the product P in the enzyme layer can be described by a system of reaction-diffusion equations ($t > 0$),

$$\begin{aligned} \frac{\partial s_e}{\partial t} &= d_{S_e} \frac{\partial^2 s_e}{\partial x^2} - \frac{v_{\max} s_e}{k_M + s_e}, \\ \frac{\partial p_e}{\partial t} &= d_{P_e} \frac{\partial^2 p_e}{\partial x^2} + \frac{v_{\max} s_e}{k_M + s_e}, \quad x \in (0,\ l_e), \end{aligned} \tag{4}$$

where x and t stand for space and time, $s_e(x, t)$ and $p_e(x, t)$ are the concentrations of the substrate and the product in the enzyme layer, d_{S_e} and d_{P_e} are the diffusion coefficients, $v_{\max}$ is the maximal enzymatic rate, $v_{\max} = k_2 e_0$, e_0 is the enzyme concentration, k_M is the Michaelis constant, $k_M = (k_{-1} + k_2)/k_1$ [23,24].

Outside the enzyme membrane only the mass transport by diffusion of both compounds takes place ($t > 0$),

$$\begin{aligned} \frac{\partial s_m}{\partial t} &= d_{S_m} \frac{\partial^2 s_m}{\partial x^2}, \\ \frac{\partial p_m}{\partial t} &= d_{P_m} \frac{\partial^2 p_m}{\partial x^2}, \quad x \in (l_e,\ l_e + l_m), \end{aligned} \tag{5}$$

where $s_m(x, t)$ and $p_m(x, t)$ are the concentrations of the substrate and the reaction product, d_{S_m}, d_{P_m} are the diffusion coefficients. The diffusion layer can be also treated as the Nernst diffusion layer - a thin layer of stagnant solution [4,22,26].

2.2.2. Initial and boundary conditions

The biosensor operation starts when the substrate appears in the buffer solution and contacts the outer surface of the membrane ($t = 0$),

$$\begin{aligned} &s_e(x, 0) = 0, \quad p_e(x, 0) = 0, \quad x \in [0,\ l_e], \\ &s_m(x, 0) = 0, \quad p_m(x, 0) = 0, \quad x \in [l_e + l_m), \\ &s_m(l_e + l_m,\ 0) = s_0, \quad p_m(l_e + l_m, 0) = 0, \end{aligned} \tag{6}$$

where s_0 is the concentration of the substrate in the buffer solution.

Due to the electrode polarization the concentration of the reaction product at the electrode surface is permanently reduced to zero, while no substrate concentration flux at that surface is assumed [7,27],

$$p_e(0, t) = 0, \quad d_{S_e} \frac{\partial s_e}{\partial x}\Big|_{x=0} = 0, \quad t > 0. \tag{7}$$

Away from the outer membrane the concentrations of the substrate and the product remain constant ($t > 0$),

$$s_m(l_e + l_m, t) = s_0, \quad p_m(l_e + l_m,\ t) = 0. \tag{8}$$