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COMPUTATIONAL MODELLING OF COMPLEX BIOCATALYTIC PROCESSES IN BIOSENSORS

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VILNIAUS UNIVERSITETAS

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KOMPIUTERINIS SUDĖTINGŲ BIOKATALIZĖS PROCESŲ BIOJUTIKLIUOSE MODELIAVIMAS

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Introduction

Research Object and Relevance of the Problem

A chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. Chemical sensors usually contain two basic components connected in series: a chemical (molecular) recognition system (receptor) and a physicochemical transducer. Biosensors are chemical sensors in which the recognition system utilizes a biochemical mechanism [1, 2]. Biosensors are suitable for fast quantitative analysis or continual monitoring of substance concentrations. [3–5].

Biosensor recognition system is usually enzyme-based. Enzymes are known for their characteristic trait to catalyze only one specific chemical reaction which provides biosensors with high specificity. Also enzymes are known to be a very efficient catalysts ensuring high biosensor sensitivity [3, 6–10].

Biosensors are known to be cheap and reliable devices which may often substitute expensive and slow quantitative analysis in a laboratory. Biosensors are widely used for environment monitoring, food analysis, clinical diagnostics, drug analysis and some other purposes [4, 11–15].

Biosensors are cheap, but their development and calibration requires a lot of effort. In order to reduce the number of required physical experiments, mathematical modelling should be used. With the help of mathematical modelling it is possible to predict properties of biosensors prior to conducting physical experiments in a laboratory. This way the number of physical experiments as well as expenses of creation of a new biosensor may be significantly reduced.

Mathematical models of biosensors may take a form of non-linear partial differential equations [16–18]. Equations usually consist of linear term representing diffusion and non-linear term representing chemical kinetics. Analytical solutions of these equations are known for special cases only: when biosensor structure is simple and concentrations of substances are very high or very low [16–18]. These equations may be solved in general case by using numerical methods. In this thesis systems of partial differential equations were solved using finite difference technique [19–21].

Objectives and Tasks of the Research

The objective of the dissertation is the automatization of computational biosensor model creation. Models of interest are of biosensors with complex biocatalytic processes: substrate and product inhibition and substrate synergy. Another objective is the investigation of biosensor properties and behaviour using created biosensor models. To achieve the objectives of the dissertation the following tasks were formulated:

1. Propose the model of an amperometric biosensor with substrate and product inhibition.
2. Propose the model of an amperometric biosensor with synergistic substrates.
3. Create generalized model of a biosensor with substrate and product inhibition which provides means for flexible description of a biosensor structure.
4. Create universal model of a biosensor which may be used to model a wide set of biosensors. This set should include biosensors with substrate and product inhibition as well as biosensors with synergistic substrates. The model should provide means for flexible description of a biosensor structure and chemical kinetics.

5. Create a software which automates the creation of computational models based on generalized model of a biosensor with substrate and product inhibition and computational models based on universal model of a biosensor.

6. Validate mathematical models of biosensors and investigate properties and behaviour of biosensors using the developed software.

Scientific Novelty and Practical Significance

Generalized model of a biosensor with substrate and product inhibition which provides means for flexible description of a biosensor structure was created.

Universal model of a biosensor which may be used to model a wide set of biosensors was created. This set includes biosensors with substrate and product inhibition as well as biosensors with synergistic substrates. The model provides means for flexible description of a biosensor structure and chemical kinetics.

The software which automates the creation of computational biosensor models based on mathematical models described in previous two paragraphs was developed.

Properties and behaviour of a biosensor with substrate and product inhibition were thoroughly investigated using the developed software. The developed software provides means for flexible description of a biosensor structure, thus the software may be used for investigation of a wide set of biosensors which were not investigated in this dissertation.

Properties and behaviour of a biosensor with synergistic substrates were thoroughly investigated using the developed software. The mechanism of substrate synergy was investigated. The conditions required for synergy to occur were determined. The software may be used for investigation of much wider set of biosensors than were investigated in this dissertation. This set includes biosensors of complex structure as well as biosensors based on chemical reactions with complex kinetics.

It is possible to optimize the configuration of a biosensor prior to a creation phase of a physical device using the developed software. More efficient biosensors may be created using results of computational investigation.

The results of the research conducted in the course of the thesis preparation were used in two scientific projects: "Development of bioelectrocatalysis for synthesis and analysis (BIOSA)" funded by a grant (No. PBT-04/2010) from the Research Council of Lithuania and "Developing computational techniques, algorithms and tools for efficient simulation and optimization of biosensors of complex geometry" funded by the European Social Fund under Measure VP1-3.1-ŠMM-07-K "Support to Research of Scientists and Other Researchers (Global Grant)".

Statements Presented for Defense

1. The generalized model of a biosensor with substrate and product inhibition provides means for flexible description of a biosensor structure. The developed software may be used to investigate biosensors described by this model.

2. The set of biosensors described by the universal biosensor model includes biosensors with substrate and product inhibition as well as biosensors with synergistic substrates. The universal biosensor model provides means to define complex structure of biosensors as well as complex kinetics of chemical reactions which take place in biosensors. The biosensors described by the universal model may be modelled using the developed software.

3. The measurement of both steady-state and maximal biosensor current in biosensor with substrate inhibition allows the determination of substrate concentration in a wider range of substrate concentrations than using the sole current. This concentration range is wider than one of a biosensor without substrate inhibition when the rest of biosensor parameters coincide.

4. The intrinsic mechanism of substrate synergy may be explained by using computational modelling of biosensors.

1. Mathematical Models of Biosensors Based on Complex Biocatalytic Processes

1.1. Modelling of Biosensors in the Case of Substrate and Product Inhibition

1.1.1. Chemical Reactions in Biosensor

Usually biosensors operate following such biocatalytic reaction scheme [3, 6, 22],

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

where E is the enzyme, S is the substrate, ES is the enzyme and substrate complex, and P is the reaction product, k_i is the reaction rate constant, $i = -1, 1, 2$. However, very often the kinetics of enzyme-catalysed reactions is much more complex. An inhibition, an activation, an allostery and other types of non-Michaelis-Menten kinetics are known for the diversity of enzymes [23–28].

The inhibition is a process when a substance (inhibitor) diminishes the rate of a biochemical reaction [25]. In enzyme-catalysed reactions, the inhibitor frequently acts by binding to the enzyme. In this dissertation, a specific case of the non-Michaelis-Menten behaviour is investigated. It is the case when the enzyme-substrate complex ES interacts with one more substrate molecule producing a non-active complex ESS (the substrate inhibition) as follows:

$$\mathrm{ES} + \mathrm{S} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{ESS}. \tag{2}$$

In addition, an enzyme molecule interacts with the product molecule producing another non-active complex EP (the product inhibition),

$$\mathrm{E} + \mathrm{P} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \mathrm{EP}. \tag{3}$$

1.1.2. Biosensor Structure

The amperometric biosensor is considered as an electrode and a relatively thin layer of an enzyme (enzyme membrane) applied onto the electrode surface. The model involves three regions: the enzyme layer where the enzymatic reaction as well as the mass transport by diffusion takes place, a diffusion limiting region where only the mass transport by diffusion takes place and a convective region where the analyte concentration is maintained constant. Assuming the symmetrical geometry

of the electrode and a homogeneous distribution of the immobilized enzyme in the enzyme membrane, the mathematical model of the biosensor action can be defined in a one-dimensional-in-space domain [16, 17].

1.1.3. Governing Equations

The governing equations for a chemical reaction network can be formulated by the law of mass action [6, 29]. Coupling the enzyme-catalysed reaction in the enzyme layer with the one-dimensional-in-space diffusion, described by Fick's law, leads to the following equations of the reaction–diffusion type ($t > 0$):

$$\frac{\partial s_e}{\partial t} = D_{s_e}\frac{\partial^2 s_e}{\partial x^2} - k_1\, e_e\, s_e + k_{-1}\, e_{es} - k_3\, e_{es}\, s_e + k_{-3}\, e_{ess}\,, \tag{4a}$$

$$\frac{\partial p_e}{\partial t} = D_{p_e}\frac{\partial^2 p_e}{\partial x^2} + k_2\, e_{es} - k_4\, e_e\, p_e + k_{-4}\, e_{ep}\,, \tag{4b}$$

$$\frac{\partial e_e}{\partial t} = -k_1\, e_e\, s_e + k_{-1}\, e_{es} + k_2\, e_{es} - k_4\, e_e\, p_e + k_{-4}\, e_{ep}\,, \tag{4c}$$

$$\frac{\partial e_{es}}{\partial t} = k_1\, e_e\, s_e - k_{-1}\, e_{es} - k_2\, e_{es} - k_3\, e_{es}\, s_e + k_{-3}\, e_{ess}\,, \tag{4d}$$

$$\frac{\partial e_{ess}}{\partial t} = k_3\, e_{es}\, s_e - k_{-3}\, e_{ess}\,, \tag{4e}$$

$$\frac{\partial e_{ep}}{\partial t} = k_4\, e_e\, p_e - k_{-4}\, e_{ep}\,, \quad 0 < x < d_e\,, \tag{4f}$$

where x and t stand for space and time, respectively, $s_e(x,t)$, $p_e(x,t)$, $e_e(x,t)$, $e_{es}(x,t)$, $e_{ess}(x,t)$ and $e_{ep}(x,t)$ are the molar concentrations of the substrate S, the product P, the enzyme E, the ES complex, the ESS complex and the EP complex, respectively, d_e is the thickness of the enzyme layer, D_{s_e} and D_{p_e} are the diffusion coefficients of the substrate and the reaction product, respectively. The enzyme and the formed ES, ESS and EP complexes are immobilized, and therefore there are no diffusion terms in the corresponding equations.

Outside the enzyme layer only the mass transport by diffusion of the substrate and the product takes place. We assume that the external mass transport obeys a finite diffusion regime,

$$\frac{\partial s_d}{\partial t} = D_{s_d}\frac{\partial^2 s_d}{\partial x^2}, \tag{5a}$$

$$\frac{\partial p_d}{\partial t} = D_{p_d}\frac{\partial^2 p_d}{\partial x^2}, \quad d_e < x < d_e + d_d, \quad t > 0, \tag{5b}$$

where $s_d(x,t)$ and $p_d(x,t)$ stand for concentrations of the substrate and the product in the diffusion layer, d_d is the thickness of the external diffusion layer, D_{s_d} and D_{p_d} are the diffusion coefficients.

1.1.4. Initial and Boundary Conditions

Let $x = 0$ represent the electrode surface, $x = d_e$ is the boundary between the enzyme and the diffusion layers, and $x = d_e + d_d$ is the boundary between the diffusion layer and the bulk solution. The biosensor operation starts when some

substrate appears in the bulk solution ($t = 0$),

$$s_e(x,0) = 0, \quad p_e(x,0) = 0, \quad 0 \le x \le d_e\,, \tag{6a}$$
$$s_d(x,0) = 0, \quad p_d(x,0) = 0, \quad d_e \le x < d_e + d_d\,, \tag{6b}$$
$$s_d(d_e + d_d, 0) = s_0, \quad p_d(d_e + d_d, 0) = 0\,, \tag{6c}$$
$$e_e(x,0) = e_0, \quad e_{es}(x,0) = 0, \quad 0 < x < d_e\,, \tag{6d}$$
$$e_{ess}(x,0) = 0, \quad e_p(x,0) = 0, \quad 0 < x < d_e\,, \tag{6e}$$

where s_0 is the concentration of the analyte (substrate) in the bulk solution, e_0 is the enzyme concentration.

Due to the electrode polarization concentration of the reaction product at the electrode surface ($x = 0$) is permanently reduced to zero [16],

$$p_e(0,t) = 0. \tag{7}$$

Since the substrate is not ionized, the substrate concentration flux on the electrode surface equals zero,

$$D_{s_e} \frac{\partial s_e}{\partial x}\bigg|_{x=0} = 0. \tag{8}$$

The external diffusion layer ($d_e < x < d_e + d_d$) is treated as the Nernst diffusion layer [30]. According to the Nernst approach the layer of the thickness d_d remains unchanged with time. It is also assumed that away from it the solution is uniform in concentration ($t > 0$),

$$s_d(d_e + d_d, t) = s_0, \quad p_d(d_e + d_d, t) = 0. \tag{9}$$

On the boundary between two regions having different diffusivities, the matching conditions have to be defined ($t > 0$),

$$D_{s_e} \frac{\partial s_e}{\partial x}\bigg|_{x=d_e} = D_{s_d} \frac{\partial s_d}{\partial x}\bigg|_{x=d_e}, \quad s_e(d_e,t) = s_d(d_e,t)\,, \tag{10a}$$
$$D_{p_e} \frac{\partial p_e}{\partial x}\bigg|_{x=d_e} = D_{p_d} \frac{\partial p_d}{\partial x}\bigg|_{x=d_e}, \quad p_e(d_e,t) = p_d(d_e,t)\,. \tag{10b}$$

According to these conditions, the substrate and the product concentration fluxes through the external diffusion layer are equal to the corresponding fluxes entering the surface of the enzyme layer. The concentrations of the substrate as well as the product from both layers are equal on the boundary between these layers.

1.1.5. Quasi-Steady-State Approximation

Some reactions in the network (1)–(3) are very fast, while others are considerably slower [3]. The large difference of timescales in the reaction network creates difficulties for simulating the temporal evolution of the network and for understanding the basic principles of its operation. To sidestep these problems, the quasi-steady-state approximation (QSSA) is often applied [31, 32],

$$\frac{\partial e_e}{\partial t} \approx \frac{\partial e_{es}}{\partial t} \approx \frac{\partial e_{ess}}{\partial t} \approx \frac{\partial e_{ep}}{\partial t} \approx 0\,. \tag{11}$$

Assuming the QSSA leads to a reduction in the dimension of the system (4),

$$\frac{\partial s_e}{\partial t} = D_{s_e}\frac{\partial^2 s_e}{\partial x^2} - \frac{k_2(e_e + e_{es} + e_{ess} + e_{ep})s_e}{\frac{k_{-1}+k_2}{k_1}\left(1 + p_e/\frac{k_{-4}}{k_4}\right) + s_e\left(1 + s_e/\frac{k_{-3}}{k_3}\right)}\,, \tag{12a}$$

$$\frac{\partial p_e}{\partial t} = D_{p_e}\frac{\partial^2 p_e}{\partial x^2} + \frac{k_2(e_e + e_{es} + e_{ess} + e_{ep})s_e}{\frac{k_{-1}+k_2}{k_1}\left(1 + p_e/\frac{k_{-4}}{k_4}\right) + s_e\left(1 + s_e/\frac{k_{-3}}{k_3}\right)}\,. \tag{12b}$$

The total sum e_0 of the concentrations of all the enzyme forms is assumed to be constant in the entire enzyme layer, $e_0 = e_e + e_{es} + e_{ess} + e_{ep}$.

In order to reduce the number of the main governing parameters of the mathematical model, the following parameters are introduced:

$$V_{max} = k_2 e_0 = k_2(e_e + e_{es} + e_{ess} + e_{ep})\,, \tag{13a}$$

$$K_M = \frac{k_{-1} + k_2}{k_1}, \quad K_s = \frac{k_{-3}}{k_3}, \quad K_p = \frac{k_{-4}}{k_4}\,, \tag{13b}$$

where V_{max} is the maximal enzymatic rate, K_M is the Michaelis constant, K_s is the substrate inhibition rate, and K_p is the product inhibition rate [3, 6, 31].

Finally, the governing equations (4) at the QSSA reduce to the following equations ($t > 0$):

$$\frac{\partial s_e}{\partial t} = D_{s_e}\frac{\partial^2 s_e}{\partial x^2} - v(s_e, p_e), \tag{14a}$$

$$\frac{\partial p_e}{\partial t} = D_{p_e}\frac{\partial^2 p_e}{\partial x^2} + v(s_e, p_e), \quad 0 < x < d_e, \tag{14b}$$

where $v(s_e, p_e)$ is the quasi-steady-state reaction rate,

$$v(s_e, p_e) = \frac{V_{max} s_e}{K_M\,(1 + p_e/K_p) + s_e\,(1 + s_e/K_s)}\,. \tag{15}$$

In the case of the Michaelis-Menten kinetics, the condition for the QSSA to be valid is $e_0 \ll s_0 + K_M$ [32, 33].

1.1.6. Characteristics of the Biosensor Response

The electric current is measured as a response of a biosensor in a physical experiment. The current depends on a flux of reaction product at an electrode surface. Thus the density j of the current at time t is proportional to the gradient of the product at the electrode surface, i.e. at the border $x = 0$, as described by Faraday's law,

$$j(t) = n_e F D_{p_e}\left.\frac{\partial p_e}{\partial x}\right|_{x=0}\,, \tag{16}$$

where n_e is a number of electrons involved in the electrochemical reaction, and F is Faraday's constant ($F = 96\,486$ C/mol) [3, 16].

We assume that the system (5)-(10), (14) approaches a steady-state as $t \to \infty$,

$$j_{st} = \lim_{t\to\infty} j(t)\,, \tag{17}$$

where j_{st} is assumed as the density of the steady-state biosensor current.

The sensitivity is also one of the most important characteristics of the biosensors. The biosensor sensitivity is usually expressed as the gradient of the biosensor current with respect to the concentration s_0 of the substrate in the bulk. Since the biosensor current as well as the substrate concentration varies even in orders of magnitude, a dimensionless expression of the sensitivity is preferable [3, 34]. Two kinds of the dimensionless biosensor sensitivity have been investigated in this dissertation. The steady-state current is used in the case of the first kind, while the maximal current is used for the second kind,

$$B_{st}(s_0) = \frac{\mathrm{d}j_{st}(s_0)}{\mathrm{d}s_0} \times \frac{s_0}{j_{st}(s_0)}, \tag{18a}$$

$$B_{max}(s_0) = \frac{\mathrm{d}j_{max}(s_0)}{\mathrm{d}s_0} \times \frac{s_0}{j_{max}(s_0)}, \tag{18b}$$

where B_{st} and B_{max} stand for the dimensionless sensitivities of the amperometric biosensor, $j_{st}(s_0)$ is the density of the steady-state biosensor current calculated at the substrate concentration s_0, and $j_{max}(s_0)$ is the maximal value of the density of the biosensor current calculated at the concentration s_0.

1.1.7. Dimensionless Model

In order to define the main governing parameters of the mathematical model, the dimensionless mathematical model has been derived.

For simplicity, the concentrations s and p of the substrate and the product, respectively, can be defined in entire domain $x \in [0, d_e + d_d]$ as follows ($t \geq 0$):

$$s = \begin{cases} s_e, & 0 \leq x \leq d_e\,, \\ s_d, & d_e < x \leq d_e + d_d \end{cases} \tag{19a}$$

$$p = \begin{cases} p_e, & 0 \leq x \leq d_e\,, \\ p_d, & d_e < x \leq d_e + d_d\,. \end{cases} \tag{19b}$$

Both concentration functions (s and p) are continuous in the entire domain $x \in [0, d_e + d_d]$. Table 1 presents all the dimensionless parameters of the model.

The governing equations in enzyme layer (14) in dimensionless coordinates are expressed as follows:

$$\frac{\partial S}{\partial T} = \frac{\partial^2 S}{\partial X^2} - \sigma^2 \frac{S}{\left(1 + P/\hat{K}_p\right) + S\left(1 + S/\hat{K}_s\right)}, \tag{20a}$$

$$\frac{\partial P}{\partial T} = \frac{D_{p_e}}{D_{s_e}} \frac{\partial^2 P}{\partial X^2} + \sigma^2 \frac{S}{\left(1 + P/\hat{K}_p\right) + S\left(1 + S/\hat{K}_s\right)}, \tag{20b}$$

where

$$\sigma^2 = \frac{V_{max} d_e^2}{D_{s_e} K_M}, \quad 0 < X < 1, \quad T > 0. \tag{21}$$

Assuming the same diffusivities for both species, the substrate and the product, the dimensionless model contains only six following parameters: δ_d - the thickness

1 Table. Dimensional and dimensionless parameters

Parameter	Dimensional	Dimensionless
Time	t, s	$T = tD_{s_e}/d_e^2$
Membrane thickness	d_e, cm	$\delta_e = d_e/d_e = 1$
Diffusion layer thickness	d_d, cm	$\delta_d = d_d/d_e$
Distance from electrode	x, cm	$X = x/d_e$
Substrate concentration	s, mol/l	$S = s/K_M,\ S_0 = s_0/K_M$
Product concentration	p, mol/l	$P = p/K_M$
Michaelis constant	K_M, mol/l	$\hat{K}_M = K_M/K_M = 1$
Substrate inhibition constant	K_s, mol/l	$\hat{K}_s = K_s/K_M$
Product inhibition constant	K_p, mol/l	$\hat{K}_p = K_p/K_M$
Current density	j, A cm^{-2}	$J = jd_e/(n_eFD_{p_e}K_M)$

of the diffusion layer, S_0 - the concentration of the substrate in the bulk, $\hat{K}_s$ - the substrate inhibition constant, $\hat{K}_p$ - the product inhibition constant, σ^2 - the diffusion modulus, and $D_{rel} = D_{s_d}/D_{s_e} = D_{p_d}/D_{p_e}$ - the ratio of the diffusivity in the diffusion layer to the diffusivity in the enzyme layer.

(The full dimensionless model is provided in the doctoral dissertation.)

1.2. Modelling of Biosensor with Synergistic Substrates

Oxidases are the kind of enzymes commonly used in biosensors. In the case of oxidases, enzyme molecules are reduced to their intermediate state and then oxidised by electron acceptors [6, 35]. The reduction process as a rule is a specific reaction, while the oxidation of the reduced enzyme by electron acceptors is less specific. Many electron acceptors can be employed in this process [36].

The kinetic analysis of the reaction of a mixture of low and high reactive electron acceptors shows that the reduction of a low reactive electron acceptor may substantially increase if the rate of the cross reaction between these substances is high enough. This phenomenon is employed in biosensors for a high sensitivity determination of synergistic substrates [37–39].

1.2.1. Chemical Reactions in Biosensor

We consider the following kinetic scheme used for the determination of synergistic substrates [37]:

$$\mathrm{E}_{ox} + \mathrm{R} \xrightarrow{k_1} \mathrm{E}_{red} + \mathrm{P} \tag{22a}$$

$$\mathrm{E}_{red} + \mathrm{S}_1 \xrightarrow{k_2} \mathrm{E}_{ox} + \mathrm{P}_1 \tag{22b}$$

$$\mathrm{E}_{red} + \mathrm{S}_2 \xrightarrow{k_3} \mathrm{E}_{ox} + \mathrm{P}_2 \tag{22c}$$

$$\mathrm{S}_1 + \mathrm{P}_2 \xrightarrow{k_4} \mathrm{P}_1 + \mathrm{S}_2 \tag{22d}$$

$$\mathrm{P}_1 \longrightarrow \mathrm{S}_1 + e^- \tag{22e}$$

$$\mathrm{P}_2 \longrightarrow \mathrm{S}_2 + e^- \tag{22f}$$

where E_{ox} and E_{red} stand for oxidized and reduced enzyme forms, respectively, R is the enzyme reducer, S_1 and S_2 are the substrates, P, P_1 and P_2 are the products of the reactions, and k_1, k_2, k_3, k_4 are the reaction rate constants.

The reactions (22a) and (22b) may be considered as the reduction of a low reactive electron acceptor S_1 by R catalyzed by the enzyme. In the reaction (22a), the enzyme is reduced by the reducer R, and an intermediate form of enzyme (E_{red}) is formed. In the reaction (22b), the electron acceptor S_1 is reduced by the E_{red}, the product P_1 is formed and enzyme molecules regain their primary oxidized form E_{ox}.

When the high reactive electron acceptor S_2 is introduced, two more reactions, (22c) and (22d), start. The reaction (22c) represents the enzyme oxidation by the high reactive electron acceptor S_2. The reaction (22d) is called a cross reaction. In the latter reaction, the substrate S_1 oxidizes the product P_2 and regenerates the substrate S_2. This reaction also produces the product P_1.

Reactions (22e) and (22f) are electrochemical reactions that take place on the biosensor electrode. Both reactions are assumed fast and irreversible due to the high electrode potential.

$[\mathrm{Fe(CN)_6}]^{3-}$ ion may be employed as the low reactive electron acceptor S_1 [37, 39]. In this case, $[\mathrm{Fe(CN)_6}]^{4-}$ ion would be the reaction product P_1. Several examples of high reactive electron acceptors are proposed in the arcticle of Kulys and Tetianec [37].

1.2.2. Biosensor Structure

The biosensor is considered as an electrode with a relatively thin layer of enzyme solution trapped on the surface of the electrode by applying a dialysis membrane [37]. The biosensor model involves four regions: the enzyme layer where the enzymatic and chemical reactions as well as the mass transport by diffusion take place, a dialysis membrane, a diffusion limiting region where only chemical reactions as well as the mass transport by diffusion take place, and a convective region where the analyte concentration is maintained constant, see Figure 1. Assuming a homogeneous distribution of the enzyme in the enzyme layer of the uniform thickness and symmetrical

geometry of the dialysis membrane leads to the mathematical model of the biosensor action defined in a one-dimensional-in-space domain [16, 17].

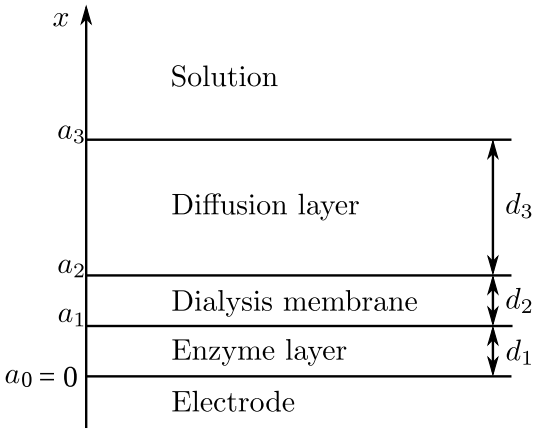

Figure 1. Principal structure of a biosensor.

Let us define d_1, d_2 and d_3 as the thicknesses of the enzyme, dialysis membrane and diffusion layers, respectively. We will also need values representing the distances between the electrode surface and the boundaries of the regions. Let a_1, a_2 and a_3 be the distances between the electrode surface and one of those boundaries shown in Figure 1.

The diffusion layer ($a_2 < x < a_3$) may be treated as the Nernst diffusion layer [30]. According to the Nernst approach a layer of thickness d_3 remains unchanged with time. It was assumed that away from it the solution is uniform in concentration.

1.2.3. Governing Equations

The governing equations for a chemical reaction network can be formulated by the law of mass action [6, 29]. Coupling reactions in the enzyme layer with the one-dimensional-in-space diffusion, described by the Fick‘s second law, leads to the following equations of the reaction–diffusion type ($0 < x < a_1$, $t > 0$):

$$\frac{\partial e_{ox}}{\partial t} = -k_1 e_{ox} r_1 + k_2 e_{red} s_{11} + k_3 e_{red} s_{21}, \tag{23a}$$

$$\frac{\partial e_{red}}{\partial t} = k_1 e_{ox} r_1 - k_2 e_{red} s_{11} - k_3 e_{red} s_{21}, \tag{23b}$$

$$\frac{\partial r_1}{\partial t} = D_{R1} \frac{\partial^2 r_1}{\partial x^2} - k_1 e_{ox} r_1, \tag{23c}$$

$$\frac{\partial s_{11}}{\partial t} = D_{S_11} \frac{\partial^2 s_{11}}{\partial x^2} - k_2 e_{red} s_{11} - k_4 s_{11} p_{21}, \tag{23d}$$

$$\frac{\partial s_{21}}{\partial t} = D_{S_21} \frac{\partial^2 s_{21}}{\partial x^2} - k_3 e_{red} s_{21} + k_4 s_{11} p_{21}, \tag{23e}$$

$$\frac{\partial p_{11}}{\partial t} = D_{P_11} \frac{\partial^2 p_{11}}{\partial x^2} + k_2 e_{red} s_{11} + k_4 s_{11} p_{21}, \tag{23f}$$

$$\frac{\partial p_{21}}{\partial t} = D_{P_21} \frac{\partial^2 p_{21}}{\partial x^2} + k_3 e_{red} s_{21} - k_4 s_{11} p_{21}, \tag{23g}$$

where x stands for space, t is time, $e_{ox}(x,t)$ and $e_{red}(x,t)$ correspond to concentrations of oxidized (E_{ox}) and reduced (E_{red}) enzyme, respectively; $s_{11}(x,t)$ and $s_{21}(x,t)$

($p_{11}(x,t)$ and $p_{21}(x,t)$) are the concentrations of substrates (products) in the enzyme layer; $r_1(x,t)$ is the reducer concentration in the enzyme layer, and D_{R1}, D_{S_11}, D_{S_21}, D_{P_11}, D_{P_21} are the diffusion coefficients of the corresponding substances defined by the subscript. In the definition of the concentrations and diffusion coefficients, here and later the last numeric subscript label denotes the region of the model, particularly, 1 stands for the enzyme layer. The molecules of both enzyme forms, E_{ox} and E_{red}, are considered as immobilized, and therefore there are no diffusion terms in the corresponding equations.

The product P does not act as a reactant in any reaction, so its concentration is not used in any further calculations. Therefore, equation system (23) contains no equation for the product P.

No enzyme molecules appear in the dialysis membrane as well as in the diffusion layer. Only the reaction (22d) as well as the mass transport by diffusion of the reducer, substrates and products take place in these two regions. The governing equations for both these layers are represented as follows ($a_{i-1} < x < a_i$, $t > 0$, $i = 2, 3$):

$$\frac{\partial r_i}{\partial t} = D_{Ri}\frac{\partial^2 r_i}{\partial x^2}; \tag{24a}$$

$$\frac{\partial s_{1i}}{\partial t} = D_{S_1 i}\frac{\partial^2 s_{1i}}{\partial x^2} - k_4 s_{1i} p_{2i}, \tag{24b}$$

$$\frac{\partial s_{2i}}{\partial t} = D_{S_2 i}\frac{\partial^2 s_{2i}}{\partial x^2} + k_4 s_{1i} p_{2i}, \tag{24c}$$

$$\frac{\partial p_{1i}}{\partial t} = D_{P_1 i}\frac{\partial^2 p_{1i}}{\partial x^2} + k_4 s_{1i} p_{2i}, \tag{24d}$$

$$\frac{\partial p_{2i}}{\partial t} = D_{P_2 i}\frac{\partial^2 p_{2i}}{\partial x^2} - k_4 s_{1i} p_{2i}, \tag{24e}$$

where $i = 2$ corresponds to the dialysis membrane, and $i = 3$ corresponds to the diffusion layer.

1.2.4. Initial Conditions

Let $x = 0$ represents the electrode surface, while $x = a_1$, $x = a_2$ and $x = a_3$ represent the boundaries between the adjacent regions as described in section 1.2.2 and shown in Figure 1. The biosensor operation starts when the reducer and the substrates appear in the bulk solution. This is used in the initial conditions ($t = 0$),

$$r_1(x,0) = s_{i1}(x,0) = p_{i1}(x,0) = 0, \quad 0 \le x \le a_1, \quad i = 1{,}2, \tag{25a}$$

$$e_{red}(x,0) = 0, \quad e_{ox}(x,0) = e_0, \quad 0 < x < a_1, \tag{25b}$$

$$r_2(x,0) = s_{i2}(x,0) = p_{i2}(x,0) = 0, \quad a_1 \le x \le a_2, \quad i = 1{,}2, \tag{25c}$$

$$p_{i3}(x,0) = 0, \quad a_2 \le x \le a_3, \quad i = 1{,}2, \tag{25d}$$

$$r_3(x,0) = s_{i3}(x,0) = 0, \quad a_2 \le x < a_3, \quad i = 1{,}2, \tag{25e}$$

$$r_3(a_3,0) = r_0, \quad s_{13}(a_3,0) = s_{10}, \tag{25f}$$

$$s_{23}(a_3,0) = \begin{cases} 0, & T_{S_2} > 0, \\ s_{20}, & T_{S_2} = 0, \end{cases} \tag{25g}$$

where e_0 stands for the total concentration of the enzyme in the enzyme layer ($e_0 = e_{ox}(x,t) + e_{red}(x,t), \forall x,t : x \in (0,a_1), t > 0$), r_0 is the reducer concentration in the bulk solution, s_{10} and s_{20} stand for substrate concentrations in the bulk solution, T_{S_2} is the moment of the substrate S_2 insertion into the bulk solution.

1.2.5. Matching Conditions

On the boundary between two regions having different diffusivities, matching conditions have to be defined ($t > 0$, $i = 1,2$, $m = 1,2$),

$$D_{Rm} \left.\frac{\partial r_m}{\partial x}\right|_{x=a_m} = D_{R,m+1} \left.\frac{\partial r_{m+1}}{\partial x}\right|_{x=a_m}, \quad r_m(a_m,t) = r_{m+1}(a_m,t), \tag{26a}$$

$$D_{S_im} \left.\frac{\partial s_{im}}{\partial x}\right|_{x=a_m} = D_{S_i,m+1} \left.\frac{\partial s_{i,m+1}}{\partial x}\right|_{x=a_m}, \quad s_{im}(a_m,t) = s_{i,m+1}(a_m,t), \tag{26b}$$

$$D_{P_im} \left.\frac{\partial p_{im}}{\partial x}\right|_{x=a_m} = D_{P_i,m+1} \left.\frac{\partial p_{i,m+1}}{\partial x}\right|_{x=a_m}, \quad p_{im}(a_m,t) = p_{i,m+1}(a_m,t), \tag{26c}$$

where $m = 1$ corresponds to the boundary between the enzyme layer and the dialysis membrane, whereas $m = 2$ corresponds to the boundary between the dialysis membrane and the diffusion layer.

These conditions mean that fluxes of the reducer, substrates and products through one region are equal to the corresponding fluxes entering the surface of the neighbouring region. Concentrations of the reducer, substrates and products in one region versus the neighbouring region are assumed to be equal.

1.2.6. Boundary Conditions

In the bulk solution, concentrations of the reducer, substrates and products remain constant ($t > 0$),

$$r_3(a_3,t) = r_0, \tag{27a}$$

$$s_{13}(a_3,t) = s_{10}, \tag{27b}$$

$$s_{23}(a_3,t) = \begin{cases} 0, & t < T_{S_2}, \\ s_{20}, & t \geq T_{S_2}, \end{cases} \tag{27c}$$

$$p_{i3}(a_3,t) = 0, \quad i = 1,2. \tag{27d}$$

The reaction products P_1 and P_2 take part in the electrochemical reactions (22e) and (22f), respectively, at the electrode surface ($x = 0$). Rates of those reactions are so high that the concentrations of P_1 and P_2 at the electrode surface are permanently reduced to zero ($t > 0$) [37],

$$p_{11}(0,t) = 0, \tag{28a}$$

$$p_{21}(0,t) = 0. \tag{28b}$$

Since the reaction (22e) produces as much S_1 as it consumes P_1, the concentration flux of P_1 on the electrode surface is equal to the concentration flux of S_1 but in opposite direction. The same assumption is also ussumed for the reaction (22f) and substances S_2 and P_2. These relations are expressed by the following boundary

conditions ($t > 0$):

$$D_{P_1 1} \left.\frac{\partial p_{11}}{\partial x}\right|_{x=0} = -D_{S_1 1} \left.\frac{\partial s_{11}}{\partial x}\right|_{x=0}, \tag{29a}$$

$$D_{P_2 1} \left.\frac{\partial p_{21}}{\partial x}\right|_{x=0} = -D_{S_2 1} \left.\frac{\partial s_{21}}{\partial x}\right|_{x=0}. \tag{29b}$$

The reducer R is electrode-inactive substance, thus its concentration flux on the electrode surface is equal to zero ($t > 0$),

$$D_{R1} \left.\frac{\partial r_1}{\partial x}\right|_{x=0} = 0. \tag{30}$$

1.2.7. Biosensor Response

The measured current is usually assumed as the response of an amperometric biosensor in physical experiments. When modelling the biosensor action, due to the direct proportionality of the current to the area of the electrode surface, the current is often normalized with that area [16, 34]. The biosensor current density $j(t)$ at time t was expressed explicitly from Faraday's and Fick's laws,

$$j_1(t) = n_1 F D_{P_1 1} \left.\frac{\partial p_{11}}{\partial x}\right|_{x=0}, \tag{31a}$$

$$j_2(t) = n_2 F D_{P_2 1} \left.\frac{\partial p_{21}}{\partial x}\right|_{x=0}, \tag{31b}$$

$$j(t) = j_1(t) + j_2(t), \tag{31c}$$

where $j_1(t)$ and $j_2(t)$ are the faradaic current densities generated by the electrochemical reactions (22e) and (22f), respectively, n_1 and n_2 are the numbers of electrons involved in a charge transfer at the electrode surface in reactions (22e) and (22f), respectively.

We assume that the system approaches a steady state as $t \to \infty$,

$$j_{st} = \lim_{t\to\infty} j(t), \tag{32a}$$

$$j_{1st} = \lim_{t\to\infty} j_1(t), \tag{32b}$$

$$j_{2st} = \lim_{t\to\infty} j_2(t), \tag{32c}$$

where j_{st}, j_{1st} and j_{2st} are the steady-state biosensor current densities.

Since the current is generated due to two electrochemical reactions (22e) and (22f), it is important to investigate the influence of each of them to the overall biosensor response. The contribution of the electrochemical reaction (22e) into the overall biosensor response was expressed as the ratio of the density of the steady-state current j_{1st} to the density j_{st} of the overall steady-state current,

$$\Lambda_1 = \frac{j_{1st}}{j_{st}}. \tag{33}$$

The sensitivity is also one of the most important characteristics of biosensors [3,

40]. The biosensor sensitivity is usually expressed as the gradient of the biosensor current with respect to the concentration of the substrate in the bulk. Since the biosensor current as well as the substrate concentration varies even in orders of magnitude, a dimensionless expression of the sensitivity is preferable [34, 41].

The biosensor based on synergistic reactions is designed to measure concentration of the substrate S_2 [37]. The dimensionless biosensor sensitivity B to the concentration of the substrate S_2 was defined as follows:

$$B(s_{20}) = \frac{\mathrm{d}j_{st}(s_{20})}{\mathrm{d}s_{20}} \times \frac{s_{20}}{j_{st}(s_{20})}, \tag{34}$$

where $j_{st}(s_{20})$ is the density of the steady-state biosensor current calculated at the concentration s_{20} of the substrate S_2 in the bulk. $B(s_{20})$ denotes the biosensor sensitivity at this concentration.

The chemical signal amplification caused by a synergistic effect is another important characteristic of this type of biosensors [37]. A measure Ψ of the synergistic effect was expressed as the ratio of the biosensor response to a substrate S_2–containing analyte to the response of the corresponding S_2–free analyte,

$$\Psi(s_{20}) = \frac{j_{st}(s_{20})}{j_{st}(0)}, \tag{35}$$

where $\Psi(s_{20})$ is the dimensionless ratio of the signal amplification obtained by the insertion of the substrate S_2 of the concentration s_{20} into the buffer solution [37, 42].

1.2.8. Dimensionless Model

In order to reduce the number of parameters and define the main governing parameters of the mathematical model, the dimensionless mathematical model has been derived.

For simplicity, the concentrations of substances can be defined in entire domain $x \in [0, a_3]$ as follows ($t \geq 0$, $m = 1, 2$):

$$r = \begin{cases} r_1, & 0 \leq x \leq a_1\,, \\ r_2, & a_1 < x \leq a_2\,, \\ r_3, & a_2 < x \leq a_3\,, \end{cases} \tag{36a}$$

$$s_m = \begin{cases} s_{m1}, & 0 \leq x \leq a_1\,, \\ s_{m2}, & a_1 < x \leq a_2\,, \\ s_{m3}, & a_2 < x \leq a_3\,, \end{cases} \tag{36b}$$

$$p_m = \begin{cases} p_{m1}, & 0 \leq x \leq a_1\,, \\ p_{m2}, & a_1 < x \leq a_2\,, \\ p_{m3}, & a_2 < x \leq a_3\,. \end{cases} \tag{36c}$$

Concentration functions (r, s_m ir p_m, $m = 1, 2$) are continuous in the entire domain $x \in [0, a_3]$. Table 2 presents all the dimensionless parameters of the model.

The governing equations in enzyme layer (23) in dimensionless coordinates are

2 Table. Dimensional and dimensionless parameters

Parameter	Dimensional	Dimensionless
Time	t, s	$T = tD_{R1}/d_1^2$
S_2 insertion time	T_{S_2}, s	$\hat{T}_{S_2} = T_{S_2} D_{R1}/d_1^2$
Layer thickness $m = 1..3$	d_m, cm	$\delta_m = d_m/d_1$
Layer boundary $m = 1..3$	a_m, cm	$\lambda_m = a_m/d_1$
Distance from electrode	x, cm	$X = x/d_1$
E_{ex} concentration	$e_{ox}, \mathrm{mol/l}$	$\hat{E}_{ox} = e_{ox}/e_0$
E_{red} concentration	$e_{red}, \mathrm{mol/l}$	$\hat{E}_{red} = e_{red}/e_0$
R concentration	$r, \mathrm{mol/l}$	$\hat{R} = r/e_0, \hat{R}_0 = r_0/e_0$
S_m concentration $m = 1, 2$	$s_m, \mathrm{mol/l}$	$\hat{S}_m = s_m/e_0, \hat{S}_{m0} = s_{m0}/e_0$
P_m concentration $m = 1, 2$	$p_m, \mathrm{mol/l}$	$\hat{P}_m = p_m/e_0$
Current density	$j, \mathrm{A\,cm^{-2}}$	$J = jd_1/(n_e F D_{P_1 1} e_0)$
Steady-state current density	$j_{st}, \mathrm{A\,cm^{-2}}$	$J_{st} = j_{st} d_1/(n_e F D_{P_1 1} e_0)$
Reaction rate constant $m = 1..4$	$k_m, \mathrm{l\,mol^{-1}\,s^{-1}}$	$\hat{k}_m = k_m/k_1$

expressed as follows ($0 < X < \lambda_1$, $T > 0$):

$$\frac{\partial \hat{E}_{ox}}{\partial T} = -\alpha \hat{E}_{ox} \hat{R} + \alpha \hat{k}_2 \hat{E}_{red} \hat{S}_1 + \alpha \hat{k}_3 \hat{E}_{red} \hat{S}_2, \tag{37a}$$

$$\frac{\partial \hat{E}_{red}}{\partial T} = \alpha \hat{E}_{ox} \hat{R} - \alpha \hat{k}_2 \hat{E}_{red} \hat{S}_1 - \alpha \hat{k}_3 \hat{E}_{red} \hat{S}_2, \tag{37b}$$

$$\frac{\partial \hat{R}}{\partial T} = \frac{\partial^2 \hat{R}}{\partial X^2} - \alpha \hat{E}_{ox} \hat{R}, \tag{37c}$$

$$\frac{\partial \hat{S}_1}{\partial T} = \frac{D_{S_1 1}}{D_{R1}} \frac{\partial^2 \hat{S}_1}{\partial X^2} - \alpha \hat{k}_2 \hat{E}_{red} \hat{S}_1 - \alpha \hat{k}_4 \hat{S}_1 \hat{P}_2, \tag{37d}$$

$$\frac{\partial \hat{S}_2}{\partial T} = \frac{D_{S_2 1}}{D_{R1}} \frac{\partial^2 \hat{S}_2}{\partial X^2} - \alpha \hat{k}_3 \hat{E}_{red} \hat{S}_2 + \alpha \hat{k}_4 \hat{S}_1 \hat{P}_2, \tag{37e}$$

$$\frac{\partial \hat{P}_1}{\partial T} = \frac{D_{P_1 1}}{D_{R1}} \frac{\partial^2 \hat{P}_1}{\partial X^2} + \alpha \hat{k}_2 \hat{E}_{red} \hat{S}_1 + \alpha \hat{k}_4 \hat{S}_1 \hat{P}_2, \tag{37f}$$

$$\frac{\partial \hat{P}_2}{\partial T} = \frac{D_{P_2 1}}{D_{R1}} \frac{\partial^2 \hat{P}_2}{\partial X^2} + \alpha \hat{k}_3 \hat{E}_{red} \hat{S}_2 - \alpha \hat{k}_4 \hat{S}_1 \hat{P}_2, \tag{37g}$$

where $\alpha = k_1 e_0 d_1^2 / D_{R1}$.

(The full dimensionless model is provided in the doctoral dissertation.)

2. Automated Modelling of Biosensors

The modelling of biosensors consists of several steps. First of all the mathematical model is created, then numerical model is derived from the mathematical model and finally the software solving the problem should be created. The software is then used to investigate properties and behaviour of a particular biosensor. It is desirable to automate a part of the work in order to create a computational model of a new biosensor easier and faster.

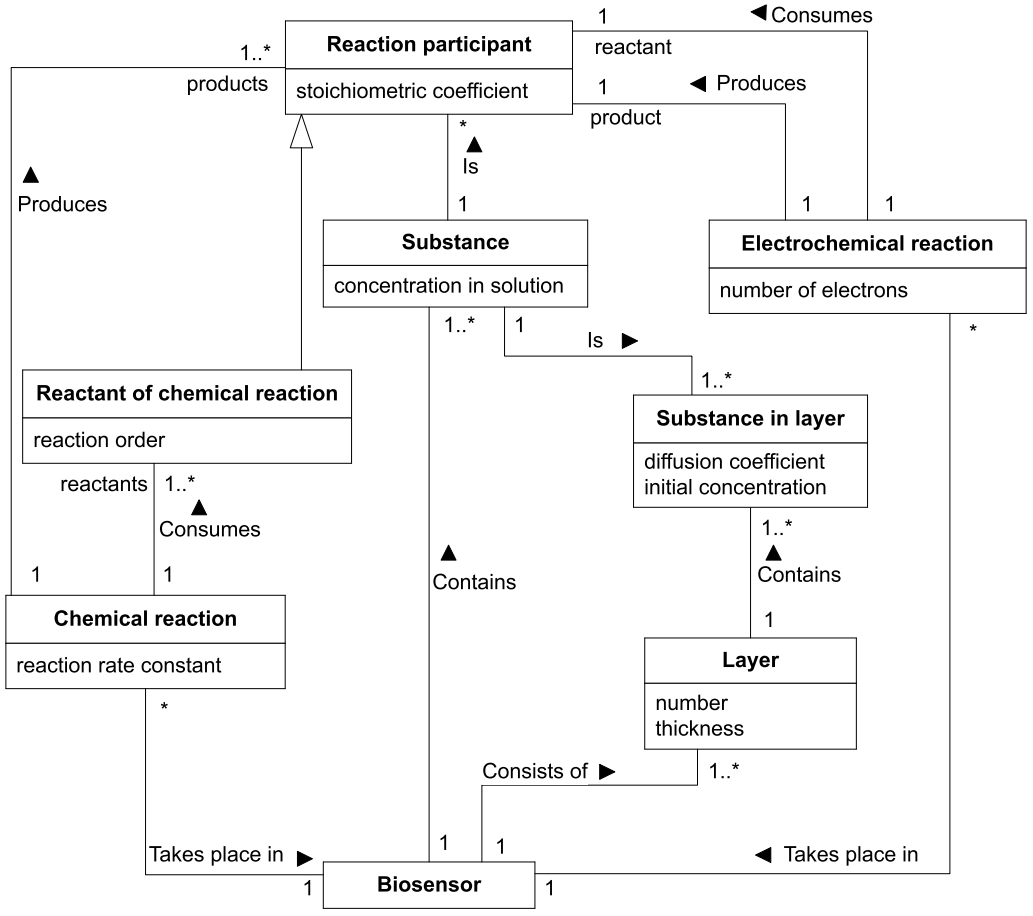

Figure 2. The universal domain model of a biosensor.

To meet this need a software was developed which automates the first three steps of biosensor modelling. The user enters data about the biosensor being modelled and the software performs necessary calculations.

During a design phase of the software development, the universal domain model (conceptual model) of a biosensor was created [43]. The universal biosensor model defines biosensors with substrate and product inhibition as well as biosensors with synergistic substrates. The model provides means for flexible definition of a biosensor structure and chemical kinetics. Model may define a custom number of biosensor layers which may contain initial concentrations of substances. The model allows definition of stoichiometric coefficients as well as reaction orders with respect to any reactant. The domain model was visualized by UML class diagram depicted in Figure 2.

Using the universal biosensor domain model, the universal mathematical model was derived.

Let us consider that there are P substances that participate in chemical or electrochemical reactions in biosensor (both reactants and products are called reaction participants).

Let su denote these substances M_1, M_2, ..., M_P. There are R chemical reactions

that take place in a biosensor. Let us denote them R_1, R_2, ..., R_R:

$$\begin{aligned}
&\mathrm{R}_1 : (-\nu_{a1})\mathrm{M}_a + (-\nu_{b1})\mathrm{M}_b + \ldots \xrightarrow{k_1} \nu_{c1}\mathrm{M}_c + \nu_{d1}\mathrm{M}_d + \ldots ,\\
&\ldots\\
&\mathrm{R}_R : (-\nu_{eR})\mathrm{M}_e + (-\nu_{fR})\mathrm{M}_f + \ldots \xrightarrow{k_R} \nu_{gR}\mathrm{M}_g + \nu_{hR}\mathrm{M}_h + \ldots ,\\
&a, b, c, d, e, f, g, h = 1, \ldots, P,
\end{aligned} \tag{38}$$

where k_1, ..., k_R are reaction rate constants, ν_{pn} is the stoichiometric coefficient of substance M_p in the reaction R_n, stoichiometric coefficients that appear on the left side of an equation are negative and on the right side are positive. In case when a substance M_p is not a reaction R_n participant, then $\nu_{pn} = 0$.

There are some number E electrochemical reactions that take place in a biosensor. Let us denote them E_1, E_2, ..., E_E:

$$\begin{aligned}
&\mathrm{E}_1 : \theta_{a1}\mathrm{M}_a \pm n_1 e^- \rightarrow \theta_{b1}\mathrm{M}_b ,\\
&\ldots\\
&\mathrm{E}_E : \theta_{c1}\mathrm{M}_c \pm n_E e^- \rightarrow \theta_{d1}\mathrm{M}_d ,\\
&a, b, c, d = 1, \ldots, P.
\end{aligned} \tag{39}$$

where θ_{pl} is the stoichiometric coefficient of substance M_p in the electrochemical reaction E_l. In case when a substance M_p is not an electrochemical reaction E_l participant, then $\theta_{pl} = 0$. n_l is the number of electrones transferred during an electrochemical reaction E_l.

Using the second Fick's law and the law of mass action equations of reaction-diffusion type were defined:

$$\begin{aligned}
&\frac{\partial m_p^{(l)}}{\partial t} = D_{M_p}^{(l)} \frac{\partial^2 m_p^{(l)}}{\partial x^2} + \sum_{n=1}^{R} \nu_{pn} k_n \prod_{r=1}^{P} (m_r^{(l)})^{\alpha_{nr}},\\
&x \in (a_{l-1}, a_l), \quad a_l = a_{l-1} + d_l, \quad p = 1, \ldots, P, \quad l = 1, \ldots, L,
\end{aligned} \tag{40}$$

where x and t stand for space and time, respectively, $m_p^{(l)}(x,t)$ is the concentration of the substance M_p in the l-th layer, d_l is the thickness of l-th layer, a_l is the interface of l-th and (l+1)-th layers, $a_0 = 0$. $D_{M_p}^{(l)}$ is the diffusion coefficient of a substance M_p in the l-th layer, L is the number of biosensor layers, α_{np} is the reaction order of reaction R_n with respect to the substance M_p. In case when substance M_p is not a reactant of reaction R_n, then $\alpha_{np} = 0$.

Using the universal biosensor mathematical model, the universal numerical model was derived. Equations of numerical model were solved using developed software.

The software consists of two parts: the calculator and the user interface. Calculator is the main part of the software. It was implemented as a dynamic library `libuniversal_calculator.so`. The dynamic library was developed in C programming language [44]. The software is open source project and is available to download at https://github.com/dainiussimelevicius/Universal-Calculator-Library.git.

3. Computational Investigation of Biosensor Properties

Properties and behaviour of biosensors were investigated. The results of computational experiments are provided in this section. Experiments were performed using the software introduced in section 2.

3.1. Computational Investigation of Biosensor Properties in the Case of Substrate and Product Inhibition

3.1.1. Computational Experiments

The biosensor response time in computational experiments was calculated using the following formula:

$$t_r = \min_{t \geq t_{min}} \left\{ t : \frac{t}{j(t)} \left| \frac{\mathrm{d}j(t)}{\mathrm{d}t} \right| < \varepsilon \right\}, \quad j(t_r) \approx j_{st}, \tag{41}$$

where t_r is biosensor response time, t_{min} - minimal response time, ε is the decay constant.

In all the numerical experiments the following values were kept constant:

$$\begin{aligned} &D_{s_e} = D_{p_e} = 100\,\mu\mathrm{m}^2/\mathrm{s}, \quad D_{s_d} = D_{p_d} = 200\,\mu\mathrm{m}^2/\mathrm{s}, \\ &K_M = 0{,}01\,\mathrm{mol/l}, \quad d_e = 10\,\mu\mathrm{m}, \quad n_e = 1, \\ &t_{min} = 5000s, \quad \varepsilon = 10^{-3}. \end{aligned} \tag{42}$$

3.1.2. Biosensor Sensitivity vs. Substrate Concentration

To investigate the dependence of the biosensor sensitivity on the substrate concentration, the biosensor response was simulated at two values of the substrate as well as the product inhibition rates and a wide range of substrate concentrations ($0.01 \leq S_0 \leq 100$). Having the simulated responses, both kinds of the biosensor dimensionless sensitivity, B_{max} and B_{st}, were calculated. Calculation results are depicted in Figure 3.

Looking at Figure 3 one can notice several distinctly different shapes of curves. Curves 1 and 2 corresponding to the biosensors with no substrate inhibition contain noticeably wider segments of the very high sensitivity (B_{max} and B_{st} equal approximately 1) than those corresponding to the biosensors with the substrate inhibition (curves 3 and 4). The substrate inhibition of the rate $\hat{K}_s = 0.1$ leads to an approximately tenfold decrease in the upper boundary of the substrate concentrations at which the biosensor operation is highly sensitive. Both kinds of the sensitivity (B_{max} and B_{st}) of the biosensors with the substrate inhibition reach zero at the substrate concentration S_0 between 2.1 and 2.5, while the biosensors with no substrate inhibition show a fairly good (not less than 0.5) sensitivity up to the concentration of $S_0 = 16$ when $B_{max} \approx 0.5$. The effect of the product inhibition on the biosensor sensitivity (B_{max} as well as B_{st}) is rather low.

One can see in Figure 3 that the sensitivity of the biosensors with the substrate inhibition can be even negative. A negative biosensor sensitivity means that the maximal (in the case of B_{max}) or the steady-state (in the case of B_{st}) current decreases with an increase in the substrate concentration. In the case of the sensitivity based on the maximal response (Figure 3a), negative values of B_{max} remains near zero ($0.02 < |B_{max}| < 0.06$). The biosensors acting under the substrate inhibition of $\hat{K}_s = 0.1$ and

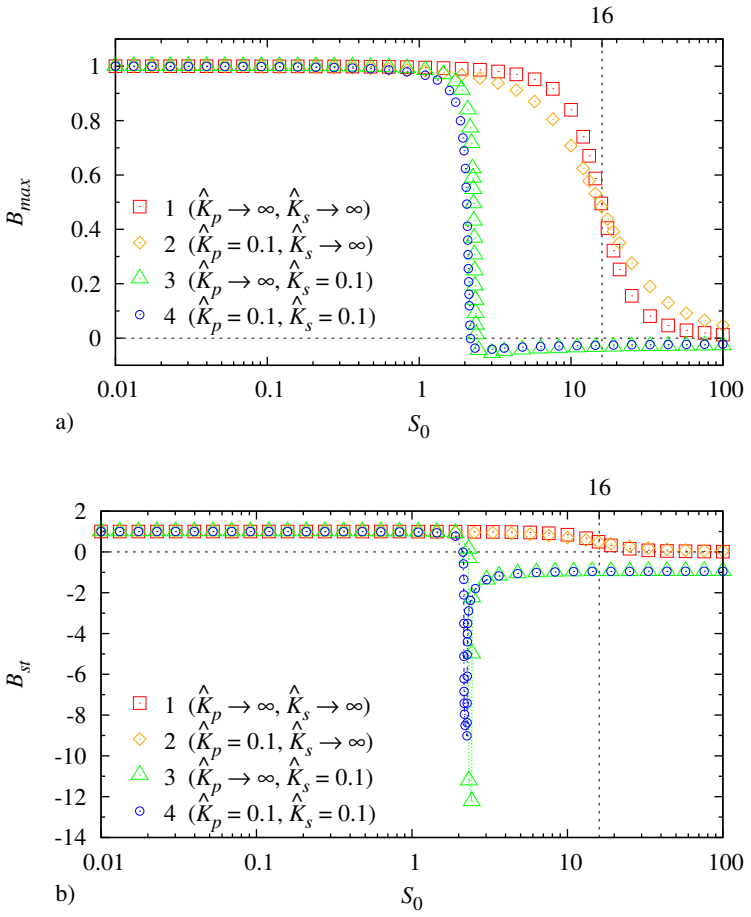

Figure 3. The dependence of biosensor sensitivity B_{max} (a) as well as B_{st} (b) on the substrate concentration S_0; $\delta_d = 30$.

measuring only the maximal current are practically inapplicable to the prediction of the substrate concentrations higher than about $2K_M$ ($S_0 > 2$, $s_0 > 2K_M$).

When comparing the sensitivity B_{st} with the sensitivity B_{max} of the biosensors with the substrate inhibition, one can see noticeable difference in the shape of the curves presenting both kinds of the biosensor sensitivity. However, the difference is only observed at relatively high substrate concentrations ($S_0 > 2$) when both sensitivities, B_{max} and B_{st}, are negative.

The biosensors acting under the substrate inhibition and supporting the sensitivity B_{st} can be successfully applied to predict the substrate concentrations in the range where B_{st} is negative. Such intelligent biosensor should support two calibration curves, one for the concentrations at which the response is directly proportional to the substrate concentration, and the other for the concentrations at which the response is inversely proportional to the substrate concentration.

In the case with only the substrate inhibition (curve 3 in Figure 3b), the biosensor sensitivity B_{st} reaches its negative peak at $S_0 = 2.4$ ($B_{st} = -12.2$). In the case with the substrate and the product inhibition (curve 4), the B_{st} reaches its minimal value at $S_0 = 2.3$ ($B_{st} = -9.0$). The points where the sensitivity curves show minimal values are the points where the biosensor steady-state response curves are the most steeply declined (see Figure 4). At higher substrate concentrations up to $S_0 = 100$, the biosensor shows also very good values of sensitivity (B_{st} steadies at about -0.9,

see Figure 3b).

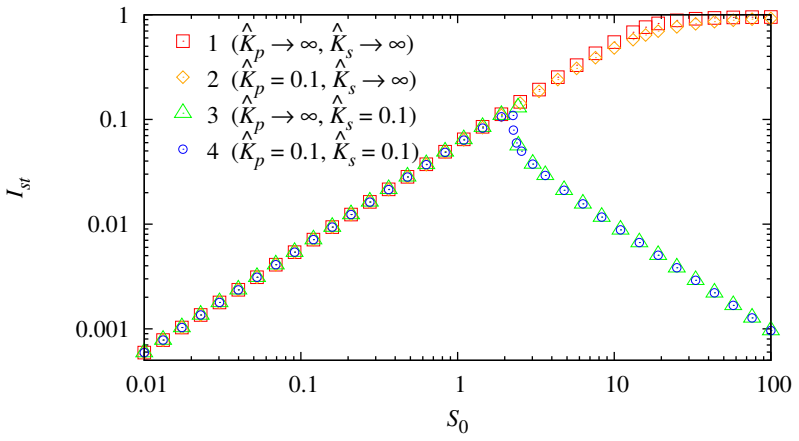

Figure 4. The dependence of the steady-state dimensionless current I_{st} on the substrate concentration S_0; $\delta_d = 30$.

However regardless high absolute values of the sensitivity B_{st}, the biosensors with the substrate inhibition has a serious drawback. The measured steady-state current is not enough for the substrate concentration prediction. As one can clearly see in Figure 4 that the steady-state current I_{st} is a non-monotonous function of the substrate concentration S_0. Because of this, an additional information is required to predict S_0 unambiguously. For instance, in addition to the steady-state current I_{st}, an intelligent biosensor could also take into consideration the maximal current I_{max}. If $I_{st} < I_{max}$ then the biosensor should use the calibration curve where I_{st} is inversely proportional to S_0. Otherwise, the concentration S_0 can be predicted under assumption of the proportionality of I_{st} to S_0. The ambiguity in the concentration prediction can be also solved if the substrate concentration S_0 is approximately known prior to the biosensor action.

After the examination of curves in Figure 3 we can conclude that the measurement of the steady-state biosensor current is more useful than the measurement of the maximal current. In the case of the absence of the substrate inhibition, the measurement of the steady-state or maximal current makes no difference. However, in the case of the substrate inhibition, the measurement of steady-state current I_{st} could be more useful because of the possibility to measure higher substrate concentrations.

3.2. Computational Investigation of Biosensor with Synergistic Substrates

3.2.1. Computational Experiments

The biosensor response time in computational experiments was calculated using the following formula:

$$t_r = \min_{t>0} \left\{ t : \frac{t}{j(t)} \left| \frac{\mathrm{d}j(t)}{\mathrm{d}t} \right| < \varepsilon \right\}, \quad j(t_r) \approx j_{st}, \tag{43}$$

In all the numerical experiments the following values were kept constant:

$$
\begin{aligned}
&k_1 = 1{,}25 \times 10^4\,\mathrm{l\,mol^{-1}\,s^{-1}}, \quad k_2 = 1{,}2 \times 10^2\,\mathrm{l\,mol^{-1}\,s^{-1}},\\
&e_0 = 0{,}04\,\mathrm{mmol/l}, \quad r_0 = 40\,\mathrm{mmol/l},\\
&D_{R1} = D_{S_i1} = D_{P_i1} = 3{,}15 \times 10^{-6}\,\mathrm{cm^2/s}, \quad i = 1, 2,\\
&D_{R2} = D_{S_i2} = D_{P_i2} = 4{,}2 \times 10^{-7}\,\mathrm{cm^2/s}, \quad i = 1, 2,\\
&D_{R3} = D_{S_i3} = D_{P_i3} = 6{,}3 \times 10^{-6}\,\mathrm{cm^2/s}, \quad i = 1, 2,\\
&d_1 = 23{,}3\,\mu\mathrm{m}, \quad d_2 = 18{,}6\,\mu\mathrm{m},\\
&n_1 = n_2 = 1, \quad \varepsilon = 10^{-3}.
\end{aligned}
$$

3.2.2. Simulating the Synergistic Effect

Understanding the nature of the synergistic effect is of crucial importance for a preparation of highly sensitive biosensors [37, 39, 45]. For a kinetic analysis of the biosensor operation, let us introduce $\bar{v}_1$, $\bar{v}_2$, $\bar{v}_3$, $\bar{v}_4$ as the average rates of the reactions (22a), (22b), (22c), (22d) taking place in the enzyme layer,

$$\bar{v}_1 = k_1 \bar{e}_{ox} \bar{r}, \tag{44a}$$
$$\bar{v}_2 = k_2 \bar{e}_{red} \bar{s}_1, \tag{44b}$$
$$\bar{v}_3 = k_3 \bar{e}_{red} \bar{s}_2, \tag{44c}$$
$$\bar{v}_4 = k_4 \bar{s}_1 \bar{p}_2, \tag{44d}$$

where $\bar{r}$, $\bar{s}_1$, $\bar{s}_2$, $\bar{p}_1$, $\bar{p}_2$, $\bar{e}_{ox}$ and $\bar{e}_{red}$ stand for the average concentrations of R, S_1, S_2, P_1, P_2, E_{ox} and E_{red} in the enzyme layer.

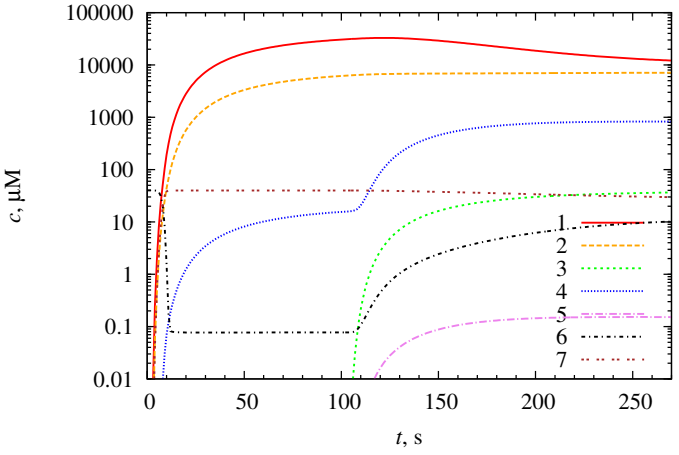

Figure 5. Average concentrations $\bar{r}$ (1), $\bar{s}_1$ (2), $\bar{s}_2$ (3), $\bar{p}_1$ (4), $\bar{p}_2$ (5), $\bar{e}_{ox}$ (6) and $\bar{e}_{red}$ (7); $d_3 = 223\,\mu\mathrm{m}$, $k_3 = 1{,}4 \times 10^6\,\mathrm{l\,mol^{-1}\,s^{-1}}$, $k_4 = 1{,}4 \times 10^6\,\mathrm{l\,mol^{-1}\,s^{-1}}$, $s_{10} = 8\,\mathrm{mmol/l}$, $s_{20} = 39\,\mu\mathrm{mol/l}$.

Figures 5 and 6 show how the synergistic effect occurs. When S_2 is introduced into the bulk solution, a fast reaction (22c) starts, and a large amount of E_{ox} and P_2 molecules are produced (see Figure 5). In the meantime the increased concentration of E_{ox} initiates an increase in the rate of the reaction (22a), molecules of P_2 start to react with S_1 in reaction (22d) (see Figure 6). A decrease of the reducer R concentration is the second indication of the increase in the reaction (22a) rate as

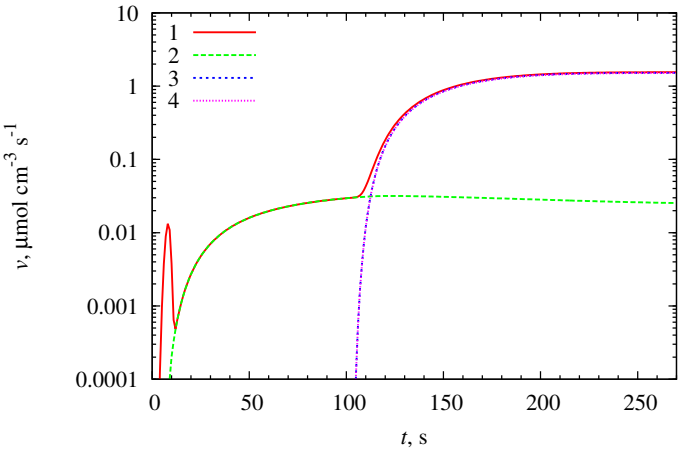

Figure 6. Average rates $\bar{v}_1$ (1), $\bar{v}_2$ (2), $\bar{v}_3$ (3), $\bar{v}_4$ (4) of the reactions (22a)-(22d) taking place in the enzyme layer (curves 3 and 4 coincide); $d_3 = 223\,\mu\text{m}$, $k_3 = 1{,}4 \times 10^6\,\text{l}\,\text{mol}^{-1}\,\text{s}^{-1}$, $k_4 = 1{,}4 \times 10^6\,\text{l}\,\text{mol}^{-1}\,\text{s}^{-1}$, $s_{10} = 8\,\text{mmol/l}$, $s_{20} = 39\,\mu\text{mol/l}$.

the reaction consumes more reactant molecules (see Figure 5). The reactions (22a) and (22c) are very strongly interrelated because E_{ox} is the product of reaction (22c), and at the same time it is the reactant of the reaction (22a) and vice versa with E_{red} molecule. Similar situation appears with the reactions (22c) and (22d) (molecules of P_2 and S_2).

The rate of the reaction (22c) is controlled by the concentration of S_2. Reactions (22a) and (22d) are accelerated by the reaction (22c) and their rates tend to reach the rate of the reaction (22c). Hence the rates of reactions (22a), (22c) and (22d) are approximately equal during almost all the time of biosensor operation as seen in Figure 6. The rate of the reaction (22b) remains almost unaffected.

The reactions (22b) and (22d) produce the product P_1 which is responsible for the biosensor current. Since the rate of the reaction (22b) remains almost unchanged and the rate of the reaction (22d) is greater than the rate of (22b) by two orders of magnitude, the biosensor response is also increased by two orders of magnitude. In other words, the signal is amplified chemically. This is the desirable outcome of synergistic effect in this type of biosensors [37].

If the synergistic effect would not occur during the biosensor operation, that would mean that biosensor signal is not chemically amplified. Response of a biosensor with no chemical amplification could be too weak for reliable measurement. That could mean the narrower range of biosensor application because biosensor would only work at relatively high concentrations of substrate. So, it is important to choose the correct biosensor parameters that support the occurrence of the synergistic effect.

3.2.3. The Composition of Biosensor Response

The biosensor response is determined by two electrochemical reactions (22e) and (22f). It is important to understand the role of each process at certain values of the biosensor parameters.

The dependence of the biosensor response composition on the reaction rate constant $\hat{k}_4$ was investigated at different diffusion layer thicknesses (δ_3) as well as at different values of the substrate concentration $\hat{S}_{20}$. Results of the numerical simulation are depicted in Figure 7.

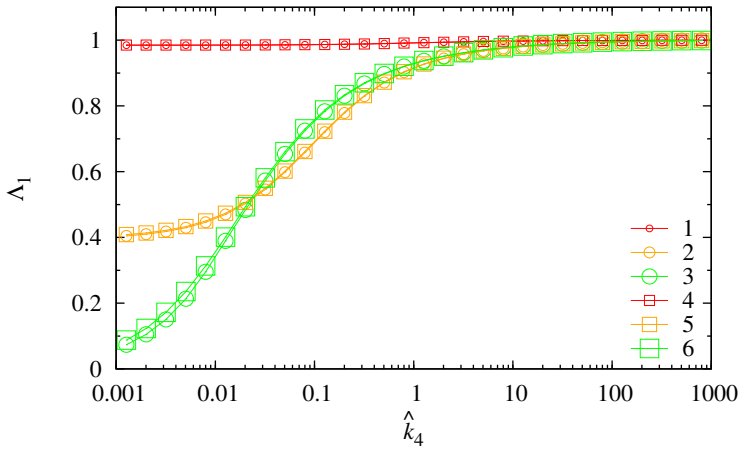

Figure 7. The dependence of the ratio Λ_1 on the reaction rate constant $\hat{k}_4$ at two thicknesses of the diffusion layer δ_3 (5 (1, 2, 3 curves) and 10 (4, 5, 6 curves)) and three concentrations $\hat{S}_{20}$ of the substrate S_2 (0,0025 (1, 4), 0,25 (2, 5) ir 25 (3, 6)); $\hat{k}_3 = 112$, $\hat{S}_{10} = 200$.

As one can see in Figure 7, the ratio Λ_1 of the density j_{1st} of the steady-state current generated by the electrochemical reactions (22e) to the density j_{st} of the overall steady-state current is directly proportional to the reaction rate constant $\hat{k}_4$. This can be explained by the reaction (22d). The faster the reaction (22d) the more P_2 it consumes, and molecules of P_2 do not reach electrode surface.

At low $\hat{S}_{20}$ concentrations, a value of $\hat{k}_4$ only slightly influences the composition of the biosensor response (curves 1 and 4). The biosensor response is almost entirely generated by the faradaic process (22e) at such concentrations of S_2. As one can see from the reaction scheme (22) that a low concentration of S_2 directly cause a low concentration of P_2. However at a high concentration $\hat{S}_{20}$ of S_2, a value of the reaction rate constant $\hat{k}_4$ makes a major impact on the composition of the biosensor response. When $\hat{k}_4$ is small enough, the ratio Λ_1 is less than 0.5, i.e., the main part of the biosensor response is generated by the faradaic process (22f).

Figure 7 also shows that the thickness δ_3 of the diffusion layer only slightly influences the composition of the biosensor response.

Conclusions

1. The computational modelling of biosensors with substrate and product inhibition as well as of biosensors with synergistic substrates may be automated. With the help of the developed software the biosensor structure and physicochemical processes occuring inside of a biosensor may be defined using domain notions, skipping the creation of mathematical and numerical models.

2. Computational modelling showed that an amperometric biosensor with substrate inhibition can measure substrate concentration at a wider concentration range than one without substrate inhibition if other biosensor parameters are equal.

3. Computational modelling may explain the significance of physical or chemical processes for biosensor operation:

(a) at certain parameter values of biosensor with synergistic substrates, biosensor response is determined by the first electrochemical reaction solely;

(b) in case when the rate of reaction producing the product responsible for biosensor response is much higher than one of another reaction producing the same product, the effect of substrate synergy amplifies a biosensor response dramatically.

Publications by the Author

1A. D. Šimelevičius, R. Baronas. Computational modelling of amperometric biosensors in the case of substrate and product inhibition. *Journal of Mathematical Chemistry*, 47(1):430–445, 2010. ISSN 0259-9791.

2A. D. Šimelevičius, R. Baronas. Amperometrinių biojutiklių su sinerginių substratų stiprinimu kompiuterinis modeliavimas. *Informacijos mokslai*, 56:174–181, 2011. ISSN 1392-0561.

3A. D. Simelevicius, R. Baronas, J. Kulys. Modelling of amperometric biosensor used for synergistic substrates determination. *Sensors*, 12(4):4897–4917, 2012. ISSN 1424-8220.

4A. D. Šimelevičius, R. Baronas. Modelling of amperometric biosensors in the case of substrate and product inhibition. *In:* A. Targamadzė, R. Butleris and R. Butkienė, eds., *Information Technologies'2008 - Proceedings of 14th International Conference on Information and Software Technologies*, pp. 125–130. Kaunas University of Technology, 2008. ISSN 2029-0020.

5A. D. Simelevicius, R. Baronas. Mechanisms controlling the sensitivity of amperometric biosensors in the case of substrate and product inhibition. *In:* A. Omerovic, D. A. Simoni and G. Bobashev, eds., *SIMUL 2011: The Third International Conference on Advances in System Simulation*, pp. 69–74. IARIA, 2011. ISBN 978-1-61208-169-4.

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Reziumė

Tiriamoji problema ir jos aktualumas

Cheminiai jutikliai yra prietaisai, transformuojantys cheminę informaciją, tokią kaip specifinės medžiagos koncentracija ar net visa medžiagų mišinio sudėtis, į kitą analizei patogų signalą. Cheminiai jutikliai dažniausiai susideda iš dviejų pagrindinių dalių: cheminio atpažinimo sistemos (receptoriaus) ir fizikocheminio keitiklio. Biojutikliai yra cheminiai jutikliai, kurių cheminio atpažinimo sistema yra paremta biocheminiu mechanizmu [1, 2]. Biojutikliai gerai tinka sparčiai kiekybinei analizei atlikti ar nuolatiniam medžiagų koncentracijų stebėjimui [3–5].

Dažnai biojutiklio atpažinimo sistemoje yra naudojami fermentai. Fermentų savybė katalizuoti tik kažkurią vieną specifinę reakciją užtikrina biojutikliams didelį selektyvumą, o tai, kad fermentai yra labai efektyvūs katalizatoriai, užtikrina didelį biojutiklių jautrumą [3, 6–10].

Biojutikliai yra pigūs ir patikimi prietaisai, dažnai pakeičiantys brangią ir lėtą kiekybinę analizę laboratorijoje. Jie plačiai naudojami įvairiose srityse: medicinoje, maisto pramonėje, aplinkosaugoje, narkotikų aptikimui [4, 11–15].

Nors biojutikliai yra palyginti pigūs, tačiau jų sukūrimui bei kalibravimui tenka įdėti nemažai pastangų, sunaudoti daug medžiagų. Tam, kad reikėtų atlikti kuo mažiau fizinių eksperimentų, galima pasinaudoti matematiniu modeliavimu. Naudojantis matematiniu biojutiklio modeliu galima numatyti biojutiklio savybes dar prieš atliekant eksperimentus laboratorijoje. Tokiu būdu galima smarkiai sumažinti eksperimentų skaičių ir sumažinti naujo biojutiklio kūrimo kaštus.

Biojutiklių matematinius modelius galima aprašyti netiesinėmis diferencialinėmis lygtimis dalinėmis išvestinėmis [16–18]. Diferencialines lygtis dažniausiai sudaro tiesinis narys, aprašantis medžiagų difuziją, ir netiesinis narys, aprašantis cheminę kinetiką. Šių lygčių tikslūs analiziniai sprendiniai yra žinomi tik atskirais atvejais, kai biojutiklio struktūra nesudėtinga, o medžiagų koncentracijos yra labai mažos arba labai didelės [16–18]. Bendruoju atveju šios diferencialinės lygtys yra sprendžiamos skaitiniais metodais, pasitelkus kompiuterį. Šioje disertacijoje diferencialinių lygčių sistemoms spręsti buvo naudojamas baigtinių skirtumų metodas [19–21].

Darbo tikslas ir sprendžiami uždaviniai

Šios disertacijos tikslas yra automatizuoti biojutiklių, kuriuose vyksta sudėtingi biokatalizės procesai: inhibicija substratu ir produktu bei substratų sinergija, kompiuterinių modelių kūrimą. Taikant kompiuterinį modeliavimą, ištirti biojutiklių savybes bei elgseną. Disertacijos tikslui įgyvendinti buvo suformuluotos tokios užduotys:

1. Pasiūlyti amperometrinio biojutiklio, kuriame vyksta inhibicija substratu ir produktu, modeliavimo būdą.
2. Pasiūlyti amperometrinio biojutiklio, kuriame vykta substratų sinergija, modeliavimo būdą.
3. Sukurti apibendrintąjį biojutiklio su inhibicija substratu ir produktu modelį, galintį lanksčiai modeliuoti biojutiklio struktūrą.
4. Sukurti universalųjį biojutiklio modelį, galintį modeliuoti plačią biojutiklių aibę. Ši aibė turi apimti biojutiklius su inhibicija substratu ir produktu bei biojutiklius

su substratų sinergija. Modelis turi leisti lanksčiai pasirinkti biojutiklio struktūrą bei jame vykstančių cheminių reakcijų kinetiką.

5. Sukurti programinę įrangą, automatizuojančią kompiuterinių modelių kūrimą pagal apibendrintąjį biojutiklio su inhibicija substratu ir produktu matematinį modelį bei pagal universalųjį biojutiklio matematinį modelį.

6. Naudojant sukurtą programinę įrangą, patvirtinti matematinių modelių adekvatumą, ištirti biojutiklių savybes bei elgseną.

Darbo naujumas ir reikšmė

Sudarytas amperometrinio biojutiklio su inhibicija substratu ir produktu apibendrintasis modelis, galintis lanksčiai aprašyti biojutiklio struktūrą.

Sudarytas universalusis biojutiklio matematinis modelis, galintis apibrėžti plačią biojutiklių aibę. Ši aibė apima biojutiklius su inhibicija substratu ir produktu, o taip pat biojutiklius su substratų sinergija. Modelis suteikia galimybę apibrėžti plačią cheminių reakcijų aibę ir lanksčiai parinkti biojutiklio struktūrą.

Sukurta programinė įranga, automatizuojanti kompiuterinių modelių kūrimą pagal minėtuosius matematinius modelius.

Taikant sukurtą programinę įrangą, detaliai ištirtos biojutiklio su substrato ir produkto ihibicija savybės ir elgsena. Programine įranga galima lanksčiai parinkti biojutiklio struktūrą, todėl galima atlikti kompiuterinio modeliavimo tyrimus ir tokiems biojutikliams, kurie šioje disertacijoje nebuvo tirti.

Taikant sukurtą programinę įrangą, detaliai ištirtos biojutiklio su substratų sinergija savybės bei elgsena. Ištirtas substratų sinergijos efekto biojutiklyje mechanizmas, nustatytos sąlygos būtinos sinergijos efektui pasireikšti. Programine įranga galima tirti žymiai platesnę biojutiklių aibę nei buvo tirta šioje disertacijoje. Ši aibė apima ne tik biojutiklius su sudėtinga struktūra, bet ir biojutiklius, kuriuose vyksta cheminės reakcijos su sudėtinga reakcijų kinetika.

Naudojant programinę įrangą, galima optimizuoti biojutiklio konfigūraciją dar prieš kuriant fizinį biojutiklį. Remiantis atliktų biojutiklių savybių tyrimų rezultatais galima kurti efektyvesnius biojutiklius.

Disertacijos rengimo metu gauti rezultatai buvo panaudoti įgyvendinant dvejus mokslinius projektus: „Bioelektrokatalizė sintezėje ir analizėje (BIOSA)“, finansuotą Lietuvos mokslo tarybos (sutarties Nr. PBT-04/2010) ir „Kompiuterinių metodų, algoritmų ir įrankių efektyviam sudėtingos geometrijos biojutiklių modeliavimui ir optimizavimui sukūrimas“ finansuojamą Europos socialinio fondo lėšomis pagal priemonę „Parama mokslininkų ir kitų tyrėjų mokslinei veiklai (visuotinė dotacija)“ VP1-3.1-ŠMM-07-K (sutarties Nr. VP1-3.1-ŠMM-07-K-01-073/MTDS-110000-583).

Ginamieji teiginiai

1. Sudarytu apibendrintuoju biojutiklio su inhibicija substratu ir produktu modeliu galima lanksčiai aprašyti biojutiklio struktūrą. Sukurta programine įranga galima atlikti šiuo modeliu aprašomų biojutiklių kompiuterinį modeliavimą.

2. Sudarytu universaliuoju biojutiklio modeliu aprašomų biojutiklių aibė apima tiek biojutiklius su inhibicija substratu ir produktu, tiek biojutiklius su substratų sinergija. Universaliuoju biojutiklio modeliu galima lanksčiai aprašyti biojutiklio struktūrą ir cheminių reakcijų kinetiką. Biojutiklius, patenkančius į modeliu

aprašomų biojutiklių aibę, galima modeliuoti, pasitelkiant sukurtą programinę įrangą.

3. Amperometriniame biojutiklyje, kuriame vyksta inhibicija substratu, matuojant tiek stacionariąją, tiek maksimaliąją biojutiklio sroves, galima išmatuoti substrato koncentraciją platesniame koncentracijų intervale nei naudojant tik kažkurią vieną srovę. Tai pat šis koncentracijų intervalas yra platesnis už biojutiklio, kuriame inhibicija substratu nevyksta, darbinių koncentracijų intervalą, jeigu kiti biojutiklio parametrai sutampa.

4. Kompiuterinio modeliavimo būdu galima paaiškinti biojutikliuose pasireiškiančio substratų sinergijos efekto vidinį mechanizmą.

Išvados

1. Biojutiklio su inhibicija substratu ir produktu ir biojutiklio su substratų sinergija kompiuterinį modeliavimą galima automatizuoti. Sukurta programine įranga galima apibrėžti biojutiklio struktūrą ir jame vykstančius fizikocheminius procesus dalykinės srities sąvokomis ir atlikti kompiuterinio modeliavimo eksperimentus, nekuriant matematinio ir skaitinio biojutiklio modelių.

2. Kompiuterinis modeliavimas parodė, kad amperometrinis biojutiklis, kuriame vyksta inhibicija substratu, gali matuoti substrato koncentracijas platesniame koncentracijų intervale nei biojutiklis, kuriame inhibicija substratu nevyksta, kai visi kiti biojutiklių parametrai sutampa.

3. Taikant kompiuterinį modeliavimą, galima išsiaiškinti fizikinių ar cheminių procesų svarbą biojutiklio veikimui:

   (a) esant tam tikriems biojutiklio su substratų sinergija parametrams, jo atsaką gali apspręsti vien tik pirmoji elektrocheminė reakcija;

   (b) substratų sinergija, sustiprinanti biojutiklio atsaką chemiškai, ypač ryškiai pasireiškia tuo atveju, kai reakcija, gaminanti produktą atsakingą už biojutiklio srovę, vyksta žymiai greičiau nei kita reakcija, gaminanti šį produktą.

References

1. D. R. Thévenot, K. Toth, R. A. Durst, G. S. Wilson. Electrochemical biosensors: Recommended definitions and classification. Tech. Rep. 12, Pure and Applied Chemistry, 1999.

2. IUPAC. *Compendium of chemical terminology. Gold book.* Blackwell Scientific Publications, Oxford, 2nd edn., 1997.

3. F. W. Scheller, F. Schubert. *Biosensors*, vol. 11 of *Techniques and Instrumentation in Analytical Chemistry*. Elsevier, Amsterdam, 1992.

4. K. Rogers. Recent advances in biosensor techniques for environmental monitoring. *Analytica Chimica Acta*, 568(1-2):222–231, 2006.

5. S. Rodriguez-Mozaz, M. J. L. de Alda, M.-P. Marco, D. Barceló. Biosensors for environmental monitoring: A global perspective. *Talanta*, 65(2):291–297, 2005.

6. H. Gutfreund. *Kinetics for the Life Sciences: Receptors, Transmitters and Catalysts*. Cambridge University Press, Cambridge, 1995.

7. B. Eggins. *Chemical Sensors and Biosensors*. Analytical Techniques in the Sciences. Wiley, 2002.

8. M. Giardi, E. Piletska. *Biotechnological Applications of Photosynthetic Proteins: Biochips, Biosensors, and Biodevices*. Biotechnology Intelligence Unit. Landes Bioscience/Eurekah.com, 2006.

9. R. S. Marks, C. R. Lowe, D. C. Cullen, H. H. Weetall, I. Karube. *Handbook of Biosensors and Biochips*. John Wiley & Sons, 2007.

10. L. L. Looger, M. A. Dwyer, J. J. Smith, H. W. Hellinga. Computational design of receptor and sensor proteins with novel functions. *Nature*, 423(6936):185–190, 2003.

11. J. F. Liang, Y. T. Li, V. C. Yang. Biomedical application of immobilized enzymes. *Journal of Pharmaceutical Sciences*, 89(8):979–990, 2000.

12. K. R. Rogers. Biosensors for environmental applications. *Biosensors and Bioelectronics*, 10(6-7):533–541, 1995.

13. F. W. Scheller, F. Schubert, J. Fedrowitz. *Practical Applications*, vol. 2 of *Frontiers in Biosensorics*. Birkhäuser, Basel, 1997.

14. D. Yu, B. Blankert, J.-C. Virè, J.-M. Kauffmann. Biosensors in drug discovery and drug analysis. *Analytical Letters*, 38(11):1687–1701, 2005.

15. I. E. Tothill. Biosensors developments and potential applications in the agricultural diagnosis sector. *Computers and Electronics in Agriculture*, 30(1-3):205–218, 2001.

16. T. Schulmeister. Mathematical modelling of the dynamic behaviour of amperometric enzyme electrodes. *Selective Electrode Reviews*, 12:203–260, 1990.

17. R. Baronas, F. Ivanauskas, J. Kulys. *Mathematical Modeling of Biosensors*, vol. 9 of *Springer Series on Chemical Sensors and Biosensors*. Springer, Dordrecht, 2010.

18. J. Kulys. Development of new analytical systems based on biocatalysers. *Enzyme and Microbial Technology*, 3(4):344–352, 1981.

19. A. A. Samarskii. *The Theory of Difference Schemes*. Marcel Dekker, New York-Basel, 2001.

20. B. Kvedaras, M. Sapagovas. *Skaičiavimo metodai*. Mintis, Vilnius, 1974.

21. R. Čiegis. *Diferencialinių lygčių skaitiniai sprendimo metodai*. Technika, Vilnius, 2003.

22. J.-P. Kernevez. *Enzyme Mathematics*, vol. 10 of *Studies in Mathematics and its Applications*. North-Holland Publishing Company, Amsterdam, 1980.

23. A. Chaubey, B. Malhotra. Mediated biosensors. *Biosensors and Bioelectronics*, 17(6-7):441–456, 2002.

24. A. Cornish-Bowden. *Fundamentals of Enzyme Kinetics*. Portland Press, London, 3rd edn., 2004.

25. NC-IUB. Symbolism and terminology in enzyme kinetics. Recommendations 1981. *Biochemistry Journal*, 213:561–571, 1983.

26. R. Baronas, F. Ivanauskas, J. Kulys. The effect of diffusion limitations on the response of amperometric biosensors with substrate cyclic conversion. *Journal of Mathematical Chemistry*, 35(3):199–213, 2004.

27. J. Kulys. Biosensor response at mixed enzyme kinetics and external diffusion limitation in case of substrate inhibition. *Nonlinear Analysis: Modelling and Control*, 11(4):385–392, 2006.

28. R. S. Ochs. Understanding enzyme inhibition. *Journal of Chemical Education*, 77(11):1453, 2000.

29. P. N. Bartlett, R. G. Whitaker. Electrochemical imobilisation of enzymes: Part I. Theory. *Journal of Electroanalytical Chemistry*, 224(1-2):27–35, 1987.

30. D. Britz. *Digital Simulation in Electrochemistry*, vol. 666 of *Lecture Notes in Physics*. Springer, Berlin Heidelberg, 3rd edn., 2005.

31. B. Li, Y. Shen, B. Li. Quasi-steady state laws in enzyme kinetics. *Journal of Physical Chemistry A*, 112(11):2311–2321, 2008.

32. L. A. Segel, M. Slemrod. The quasi-steady-state assumption: a case study in perturbation. *SIAM Review*, 31(3):446–477, 1989.

33. A. Ciliberto, F. Capuani, J. J. Tyson. Modeling networks of coupled enzymatic reactions using the total quasi-steady state approximation. *PLoS Computational Biology*, 3(3):e45, 2007.

34. R. Baronas, J. Kulys. Modelling amperometric biosensors based on chemically modified electrodes. *Sensors*, 8(8):4800–4820, 2008.

35. N. J. Ronkainen, H. B. Halsall, W. R. Heineman. Electrochemical biosensors. *Chemical Society Reviews*, 39(5):1747–1763, 2010.

36. J. Kulys, L. Tetianec, I. Bratkovskaja. Pyrroloquinoline quinone-dependent carbohydrate dehydrogenase: activity enhancement and the role of artificial electron acceptors. *Biotechnology Journal*, 5(8):822–828, 2010.

37. J. Kulys, L. Tetianec. Synergistic substrates determination with biosensors. *Biosensors and Bioelectronics*, 21(1):152–158, 2005.

38. I. Bratkovskaja, R. Ivanec, J. Kulys. Mediator-assisted laccase-catalyzed oxidation of 4-hydroxybiphenyl. *Biochemistry (Moscow)*, 71:550–554, 2006.

39. J. Kulys, R. Vidziunaite. Laccase based synergistic electrocatalytical system. *Electroanalysis*, 21(20):2228–2233, 2009.

40. A. P. F. Turner, I. Karube, G. S. Wilson. *Biosensors: Fundamentals and Applications*. Oxford University Press, Oxford, 1987.

41. D. Šimelevičius, R. Baronas. Computational modelling of amperometric biosensors in the case of substrate and product inhibition. *Journal of Mathematical Chemistry*, 47(1):430–445, 2010.

42. R. Baronas, F. Ivanauskas, J. Kulys, M. Sapagovas. Computational modelling of a sensor based on an array of enzyme microreactors. *Nonlinear Analysis: Modelling and Control*, 9(3):203–218, 2004.

43. C. Larman. *Applying UML and Patterns: An Introduction to Object-Oriented Analysis and Design and the Unified Process*. Prentice Hall PTR, 2nd edn., 2001.

44. W. H. Press, S. A. Teukolsky, W. T. Vetterling, B. P. Flannery. *Numerical Recipes in C: The Art of Scientific Computing*. Cambridge University Press, Cambridge, 2nd edn., 1992.

45. J. Kulys, Ž. Dapkūnas. The effectiveness of synergistic enzymatic reaction with limited mediator stability. *Nonlinear Analysis: Modelling and Control*, 12(4):495–501, 2007.